UNIVERSIDAD AUTÓNOMA DE MADRID

PROGRAMA DE DOCTORADO EN BIOCIENCIAS MOLECULARES

ENFERMEDADES HEREDITARIAS DE LA RETINA SINDRÓMICAS: ASPECTOS CLÍNICOS Y MOLECULARES

TESIS DOCTORAL

Irene Perea Romero

Madrid, 2022

UNIVERSIDAD AUTÓNOMA DE MADRID

Facultad de Medicina

Departamento de Bioquímica

ENFERMEDADES HEREDITARIAS DE LA RETINA SINDRÓMICAS: ASPECTOS CLÍNICOS Y MOLECULARES

Memoria presentada por **Irene Perea Romero**, graduada en Biotecnología, para optar al título de Doctor por la Universidad Autónoma de Madrid

Directora de Tesis:

Dra. Carmen Ayuso García

Tutor de Tesis:

Dr. Víctor Luis Ruiz Pérez

Realizado en el Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD)

Dña. CARMEN AYUSO GARCÍA, Jefa del Servicio de Genética del Hospital Universitario Fundación Jiménez Díaz y Directora Científica del Instituto de Investigación Sanitaria Fundación Jiménez Díaz,

CERTIFICA:

Que la presente memoria titulada: "Enfermedades hereditarias de la retina sindrómicas: Aspectos clínicos y moleculares", que presenta Dña. Irene Perea Romero para obtener el Grado de Doctor, ha sido realizada bajo mi dirección, autorizándola para su presentación ante un Tribunal Calificador.

Madrid, 20/04/2022

Fdo. Dra. Carmen Ayuso García

Directora de la Tesis Doctoral

Esta Tesis Doctoral ha sido realizada gracias a la concesión por parte del Instituto de Salud Carlos III (ISCIII) de los proyectos de investigación ISCIII-FIS: PI16/00425 y PI19/00321, y el Fondo Europeo de Desarrollo Regional (FEDER).

La doctoranda ha sido financiada por el ISCIII con un contrato Predoctoral de Formación en Investigación en Salud (PFIS), FI17/00192, y a través del proyecto europeo UshTher (*EU Project* 754848).

La estancia realizada por la doctoranda en el *Institute of Molecular and Clinical Ophthalmology Basel* (Basilea, Suiza) ha sido financiada por el ISCIII con una ayuda para la movilidad del personal investigador (M-AES), MV19/00015.

"…todo está sujeto a las leyes más o menos conocidas: a la ciencia pertenece descubrirlas por medio de la observación y la experiencia…"

-Antonio Machado y Núñez-

"*Darwinismo*", Revista mensual de Filosofía, Literatura y Ciencias de Sevilla. 1872. IV, S23

AGRADECIMIENTOS

AGRADECIMIENTOS

Antes de empezar a agradecer, me gustaría pedir perdón. Perdón por si se me olvida nombrar a alguien, y, sobre todo, perdón por si he estado inaguantable durante este largo proceso llamado "Tesis".

En primer lugar, quiero dar las gracias a mi directora de Tesis, Carmen. No solo por acogerme y darme la oportunidad de formar parte de su grupo de investigación, sino por enseñarme las bases de la Genética Humana.

Gracias al Instituto de Salud Carlos III, por financiarme durante estos años.

Al IIS-FJD, en especial a Yolanda, Andrés y Oliver, por su paciencia con todos los trámites y plantillas.

Gracias a todos los compañeros de Genética por toda su ayuda y por suponer un referente desde que empecé. A quienes se han ido durante este tiempo, Esther, Rosario, Lilián, Ana A, Alejandra T, Laura, Aurora, Elvira y Perce. A Iker, por todo su trabajo previo en las sindrómicas. A Marta "delpo", por amenizarme los días. A Andrea y Lorena, por conseguir hacer esta etapa algo menos UNeasi, sin ser ellas nada de eso. A Gema, Lucía y Jennifer, por estar ahí, aunque sea casi imposible coincidir. A Fiona, Marta C, Inma y Berta, por estar siempre disponibles para ofrecerme sus consejos, revisiones y ayuda. A Loli, Olga y Lidia, por esos ratitos graciosos, que ya son para nosotras. A los bioinformáticos, Gonzalo, Ionut, Lorena, Pablo y Raquel, por su ayuda y todo su trabajo con la *pipeline*. A las chicas de la 4ª, Alejandra D, Carolina, Cristina R y Cristina V, por amenizarnos cuando bajan. A todos los demás compañeros e integrantes del Servicio de Genética, por hacer esta etapa mucho mejor: Almudena, Ana A, Ana B, Ana Isabel, Belén, Camilo, Carol, Chony, Fermina, Fernando, Inés, Isabel, Jesús, Laura, María José, Marta R, Miguel, Rocío, Rodrigo, Rosa, Ruth, Saoud y Teresa. Gracias también a las oftalmólogas de la FJD, a Blanca, Belén y Ester, por poder contar con ellas en todo momento.

Thanks to my colleagues from Basel: Carlo, for letting me be part of his group and sharing his knowledge with me, Mathieu and Virginie, for helping me during my stay, and Ji Hoon, Ansar, Francesca and Marc, for welcoming me with open arms.

Gracias también al resto de personas que han participado de alguna u otra forma en este ciclo: a Amelia y Julia, a mi tutor Víctor Ruiz, a los que trabajan en la Escuela de Doctorado y al Programa de Biociencias Moleculares, por aguantar innumerables preguntas y dudas. Gracias a los revisores externos de esta Tesis, Sandro y Marta. A los miembros del Tribunal, por aceptar la invitación.

Gracias a Lenovo, por el ordenador que ha aguantado la escritura de esta Tesis.

Pero no es todo trabajar... Gracias a Alba, Alicia, Amaia, Helen, Loreto y Rocío, por conseguir que me olvidase de los agobios con esos torneos y quedadas. A Inés y Harry, por su apoyo constante.

Gracias a los de siempre, mi familia en Madrid, Antonio, Charlotte, Laura, Manu, Pepe, Rafa B, Rafa I y Rocío, por el apoyo moral en cada momento, por tantas risas y ratos compartidos. A todos los que se han ido uniendo a esa familia, Alberto, Álvaro, Marta, Pablo y Rafa G. Gracias al resto de la Chupi, que aún desde la lejanía están siempre muy presentes, a Andrés, Ara, Lucía y Marta.

Gracias a Noe, por escucharme cuando necesitaba desahogarme, apoyarme sin condiciones y entenderme en cualquier circunstancia que para mi sea importante. Eres increíble. Te quiero.

AGRADECIMIENTOS

Gracias a mis padres, por la educación que me han dado, por enseñarme el valor del esfuerzo y el trabajo bien hecho, por todas las oportunidades que me han proporcionado, por su entrega y cariño…. En definitiva, gracias por hacerme la persona que soy hoy.

Gracias a mi hermana, Julia, por ser la mejor hermana que cualquiera podría desear y por saber sacarme una sonrisa en cualquier situación.

Y, por último, gracias de corazón a todos los pacientes y sus familiares que han participado en estos estudios, ya que sin ellos este trabajo no existiría. Al fin y al cabo, todo esto es por vosotros.

Esta Tesis va por todos. A los que están siempre y a los que vienen y van.

Gracias, ha sido una fantasía.

RESUMEN, *ABSTRACT*

Las enfermedades hereditarias de la retina (EHR) son un conjunto de trastornos que se caracterizan por la disfunción o pérdida progresiva de fotorreceptores, y por una elevada heterogeneidad, tanto a nivel clínico como genético. Se estima que afectan a 1 de cada 3000-4000 personas. La mayoría de los casos de EHR solo tienen manifestaciones oftalmológicas (no sindrómicas), no obstante, alrededor del 20%-30% presentan además manifestaciones sistémicas asociadas a la alteración visual (enfermedades hereditarias de la retina sindrómicas, ERS).

Aún con el uso de la secuenciación masiva (NGS) como enfoque estándar en el estudio de las EHR, el diagnóstico molecular de estas patologías es difícil. De hecho, entre el 30%-50% de los pacientes no son caracterizados genéticamente, por lo que profundizar en el estudio de estos casos, con el fin de encontrar el gen causante de la enfermedad, es de gran relevancia.

En esta Tesis Doctoral se ha estudiado la cohorte registrada en el Servicio de Genética de la Fundación Jiménez Díaz a lo largo de los últimos 30 años. Dicha cohorte representaría el 20%-53% de los pacientes con EHR de España y tendría una tasa de caracterización del 53% con un total de 142 genes y 1549 variantes patogénicas y probablemente patogénicas diferentes identificadas. Los genes más prevalentes fueron *ABCA4* y *USH2A*, estando implicados en el 37% de las familias caracterizadas. Mientras que las variantes más frecuentes en la población española fueron p.(Arg1129Leu) en *ABCA4* y p.(Cys759Phe) en *USH2A*.

El estudio de casos con sospecha de ERS de tipo no Usher mediante NGS señala la importancia del i) fenotipado detallado usando términos HPO y criterios clínicos establecidos; ii) el uso de paneles virtuales bien diseñados para el análisis de los datos de NGS; iii) la evaluación de las variantes en el número de copia; y iv) la realización de reanálisis bioinformáticos periódicos. Con todas estas aproximaciones se alcanza el 52% de familias/probandos caracterizados, aunque el rendimiento aumenta en los casos prospectivos (83%) o los que presentaban síndromes reconocibles (62%). Gracias a este trabajo, se pudo reclasificar clínicamente un 27% de los casos, encontrar presentaciones clínicas atípicas en síndromes ya conocidos (17% de los casos) e identificar pacientes con afectaciones duales, ya sea porque tienen una combinación de una EHR no sindrómica con una enfermedad no genética (6%) o porque presentan dos enfermedades genéticas distintas (gen EHR no sindrómico + reordenamiento genómico; 4%).

Asimismo, se han identificado potenciales alelos modificadores del fenotipo en un 13% de las familias informativas con diagnóstico genético final de síndrome de Bardet-Biedl (BBS). Sin embargo, no se han encontrado evidencias de la existencia de trialelismo en BBS en nuestra serie. Con este trabajo, se pone de manifiesto que el uso de herramientas *in silico* (p. ej. ORVAL o DiGePred) es una alternativa cuando no se pueden estudiar las combinaciones de variantes funcionalmente, ya que respaldan la posible existencia de herencia oligogénica en el 44% de las familias de BBS trialélicas, donde se incluye a 5 de las 6 familias con diferencias fenotípicas intrafamiliares.

Por otra parte, no se ha podido obtener una correlación genotipo-fenotipo para *MYO7A* en las 46 familias analizadas, ya que el tipo de variante no parece cambiar el inicio, la gravedad o la evolución de la enfermedad visual.

Los resultados de esta Tesis amplían el conocimiento de las bases moleculares y los posibles mecanismos hereditarios de las EHR. Además, resaltan la importancia del diagnóstico genético en el correcto manejo y pronóstico de la enfermedad en estos pacientes, así como su posible inclusión en ensayos clínicos.

Inherited retinal diseases (IRD) are a group of disorders characterized by the progressive loss or dysfunction of photoreceptors and high heterogeneity, both clinically and genetically. It is estimated that 1 in 3000-4000 people are affected. Most cases of IRD have only ophthalmic manifestations (non-syndromic). However, around 20%-30% of cases also present systemic features associated with visual alterations (syndromic retinal diseases, SRD).

Even with the use of next generation sequencing (NGS) as the standard approach in the study of IRD, the molecular diagnosis of these diseases is difficult. In fact, between 30%-50% of patients are not genetically characterized, so deepening in the studies of these cases, in order to find the gene causing the disease, is of great relevance.

In this Doctoral Thesis, the cohort registered in the Genetics Service of the Hospital Universitario Fundación Jiménez Díaz over the last 30 years has been studied. This cohort would represent 20%-53% of patients with IRD in Spain and would have a characterization rate of 53%, with a total of 142 genes and 1549 different pathogenic and likely pathogenic variants identified. The most prevalent genes were *ABCA4* and *USH2A*, being involved in 37% of the characterized families. Besides, the most frequent variants in the Spanish population were p.(Arg1129Leu) in *ABCA4* and p.(Cys759Phe) in *USH2A*.

The study of cases with suspected non-Usher SRD by means of NGS points out the importance of i) deep phenotyping using HPO terms and established clinical criteria; ii) the use of well-designed virtual panels for the analysis of NGS data; iii) the evaluation of Copy Number Variations; and iv) performing periodic bioinformatic reanalysis. With all of these approaches, a characterization rate of 52% in the studied families/probands was reached, although the yield increases in prospective cases (83%) and well-recognizable syndromes (62%). Thanks to this work, it was possible to clinically reclassify 27% of the cases, find atypical clinical presentations in already known syndromes (17%) and identify patients with dual affectations, either because they have a combination of a non-syndromic IRD with a non-genetic disease (6%) or because they have two distinct genetic diseases (non-syndromic IRD gene + genomic rearrangement; 4%).

Moreover, potential phenotype modifier alleles have been identified in 13% of the informative families with a final genetic diagnosis of Bardet-Biedl syndrome (BBS). However, no evidence of triallelism in BBS has been found in our series. With this work, it is shown that the use of *in silico* tools (e. g. ORVAL and DiGePred) is an alternative when combinations of variants cannot be studied functionally, since they support the possible existence of oligogenic inheritance in the 44% of the triallelic BBS families, including 5 of the 6 families with intra-familial phenotypic differences.

On the other hand, it has not been possible to obtain a genotype-phenotype correlation for *MYO7A* in the 46 families analysed, since the type of variant does not seem to change the onset, severity, or progression of the visual disease.

The results of this Thesis broaden the knowledge of the molecular bases and the possible hereditary mechanisms of IRD. In addition, they highlight the importance of genetic diagnosis in the correct management and prognosis of the disease in these patients, as well as its possible inclusion in clinical trials.

ÍNDICE

ÍNDICE DE FIGURAS Y TABLAS

Índice de Figuras

Índice de Tablas

CLAVE DE ABREVIATURAS

AAV	Virus adenoasociados
ACLS	Síndrome acrocalloso
ACMG	Colegio Americano de Genética y Genómica Médica
AD	Autosómico dominante
ALMS	Síndrome de Alström
APEX	Extensión de cebadores en matriz (*Arrayed Primer Extension*)
AR	Autosómico recesivo
ATS	Síndrome de Alport
BBS	Síndrome de Bardet-Biedl
CC	Cilio conector
CCG	Capa de células ganglionares
CES	Secuenciación del exoma clínico (*Clinical Exome Sequencing*)
CF	Capa de fotorreceptores
CFNO	Capa de fibras del nervio óptico
CGE	Capa granular externa
CGH	Hibridación Genómica Comparada
CGI	Capa granular interna
CLE	Capa limitante externa
CLI	Capa limitante interna
CLN	Lipofuscinosis neuronal ceroidea
CNV	Variación en el número de copia
COACH	Hipo/aplasia del vermis cerebeloso, Oligofrenia, Ataxia congénita, Coloboma ocular y Fibrosis hepática (*Cerebellar vermis hypo/aplasia, Oligophrenia, Congenital ataxia, ocular Coloboma, and Hepatic fibrosis*)
COH	Síndrome de Cohen
CPE	Capa plexiforme externa
CPI	Capa plexiforme interna
CSNB	Ceguera nocturna estacionaria congénita
DBC	Distrofia de bastones-conos
DC	Distrofia de conos
DCB	Distrofia de conos-bastones
DM	Distrofias maculares
DMT2	Diabetes mellitus de tipo 2
EHR	Enfermedad hereditaria de la retina
EPR	Epitelio pigmentario de la retina
ERS	Enfermedades hereditarias de la retina sindrómicas

CLAVE DE ABREVIATURAS

FERM	F por proteína 4.1, E por ezrina, R por radixina, M por moesina
Glu	Glutamato
GMPc	Guanosín monofosfato cíclico
GTP	Guanosín trifosfato
GTPasa	Guanosina trifosfatasa
HPO	Ontología del fenotipo humano (*Human Phenotype Ontology*)
IFTA	Complejo A del transporte intraflagelar
IFTB	complejo B del transporte intraflagelar
IQ	Isoleucina-glutamina
JBTS	Síndrome de Joubert
LCA	Amaurosis congénita de Leber
MKS	Síndrome de Meckel-Gruber
ML	Mucolipidosis
MLPA	Amplificación de la sonde dependiente de la ligadura multiplex (*Multiplex Ligation-dependent Probe Amplification*)
MORM	Retraso mental, Obesidad truncal, Distrofia de retina y Micropene (*Mental retardation, truncal Obesity, Retinal dystrophy, and Micropenis*)
MTS	"Signo del diente molar"
MyTH4	Dominio de homología de cola de miosina 4
NGS	Secuenciación masiva (*Next Generation Sequencing*)
NPHP	Nefronoptisis
OFD	Síndrome oro-facio-digital
OMIM	*Online Mendelian Inheritance in Man*
PBD	Trastornos de la biogénesis peroxisomal
PDE	Fosfodiesterasa
ROH	Región de homocigosidad
RP	Retinosis pigmentaria
RHYNS	Síndrome de RHYNS (*Retinitis pigmentosa, Hypopituitarism, Nephronopthisis, and mild Skeletal dysplasi*a)
SE	Segmento externo
SH3	Dominio de homología 3 de SRC
SI	Segmento interno
SLSN	Síndrome de Senior-Løken
SNC	Sistema nervioso central
SNP	Polimorfismo de un solo nucleótido
SNV	Variante de un solo nucleótido
SRTD	Displasia torácica de costillas cortas con o sin polidactilia

STGD	Enfermedad de Stargardt
T	Transducina
TS	Secuenciación dirigida (*Targeted Sequencing*)
USH	Síndrome de Usher
USH1	Síndrome de Usher de tipo 1
USH2	Síndrome de Usher de tipo 2
USH3	Síndrome de Usher de tipo 3
VUS	Variante de significado incierto
WES	Secuenciación del exoma completo (*Whole Exome Sequencing*)
WFS	Síndrome de Wolfram
WGS	Secuenciación del genoma completo (*Whole Genome Sequencing*)
XL	Ligado al cromosoma X

INTRODUCCIÓN

1. La visión y el ojo humano

El sentido de la vista nos permite percibir e interpretar nuestro alrededor gracias a la capacidad del ojo de participar en la captación de los estímulos luminosos en el espectro visible y transformarlos en impulsos eléctricos, gracias a los fotorreceptores. Posteriormente, estos potenciales serán transmitidos por el nervio óptico y procesados en la corteza visual del cerebro que, a su vez, los interpreta y controla el sentido de la vista.

El ojo comienza a formarse en torno al día 22 del desarrollo embrionario, con la aparición del primordio óptico en los pliegues neurales. Es un proceso que continúa durante todo el desarrollo fetal y finaliza en el 9º mes, con la formación de los vasos retinianos periféricos, la mielinización de las fibras del nervio óptico y la desaparición de la membrana pupilar. Los tejidos embrionarios del ojo se derivan de ectodermo (neuroectodermo, células de la cresta neural o ectodermo superficial) y mesodermo [1].

Anatómicamente, el globo ocular (Figura 1.A) está compuesto por 3 capas concéntricas [2]:

- La capa externa o túnica fibrosa, que incluye la esclera y la córnea.
- La capa intermedia o túnica vascular, que comprende el iris, el cuerpo ciliar y la coroides.
- La capa interna o túnica neurosensorial formada por la retina.

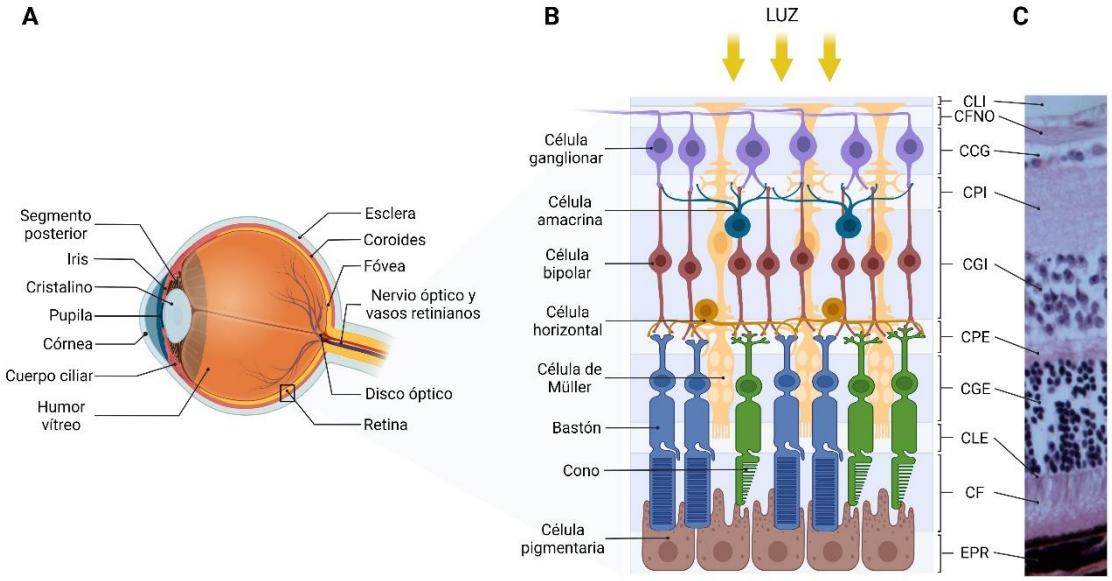


Figura 1. *El ojo y la retina humana. (**A**) Anatomía del globo ocular. (**B**) Composición celular y capas estructurales de la retina. Abreviaturas (por orden alfabético): CCG, capa de células ganglionares; CF, capa de fotorreceptores; CFNO, capa de fibras del nervio óptico; CGE, capa granular externa; CGI, capa granular interna; CLE, capa limitante externa; CLI, capa limitante interna; CPE, capa plexiforme externa; CPI, capa plexiforme interna; EPR, epitelio pigmentario de la retina. (C) Sección histológica de las capas de la retina. Imagen creada con BioRender y subapartado (C) tomado de Tsang y Sharma (2018) [3].*

1.1. Anatomía y fisiología de la retina

La retina es una delgada multicapa compuesta por células nerviosas derivadas del neuroectodermo que recubre el interior del ojo.

En 1891, Santiago Ramón y Cajal publicó la primera descripción de las neuronas retinianas [4]. Este complejo órgano presenta una estructura estratificada formada por 10 capas paralelas

(Figura 1.B-C) [5]. En la zona más cercana al humor vítreo encontraríamos la capa limitante interna (CLI), la cual es una membrana basal formada por los extremos cónicos de las células de Müller y astrocitos. La capa de fibras del nervio óptico (CFNO) está formada por los axones de las células ganglionares, vasos retinianos y células gliales. La capa de células ganglionares (CCG) contiene predominantemente el núcleo de las células ganglionares. En la capa plexiforme interna (CPI) es donde interactúan las células ganglionares, bipolares y amacrinas. La capa granular interna (CGI) alberga los núcleos de las células bipolares, horizontales, amacrinas y de Müller. En la capa plexiforme externa (CPE) es donde tienen lugar las conexiones entre los fotorreceptores y las células bipolares y donde las horizontales interactúan tanto con los fotorreceptores como con las bipolares. La capa granular externa (CGE) está formada por los núcleos de los fotorreceptores. En la capa limitante externa (CLE) se realizan tanto las uniones entre las células de Müller consigo mismas como entre las células de Müller con los fotorreceptores. La capa de fotorreceptores (CF) comprende los segmentos externos de los fotorreceptores. Por último, el epitelio pigmentario de la retina (EPR) es una monocapa de células epiteliales cuboidales cuyas funciones son permitir la difusión de nutrientes desde la coroides, secretar factores de crecimiento, reciclar los discos de los segmentos externos de los fotorreceptores y participar en el ciclo visual.

1.2. Fotorreceptores

Los fotorreceptores son células sensibles a luz que se encuentran en la parte posterior de la retina en contacto con el EPR y que se encargan de la transformación de la luz en potenciales eléctricos mediante la fototransducción. Existen dos tipos de fotorreceptores, bastones y conos (Figura 2.A), los cuales se diferencian estructural y funcionalmente. Sin embargo, ambos comparten una estructura básica: a) segmento externo (SE), es un cilio primario especializado formado por discos en los bastones y por lamelas en los conos, donde se sitúan los pigmentos visuales y comienza la fototransducción; b) cilio conector (CC), conecta el SE con el segmento interno y permite el tráfico intracelular de vesícula y otros metabolitos; c) segmento interno (SI), contiene la maquinaría biosintética y celular, incluyendo al núcleo y a las mitocondrias; y d) terminación sináptica, realiza la sinapsis con las células bipolares y horizontales [5,6].

La retina humana contiene alrededor de 120 millones de bastones y 6 millones de conos (relación 20:1) [5]. Los bastones y conos tienen una distribución retiniana única, condensándose estos últimos en la zona central de la retina (mácula, especialmente en la fóvea) y disminuyendo su densidad hacia la periferia, donde predominan los bastones [7].

Los bastones son muy sensibles a la luz y contribuyen principalmente a la visión nocturna (condiciones escotópicas). Mientras que los conos funcionan con iluminación ambiental y brillante, ya que generan respuestas rápidas frente a variaciones de intensidad lumínica y son los responsables de la visión del color y la agudeza visual (condiciones fotópicas), según el fotopigmento que presenten en las invaginaciones del SE [8,9]. Deficiencias en los bastones producen problemas de ceguera nocturna y pérdida de visión periférica, por su parte deficiencias en los conos se traducen en pérdida de la agudeza visual o discromatopsia [10].

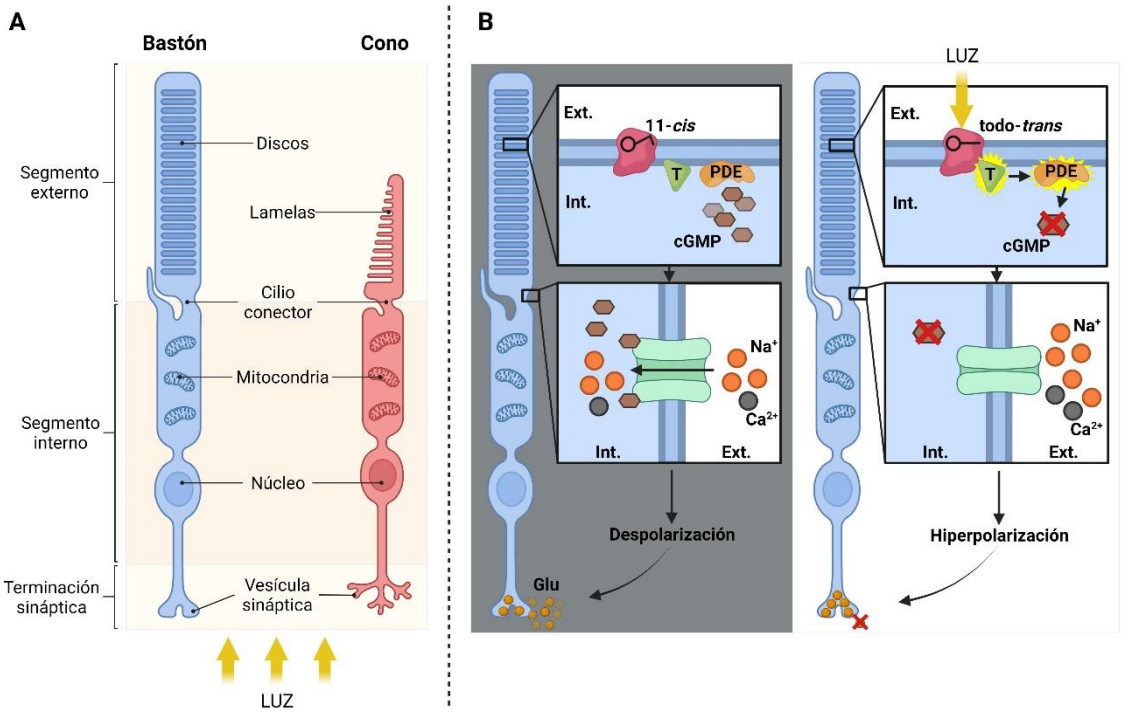


Figura 2. *Fotorreceptores. (**A**) Estructura esquemática de los bastones y conos. Imagen modificada de Wright et al. (2010) [10] con BioRender. (**B**) Esquema de la fototransducción. Abreviaturas (por orden alfabético): cGMP, guanosín monofosfato cíclico; Ext., lado externo de la membrana; Glu, glutamato; Int., lado interno de la membrana; PDE, fosfodiesterasa; T, transducina. Imagen modificada de Klapper et al. (2016) [11] con BioRender.*

1.3. Fototransducción

La fototransducción es el proceso en el cual la luz es capturada por los fotorreceptores y convertida en potenciales eléctricos mediante una serie de reacciones bioquímicas en la retina, haciendo que las células ganglionares se despolaricen y terminen transmitiendo la señal visual al núcleo geniculado lateral en el cerebro.

Los SEs de los fotorreceptores están enriquecidos en fotopigmentos formados por una opsina (proteína de membrana) a la que se une el cromóforo 11-*cis*-retinal. La fototransducción se inicia con la absorción de un fotón por parte del 11-*cis*-retinal y su consiguiente isomerización a todo-*trans*-retinal. Esta reacción genera un cambio conformacional en el fotopigmento, que desencadena una cascada de señalización en la que participa la transducina y que finaliza con la hidrólisis del guanosín monofosfato cíclico (GMPc) por la fosfodiesterasa (PDE). La reducción del GMPc intracelular da como resultado el cierre de canales iónicos y el cese en la entrada de cationes (Na^+ y Ca^{2+}), lo cual se traduce en la hiperpolarización de la membrana y la consecuente inhibición en la liberación de neurotransmisores en la sinapsis del fotorreceptor. El todo-*trans*-retinal se vuelve a convertir en 11-*cis*-retinal para su reutilización a través de una serie de reacciones bioquímicas conocidas como ciclo visual, que ocurre tanto en el SE de los fotorreceptores como en el EPR [12–14] (Figura 2.B).

2. Enfermedades hereditarias de la retina

Las enfermedades hereditarias de la retina (EHRs) son uno de los trastornos clínicos y genéticos más heterogéneos que se conocen entre todas las afecciones médicas humanas. Se caracterizan por la disfunción o degeneración de las células retinianas, lo que resulta en una discapacidad visual grave [15].

Las EHRs se clasifican de acuerdo con diferentes criterios, entre los que se incluyen el tipo de célula retiniana afectada (EPR, bastones, conos, células bipolares o células ganglionares), la edad de inicio de los primeros síntomas (desde la infancia hasta la edad adulta), la progresión de la enfermedad a lo largo del tiempo (estacionaria o progresiva) y la presencia/ausencia de características sistémicas asociadas (formas no sindrómicas o sindrómicas) [16,17].

En las EHRs se pueden encontrar todos los modos de herencia, autosómico dominante (AD), autosómico recesivo (AR) o ligado al cromosoma X (XL, por sus siglas en inglés), así como formas raras no Mendelianas (patrones de herencia mitocondrial u oligogénico) [15,16,18].

2.1. Epidemiología

Las EHRs se catalogan como enfermedades raras, aunque consideradas en conjunto, su prevalencia estimada es de aproximadamente 1 entre 3000-4000 personas [10]. La EHR más común es la retinosis pigmentaria (RP), con una prevalencia mundial de 1 entre 4000 individuos [15], aunque hay trabajos en los se reportan variaciones desde 1:750 [19] hasta 1:9000 [20], según la población estudiada.

2.2. Aspectos clínicos

Las EHRs se pueden clasificar en formas periféricas y formas centrales, según el fotorreceptor afectado [17]. Las formas periféricas comienzan con la degeneración de los bastones, lo que deriva en ceguera nocturna y pérdida del campo visual periférico (visión en túnel) [10]. Según su evolución, pueden encontrarse formas estacionarias (p. ej. ceguera nocturna estacionaria congénita, CSNB, por sus siglas en inglés) [21] o formas progresivas, donde además de la pérdida visual periférica progresiva, también se puede producir, en algunos casos, una degeneración tardía de los conos, con la consiguiente pérdida de visión central. Ejemplos de formas progresivas son la RP o la distrofia de bastones-conos (DBC) [16,17,22].

Por otra parte, las formas centrales se producen por la disfunción de los conos, que terminan generando la pérdida de la agudeza visual, del campo visual central, fotofobia o alteración de la visión del color [10]. Incluyen formas estacionarias (p. ej. acromatopsia o monocromatismo de conos azules) y formas progresivas, entre las que se incluyen distrofias de conos (DC), distrofias de conos-bastones (DCB) o algunas distrofias maculares (DM), como son la enfermedad de Stargardt (STGD, por sus siglas en inglés) o la distrofia macular viteliforme de Best [17,23,24].

Finalmente, la amaurosis congénita de Leber (LCA, por sus siglas en inglés) es la forma más severa de EHR, caracterizada por disfunción de conos y bastones, lo que genera una ceguera congénita o de inicio precoz [25].

La mayoría de los casos de EHR solo presentan manifestaciones oftalmológicas (no sindrómicas), no obstante, alrededor del 20%-30% corresponden a tipos sindrómicos [26]. Se han descrito más de 80 síndromes específicos (EHR sindrómicas, ERS), entre los que destacan las ciliopatías (ver apartado 3) y otros síndromes específicos no relacionados con los cilios (ver apartado 4) [27]. Las ERS se caracterizan por un diagnóstico clínico extremadamente complejo debido a que hay pacientes con una alta heterogeneidad clínica que no pueden ser categorizados dentro de alguno de los síndromes bien conocidos [28].

Asimismo, existen casos en los que una EHR no sindrómica puede coexistir con otras enfermedades raras no oculares (genéticas o no genéticas), lo que lleva a una sospecha clínica o a un diagnóstico erróneo de un síndrome [29].

2.3. Aspectos genéticos y diagnóstico molecular

Las EHRs presentan una elevada heterogeneidad genética (Figura 3), ya que variantes patogénicas en un mismo gen pueden producir fenotipos diferentes, así como mutaciones en genes distintos pueden dar el mismo fenotipo. Algunos ejemplos son: el gen *RPGR* (MIM *312610) que puede producir tanto RP como DCB; o los genes *USH2A* [30] (MIM *608400), *BBS1* [31] (MIM *209901) y *CEP290* (MIM *610142), que pueden asociarse a formas no sindrómicas (RP o LCA) o sindrómicas. Asimismo, también hay enfermedades homogéneas dentro de las DHR, donde la enfermedad solo es producida por mutaciones en un solo gen, como es el caso de la coroideremia (gen *CHM*; MIM *300390).

A día de hoy, existen unos 280 genes relacionados tanto con formas no sindrómicas como sindrómicas (*The Retinal Information Network*, RetNet; https://sph.uth.edu/retnet/; último acceso febrero 2022).

Todo ello hace que el diagnóstico molecular sea difícil, de hecho entre el 30%-50% de los casos de EHR no son caracterizados genéticamente [32,33].

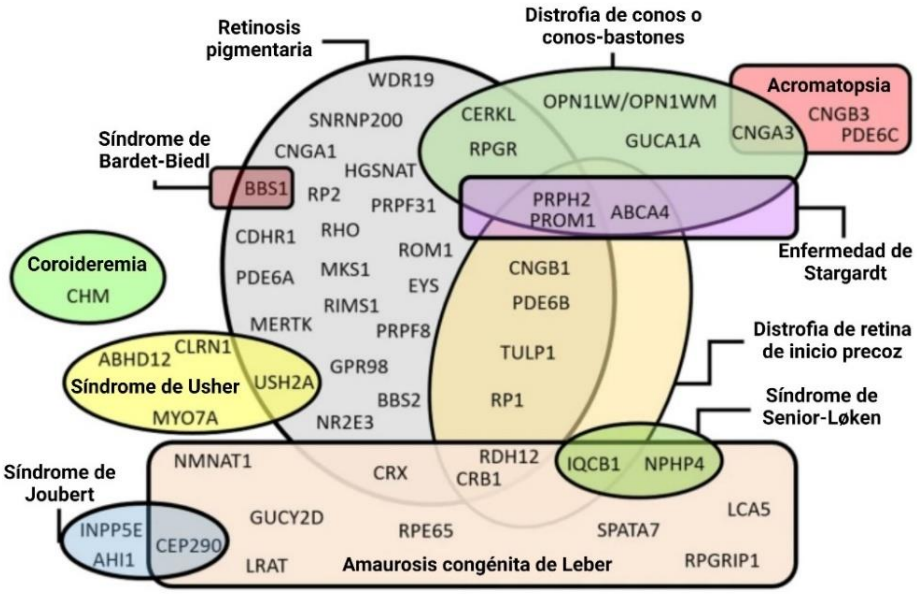


Figura 3. *Heterogeneidad genética en las enfermedades hereditarias de la retina. Las zonas solapantes indican los genes que pueden producir más de un fenotipo. Imagen modificada de Motta et al. (2018) [34] con BioRender.*

3. Ciliopatías

Las ciliopatías son un grupo de trastornos genéticos raros producidos por defectos en los cilios o en la maquinaría que participa en su ensamblaje. Estas enfermedades pueden afectar a uno o más órganos o sistemas, entre los que se incluyen retina (fotorreceptores), epitelio olfativo, sistema nervioso central, sistema cardiovascular, riñones, hígado, gónadas, sistema esquelético o tejido adiposo [35].

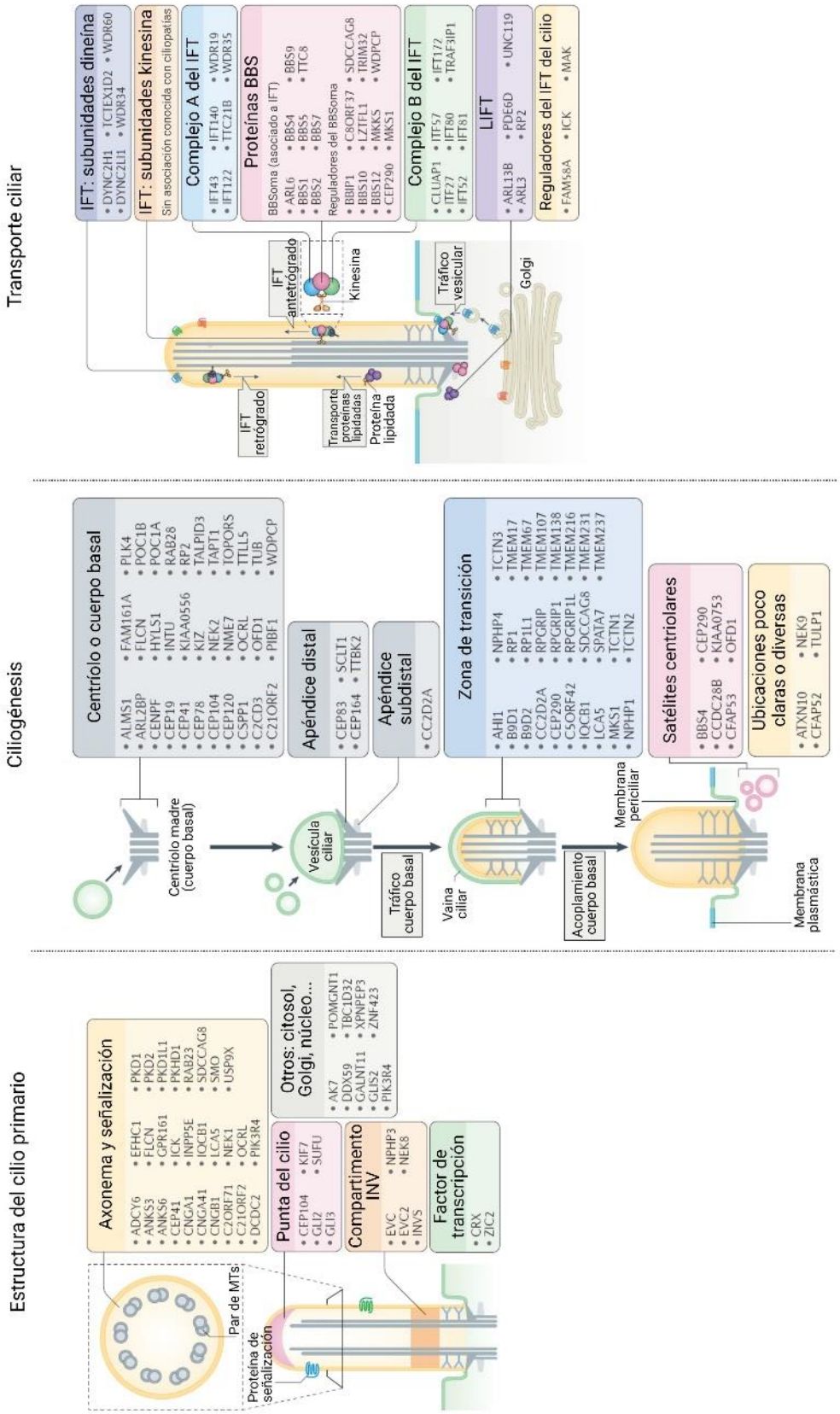


Figura 4. *La complejidad del cilio. Proteínas relacionadas con la estructura del cilio primario, la ciliogénesis y el transporte ciliar. Abreviaturas (por orden alfabético): BBS, síndrome de Bardet-Biedl; IFT, transporte intraflagelar; INV, inversina; LIFT, direccionamiento intraflagellar de proteínas lipídicas. Imagen modificada de Reiter y Leroux (2017) [36] con BioRender.*

3.1. El cilio

Los cilios son orgánulos complejos, dinámicos y muy conservados, de manera que prácticamente se pueden encontrar en todos los tipos celulares en vertebrados y, de forma ubicua, en todas las especies [37]. Se dividen en cilios móviles y no móviles, estos últimos también llamados primarios o sensoriales (Figura 4). Ambos comparten una estructura básica común, el axonema. Esta estructura citoesquelética está compuesta por numerosas proteínas y rodeada por una membrana que se une a la membrana plasmática de la célula. El interior del axonema presenta una arquitectura cilíndrica formada por nueve pares de microtúbulos (un túbulo A unido a uno B), los cuales pueden rodear a un par central de microtúbulos (conformación 9+2) o no (configuración 9+0). Asimismo, el axonema también contiene brazos de dineína internos y externos que originan la fuerza para la motilidad y proteínas radiales que participan en la regulación de la motilidad. Los cilios primarios presentan una configuración 9+0 y carecen de los brazos de dineína y las proteínas radiales [38–41].

Otras regiones importantes en los cilios son el cuerpo basal y la zona de transición [40]. El cuerpo basal se sitúa en la zona proximal de los cilios y está formado por un anillo de tripletes de microtúbulos, que actúan como base del cilio, punto de unión con la célula y como zona de acoplamiento para las proteínas que van a ser transportadas por él. Los cilios crecen desde el extremo más distal del cuerpo basal. Por su parte, la zona de transición está rodeada por la membrana periciliar y conecta los pares de microtúbulos del axonema con el cuerpo basal a través de conectores Y [38,41].

Existen numerosas proteínas relacionadas con los cilios (Figura 4) [42,43], las cuales intervienen en procesos y estructuras relacionados con ellos, como son la señalización, ciliogénesis, compartimentalización o tráfico dinámico de proteínas y vesículas. Asimismo, hay proteínas no ciliares que influyen en las funciones del cilio [44].

3.2. Ciliopatías sensoriales sindrómicas

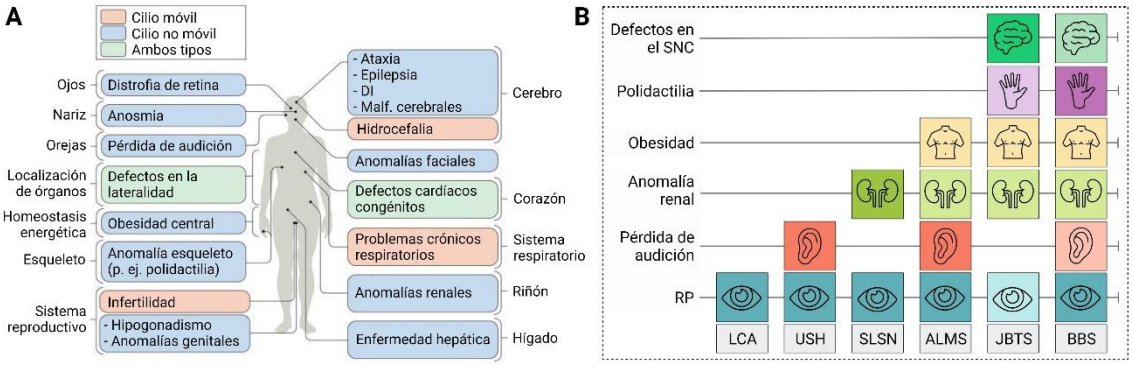


Figura 5. *Órganos diana y fenotipos asociados en las ciliopatías. (**A**) Disfunciones en los cilios móviles y primarios causan ciliopatías que afectan a numerosos órganos y sistemas. Imagen modificada de Reiter y Leroux (2017) [36] con BioRender. (**B**) Espectro fenotípico de las ciliopatías. Abreviaturas (por orden alfabético): ALMS, síndrome de Alström; BBS, síndrome de Bardet-Biedl; JBTS, síndrome de Joubert; LCA, amaurosis congénita de Leber; RP, retinosis pigmentaria; SLSN, síndrome de Senior-Løken; SNC, sistema nervioso central; USH, síndrome de Usher. Imagen modificada de Mockel et al. (2011) [45] con BioRender.*

Las ciliopatías sensoriales son un grupo de trastornos hereditarios heterogéneos producidos por defectos en la estructura o funcionamiento del cilio primario [39]. Debido a la presencia de los cilios primarios en casi todos los tejidos y órganos, este tipo de ciliopatías dan lugar a un amplio

grupo de fenotipos (Figura 5.A), que van desde la afectación de un solo órgano (ciliopatías no sindrómicas) hasta trastornos sistémicos más complejos (ciliopatías sindrómicas) (Figura 5.B) [36].

Se pueden encontrar diversos síndromes característicos dentro de las ciliopatías sindrómicas, entre los que destacan el síndrome de Usher (USH), el síndrome de Bardet-Biedl (BBS), el síndrome de Joubert (JBTS), el síndrome de Alström (ALMS), o el síndrome de Senior-Løken (SLSN). Sin embargo, las superposiciones clínicas y la gran heterogeneidad genética existente hace difícil la clasificación y diagnóstico de estas enfermedades [35,46].

3.2.1. Síndrome de Usher (USH)

El síndrome de Usher es un grupo de enfermedades hereditarias autosómicas recesivas caracterizadas por la pérdida de la visión (en forma de RP) e hipoacusia neurosensorial. En algunos casos también puede aparecer disfunción vestibular [47]. El USH fue descubierto en 1858 por el oftalmólogo Albrecht von Graefe [48], aunque no fue hasta 77 años más tarde (1935) cuando el oftalmólogo Charles Usher sentó las bases de su carácter hereditario [49].

Se estima que la prevalencia del USH va desde 3,2 a 6,2 por cada 100 000 personas [50–52]. Sin embargo, su prevalencia puede llegar hasta unos 16,7 por cada 100 000 en ciertas zonas de Estados Unidos [53].

3.2.1.1. *Manifestaciones clínicas y aspectos genéticos generales del síndrome de Usher*

De forma general, el síndrome de Usher se divide en 3 subtipos clínicos en función de la gravedad de la pérdida de audición, la presencia o ausencia de función vestibular y la edad de inicio de la RP: síndrome de Usher de tipo 1 (USH1), de tipo 2 (USH2) y de tipo 3 (USH3). Las características generales de cada uno pueden consultarse en la Tabla 1.

Tabla 1. Características clínicas y genéticas de los tres subtipos del síndrome de Usher. Tabla creada con datos de Yan y Liu (2010), Bujakowska *et al*. (2017) y Fuster-García *et al*. (2021) [47,54,55].

	Frecuencia	RP	Pérdida de audición	Función vestibular	Gen	Número MIM	Locus
USH1	33-44%	Inicio temprano	Profunda y severa Estable Prelingual	Alterada	*CDH23*	*605516	10q22.1
					CIB2	*605564	15q25.1
					MYO7A	*276903	11q13.5
					PCDH15	*605514	10q21.1
					USH1C	*605242	11p15.1
					USH1G	*607696	17q25.1
USH2	56-57%	Segunda década de vida	Moderada-severa Estable Prelingual	Normal	*ADGRV1*	*602851	5q14.3
					USH2A	*608400	1q41
					WHRN	*607928	9q32
USH3	2%	Variable	Variable Progresiva Poslingual	Variable	*CLRN1*	*606397	3q25.1

Abreviaturas (por orden alfabético): RP, retinosis pigmentaria; USH1, síndrome de Usher de tipo 1; USH2, síndrome de Usher de tipo 2; USH3, síndrome de Usher de tipo 3.

Aproximadamente el 80% de los casos con USH se diagnostican genéticamente con alguno de los 10 genes descritos en la Tabla 1 mediante métodos de secuenciación dirigida o secuenciación del exoma completo (en inglés, *Whole Exome Sequencing*, WES) [56,57]. No obstante, con el uso de distintos métodos de estudio combinados se alcanza una tasa de caracterización de más del 90% [58].

La correlación fenotipo-genotipo de los 10 genes causantes del USH no es inamovible, sino que existen trabajos que señalan la existencia de pacientes con variantes patogénicas en un gen concreto asociado a otro subtipo distinto de USH [59,60].

Sin embargo, algunos casos no cumplen los criterios para poder ser clasificados en uno de los tres subtipos de USH, son los llamados USH atípicos. Estos casos atípicos descritos en la literatura se caracterizan por presentar pérdida auditiva más DCB en vez de RP o en que el grado de gravedad de los síntomas es menor [61]. Se han propuesto 6 genes causantes en estos casos: *ABHD12* (MIM *613599), *ARSG* (MIM *610008), *CEP250* (MIM *609689), *CEP78* (MIM *617110), *ESPN* (MIM *606351) y *HARS* (MIM *142810). Aunque su asociación con USH es controvertida debido a la poca información existente y a la discordancia de los síntomas [47,61].

Se han mapeado tres nuevos loci relacionados con USH, aunque actualmente aún no se han identificado los genes candidatos. Estos loci son *USH1E* (MIM %602097), *USH1H* (MIM %612632) y *USH1K* (MIM %614990).

3.2.1.2. MYO7A

MYO7A (MIM *276903) fue el primer gen asociado al síndrome de Usher en 1995 [62]. Este gen, situado en el brazo largo del cromosoma 11, está formado por 49 exones, los cuales pueden sufrir procesos de empalme alternativo. Su isoforma principal consta de 2215 aminoácidos codificados por una región que no incluye ni el primer exón ni la mitad del segundo [63].

Codifica a la miosina 7A, que pertenece a la familia de las miosinas poco convencionales. Esta proteína motora tiene una estructura dividida en dominios funcionales (Figura 6.A): i) cabeza motora, que contiene el sitio de unión a actina y el de unión a ATP; ii) región del cuello, con cinco motivos IQ (isoleucina-glutamina) de unión a otras proteínas, como la calmodulina; y iii) región de la cola, con distintos motivos que interaccionan y se unen a la membrana plasmática y a diversas proteínas, determinando la especificidad de la miosina 7A [64].

MYO7A se expresa en el oído interno y en la retina. En el oído, se sitúa en las células ciliadas sensoriales y es fundamental en la diferenciación y desarrollo de los haces de los estereocilios [65]. Mientras que en la retina, se encuentra en la región apical del EPR y en el cilio conector y región periciliar de los fotorreceptores y participa en el transporte de las opsinas desde el segmento interno al externo de conos y bastones, y en el movimiento de melanosomas para el reciclaje de los discos (Figura 6.B) [66–68].

MYO7A es el gen más mutado en USH1, siendo la causa de más de la mitad de los casos, y el segundo más frecuentemente mutado entre todos los casos de USH [69]. Además de USH1, mutaciones en *MYO7A* causan hipoacusia neurosensorial aislada, la cual puede ser autosómica dominante o recesiva [70], y formas atípicas de USH [71].

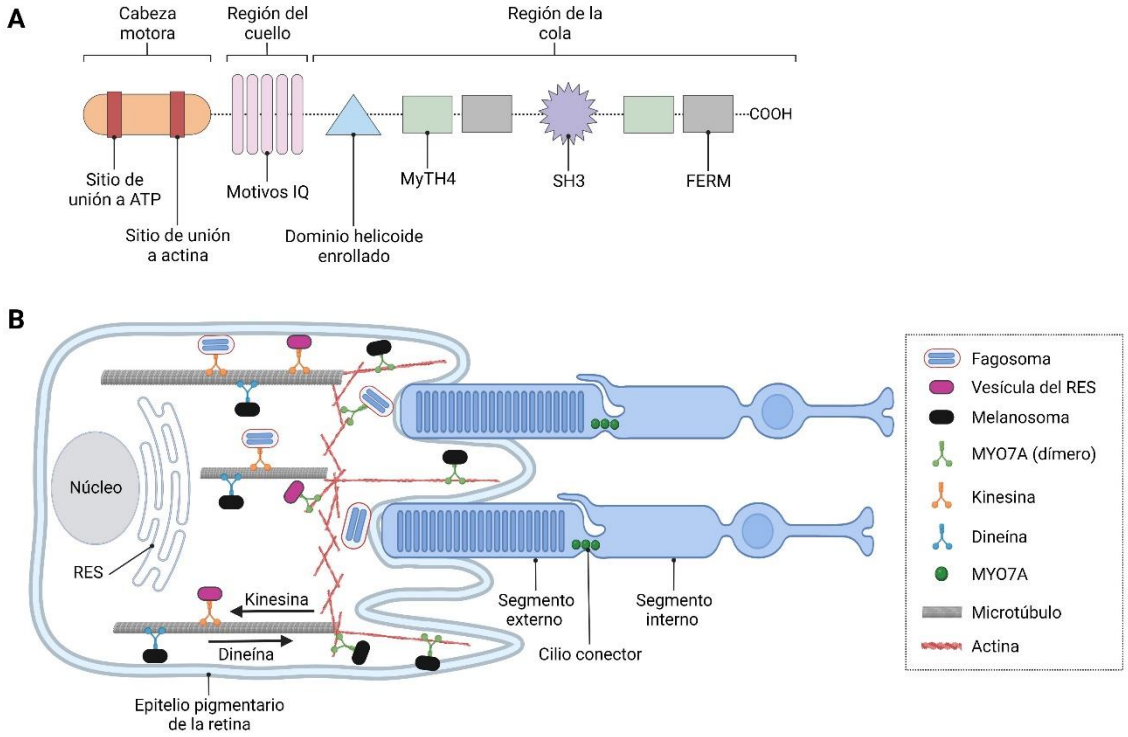


Figura 6. *Estructura y localización retiniana de la proteína MYO7A. (**A**) Representación esquemática de la estructura de la proteína MYO7A. Abreviaturas (por orden alfabético): FERM, dominio FERM (F por proteína 4.1, E por ezrina, R por radixina, M por moesina); IQ, isoleucina-glutamina; MyTH4, dominio de homología de cola de miosina 4; SH3, dominio de homología 3 de SRC. Imagen modificada de Reiners et al. (2006) [64] con BioRender. (**B**) Esquema de la localización de MYO7A en el epitelio pigmentario de la retina y los fotorreceptores. Abreviaturas (por orden alfabético): RES, retículo endoplasmático liso. Imagen modificada de Williams y Lopes (2013) [68] con BioRender.*

3.2.1.3. *Aproximaciones terapéuticas en el síndrome de Usher. Terapia génica en MYO7A*

El síndrome de Usher es un trastorno muy incapacitante, ya que afecta a los sentidos de la visión y del oído, y que actualmente no tiene cura. Si bien el problema de la pérdida de la audición puede contrarrestarse mediante el uso de audífonos o implantes cocleares [72], la degeneración visual permanece intratable. De manera general, existen diversas estrategias para tratar la pérdida de visión, según si se enfocan en un trastorno genético específico o si es independiente de la causa, como son las aproximaciones farmacológicas, terapias génicas (p. ej. suplementación génica, oligonucleótidos antisentido o edición génica), terapia celular o estrategias optogenéticas [73]. Sin embargo, los posibles tratamientos aún se encuentran en fase de investigación o de ensayo clínico.

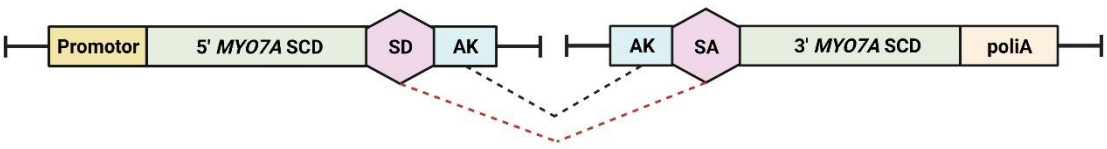


Figura 7. *Representación esquemática de los vectores duales adenoasociados AAV-MYO7A. Las líneas punteadas en negro muestran regiones superpuestas para la recombinación homóloga y las líneas punteadas en rojo representan el empalme que se produce entre SD y SA. Abreviaturas (por orden alfabético): AK, región recombinogénica del fago F1; poliA, señal de poliadenilación; SCD, secuencia codificante; SA, sitio aceptor de empalme; SD, sitio donante de empalme. Imagen modificada de Trapani et al. (2014) [74] con BioRender.*

Uno de estos ensayos clínicos es un fase I/II para evaluar la seguridad y la actividad de la terapia génica mediante el reemplazo del gen *MYO7A* (UshStat; SAR421869) para tratar la RP en

pacientes con síndrome de Usher tipo 1B (USH1B), que se inició en 2012. Los virus adenoasociados (AAV, por sus siglas en inglés) han sido una de las herramientas más estudiadas para la terapia génica de la retina. No obstante, dado el gran tamaño de la secuencia codificante de *MYO7A* (alrededor de 7 kb) y la capacidad limitada de los AAV (aproximadamente 5 kb), estos no pueden usarse para la terapia génica en el USH1B. La estrategia usada para superar este problema fue dividir la secuencia codificante del gen en dos partes y tener cada una contenida en un AAV separado (Figura 7). Una vez dentro de la célula, los dos fragmentos se unen por recombinación homóloga, formando un ARN mensajero y proteína completos [74,75].

3.2.2. Síndrome de Bardet-Biedl (BBS)

El síndrome de Bardet-Biedl es un trastorno multisistémico autosómico recesivo, caracterizado por una marcada variabilidad inter- e intrafamiliar [76,77]. Fue descrito por primera vez en los años 20 por el pediatra francés Georges Louis Bardet y el patólogo húngaro Arthur Biedl. Su prevalencia estimada varía entre las distintas poblaciones, desde 1 entre 160 000 individuos en el norte de Europa [78] hasta 1 entre 13 500 [79] o 1 entre 18 000 [80] en regiones aisladas con alta consanguinidad de Kuwait o Canadá, respectivamente.

3.2.2.1. *Manifestaciones clínicas del síndrome de Bardet-Biedl*

El BBS presenta una amplia gama de características clínicas que se dividen en primarias y secundarias. Las características primarias incluyen distrofia progresiva de retina, polidactilia postaxial, obesidad, hipogonadismo, anomalías renales y problemas de aprendizaje. Mientras que las secundarias comprenden retraso o trastorno del habla, retraso en el desarrollo, diabetes mellitus tipo 2 (DMT2), anomalías dentales, malformaciones cardíacas, braquidactilia, sindactilia, ataxia, mala coordinación, anosmia e hiposmia. Existen otros hallazgos clínicos adicionales que también pueden encontrase, como son otitis media crónica o enfermedad de Hirschprung [45,81].

Comúnmente, el diagnóstico de BBS se basa en: i) la presencia de cuatro de las seis características clínicas primarias; o ii) en aquellos casos en los que solo hay tres de ellas, se necesita adicionalmente de al menos dos rasgos clínicos secundarios [82].

3.2.2.2. Genética del síndrome de Bardet-Biedl

En el año 2000 fue descubierto el primer gen asociado al BBS tras la identificación de mutaciones en *MKKS* de forma independiente en dos familias distintas [83,84]. Actualmente, el BBS se relaciona con 24 genes distintos (Anexo 1), los cuales se han ido identificando a lo largo de las últimas dos décadas [85].

Los primeros 21 loci (BBS1-BBS21) explican aproximadamente el 80% de todos los casos diagnosticados con este síndrome [86]. *BBS1*, *BBS2* y *BBS10* son los genes más mutados, representando alrededor de la mitad de todos los pacientes diagnosticados genéticamente con BBS [82]. Por otra parte, otros genes (p. ej. *BBIP1*, *IFT27* o *WDPCP*) solo se han encontrado mutados aisladamente en una o pocas familias o pacientes [87–89]. Sin embargo, la contribución de cada locus a la carga mutacional total varía entre las distintas poblaciones, al igual que la frecuencia de alelos mutantes [90,91].

Además de los 24 genes relacionados con BBS, existen adicionalmente otros 12 genes candidatos causales y/o modificadores relacionados con BBS o con fenotipos similares a BBS (Anexo 2).

Además de esa gran heterogeneidad de loci genéticos, existen otros mecanismos genéticos que pueden explicar la complejidad subyacente al BBS, como son las variaciones en el número de copia (CNV, por sus siglas en inglés) [92], el aumento de la carga alélica (trialelismo y alelos modificadores) [93–96] o la mutagénesis mediada por transposones [97].

El modelo de herencia trialélico fue descrito por primera vez en 2001, al reportarse en los individuos afectos de 4 familias la presencia de 3 alelos mutantes en dos genes distintos (*BBS2* y *MKKS*) [93]. En estas familias eran necesarias las 3 mutaciones para causar la enfermedad, lo que permitió definir el concepto de trialelismo. Sin embargo, otros estudios posteriores no han encontrado evidencias de su existencia, por lo que han cuestionado su frecuencia y respaldado un patrón de herencia autosómico recesivo [98,99]. Independientemente del trialelismo, en otros casos, esa tercera mutación en un segundo gen se ha correlacionado con un fenotipo más severo debido a un efecto modificador. Por lo que cambios en la expresividad del fenotipo se podrían asociar a la presencia o no de alelos modificadores, generándose variabilidad intrafamiliar [100–102].

3.2.2.3. Aproximaciones terapéuticas del síndrome de Bardet-Biedl

Uno de los principales desafíos en su tratamiento es que es un trastorno multisistémico con muchos órganos afectados y, por lo tanto, los pacientes requieren de una atención multidisciplinar para tratar sintomáticamente las distintas manifestaciones [103].

El gran número de genes causantes de BBS y de mutaciones únicas en las familias hacen que el desarrollo de terapias genéticas en este síndrome sea difícil [104]. La terapia génica y otras intervenciones farmacológicas representan ahora mismo los principales focos de investigación [103–105]. Por ejemplo, en el año 2021, se concluyó un ensayo en fase 3 (NCT03746522) para evaluar la eficacia y la seguridad de la Setmelanotida, un fármaco agonista de la melanocortina, para tratar la obesidad y la hiperfagia en pacientes diagnosticados clínicamente con BBS o ALMS [106].

3.2.3. Otras ciliopatías

3.2.3.1. Síndrome de Alström (ALMS)

El ALMS es un trastorno raro monogénico autosómico recesivo, cuya prevalencia estimada varía desde 1 entre 100 000 individuos [107] hasta 1 entre 1 000 000 [108].

Presenta una amplia gama de características clínicas, unas primarias como los problemas de visión (nistagmos, ceguera legal y/o DBC); y otras secundarias como obesidad, resistencia a la insulina, DMT2, pérdida de audición, cardiomiopatía dilatada, disfunción hepática, fallo renal, estatura baja, hipogonadismo en hombres y menstruaciones irregulares y/o hiperandrogenismo en mujeres. Existen otros hallazgos clínicos adicionales que también pueden encontrase, como son: infecciones pulmonares recurrentes, retraso en el desarrollo, hiperlipidemia, escoliosis, pies planos, hipotiroidismo, hipertensión, infecciones recurrentes del tracto urinario, deficiencia de la hormona del crecimiento o alopecia [108–110]. El diagnóstico de ALMS suele ser difícil en niños, ya que muchas de las características clínicas se desarrollan con el tiempo, por lo que en el diagnóstico de ALMS hay que considerar la edad de inicio de los síntomas primarios y secundarios [109].

Los estudios genéticos se usan para confirmar el diagnóstico clínico de ALMS, ya que este síndrome solo es causado por variantes patogénicas en el gen *ALMS1* (MIM *606844), que se

encuentra situado en el brazo corto del cromosoma 2 [109,111]. Aunque la gran mayoría de las variantes en *ALMS1* solo han sido descritas en un solo paciente, existen algunas mutaciones con efecto fundador [107,112,113].

El gen *ALMS1* codifica para la proteína ALMS1, que se encuentra en el cilio primario, concretamente localiza en el centrosoma y en el cuerpo basal [114]. Esta proteína interviene en las vías de señalización ciliar, el control del ciclo celular, la diferenciación celular, el tráfico intracelular y la homeostasis del metabolismo energético [115].

3.2.3.2. Síndrome MORM

El síndrome MORM (en inglés, *Mental retardation, truncal Obesity, Retinal dystrophy, and Micropenis syndrome*) es un trastorno ultrarraro descrito en individuos afectos de una familia pakistaní [116,117]. Se manifiesta como discapacidad intelectual estática moderada, obesidad troncal, DHR no progresiva e hipogonadismo [116]. Este síndrome presenta grandes similitudes con otro trastorno, el BBS [55].

Mutaciones en el gen *INPP5E* (MIM *613037) producen JBTS y síndrome de MORM [118]. Sin embargo, hasta el año 2021 solo una mutación sin sentido en el gen era la causante del síndrome MORM [119,120]. La variante p.(Gln627*) se encuentra en el dominio C-terminal del gen, resultando en una proteína truncada, que conduce a una disminución de la estabilidad de los cilios y de las estructuras ciliares debido a su deslocalización [117,121].

3.2.3.3. *Síndrome de Joubert (JBTS)*

El JBTS es un trastorno raro del neurodesarrollo, típicamente autosómico recesivo, que afecta aproximadamente a 1 de cada 100 000 recién nacidos [122].

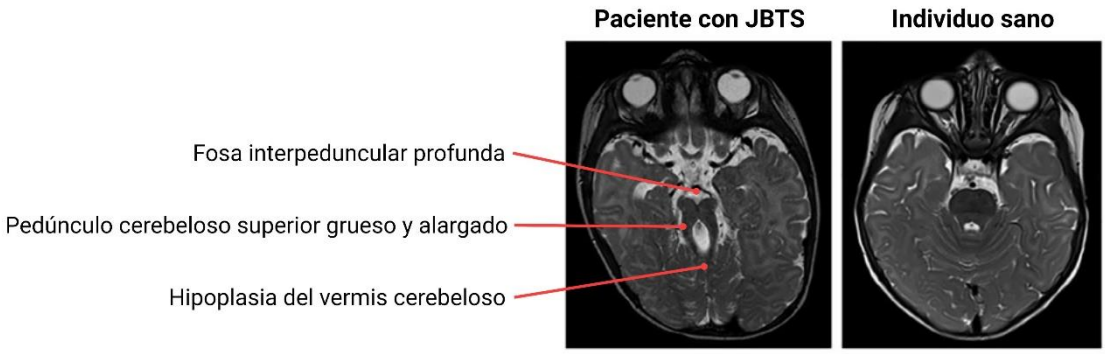


Figura 8. *"Signo del diente molar" en imágenes de resonancia magnética de un paciente con síndrome de Joubert. Imagen modificada de Bachmann-Gagescu et al. (2020) [123] con BioRender.*

El diagnóstico de JBTS se basa en una malformación patognomónica en las imágenes de la resonancia magnética conocida como "signo del diente molar" (MTS, por sus siglas en inglés), la cual consiste en la hipoplasia del vermis cerebeloso, engrosamiento y orientación horizontal de los pedúnculos cerebelosos superiores y fosa interpeduncular profunda (Figura 8). El JBTS clásico se manifiesta además con hipotonía en la infancia y discapacidad intelectual/retraso en el desarrollo, como características primarias. Adicionalmente, el JBTS puede presentar otras características secundarias, tales como problemas visuales (EHR o LCA), movimientos oculares anormales (nistagmos o apraxia oculomotora), anomalías respiratorias, encefalocele, polimicrogiria, otras malformaciones del SNC (agenesis del cuerpo calloso, malformación Dandy-Walker, o displasia cortical), ataxia, alteraciones renales (nefronoptisis o enfermedad quística renal) o polidactilia [122,124,125].

Este síndrome es genéticamente muy heterogéneo y ha sido asociado a mutaciones en 40 genes distintos (Anexo 1), todos los cuales codifican proteínas que se localizan en el cilio primario o en el cuerpo basal [126]. Además de esos 40 genes, existen adicionalmente otros 11 genes que no han sido aún asignados de manera formal al JBTS, pero que se consideran candidatos o se han encontrado variantes patogénicas en ellos junto el "signo del diente molar" (Anexo 2).

Dada la extrema heterogeneidad clínica y genética del JBTS, los estudios genéticos se basan en las correlaciones genotipo-fenotipo de los distintos genes relacionados con el JBTS [126,127].

Asimismo, mutaciones en los genes asociados con el JBTS suelen ser la principal causa también de otras ciliopatías, como la nefronoptisis (NPHP) o el síndrome de Meckel-Gruber (MKS) [128].

3.2.3.4. *Síndrome de Senior-Løken (SLSN)*

El SLSN es un trastorno raro que se superpone fenotípica y genéticamente con otras ciliopatías, como son el BBS, JBTS o NPHP [55]. Su prevalencia estimada es de 1 entre 1 000 000 de individuos [129]. Sus principales características clínicas son la nefronoptisis juvenil y EHR de aparición temprana, donde las lesiones retinianas son variables y comprenden desde LCA hasta RP [130]. De forma adicional, el SLSN puede tener en algunos casos otras características clínicas asociadas, como son el "signo del diente molar" en la resonancia magnética, hipotonía muscular, discapacidad intelectual/retraso en el desarrollo, movimientos oculares anormales (nistagmos o apraxia oculomotora), encefalocele o fibrosis hepática [122].

Actualmente, mutaciones en 9 genes se han asociado al SLSN (Anexo 1). Además, se han propuesto como posibles candidatos variantes en 2 genes adicionales (Anexo 2).

4. Síndromes no relacionados con los cilios

Dentro de las ERS también se pueden encontrar síndromes raros que no están relacionados con los cilios (Tabla 2), sino que están vinculados a otros componentes celulares, como son el aparato de Golgi, retículo endoplasmático, lisosomas, peroxisomas o mitocondria. Algunos de estos síndromes suelen conducir a errores congénitos en el metabolismo, como por ejemplo del metabolismo de los carbohidratos, de las proteínas, la oxidación de ácidos grasos o el almacenamiento de glucógeno [27,131,132].

Tabla 2. Características generales de algunos síndromes no clasificados como ciliopatías

Síndrome	Prevalencia	Gen	Componente celular afectado	Patogénesis
ML	1:37 000 – 1:450 000 [133,134]	*GNPTAB, MCOLN1*	Lisosomas	Enzimas lisosomales deficientes o con tráfico alterado, acumulación sustratos no degradados en los lisosomas [135]
ATS	1:50 000 nacidos vivos [136]	*COL4A3, COL4A4, COL4A5*	Membrana basal	Síntesis deficiente de heterotrímeros de colágeno [137]
CLN	1:12 500 – 1:100 000 [138]	*PPT1, TPP1, CLN3, CLN5, CLN6, MFSD8, CLN8, CTSD, GRN*	Lisosomas	Defectos en proteínas lisosomales o del RE producen acumulación neuronal y extraneuronal de pigmento autofluorescente [139]

Síndrome	Prevalencia	Gen	Componente celular afectado	Patogénesis
COH	~200 afectos [140]	*VPS13B*	Aparato de Golgi	Defectos en la N-glicosilación [141]
WFS	1:160 000 – 1:770 000 [142,143]	*WFS1*, *CISD2*	Retículo endoplasmático	Estrés en el retículo endoplasmático, alteración de la progresión del ciclo celular, de la homeostasis del calcio y de la dinámica mitocondrial [144]
PBD	1:50 000 – 1:500 000 [145,146]	*PEX1, PEX2, PEX5, PEX6, PEX7, PEX12, PEX13, PEX16*	Peroxisomas	Defectos en la biosíntesis, ensamblaje y funciones bioquímicas de los peroxisomas [147]

Abreviaturas (por orden alfabético): ATS, síndrome de Alport; CLN, lipofuscinosis neuronal ceroidea; COH, síndrome de Cohen; ML, mucolipidosis; PBD, Trastornos de la biogénesis peroxisomal; WFS, síndrome de Wolfram. Números MIM de los distintos síndromes: Mucolipidosis (#252600, #252650); síndrome de Alport (#203780, #301050); Lipofuscinosis neuronal ceroidea (#256730, #204500, #204200, #256731, #601780, #610951, #600143, #610127, #614706); síndrome de Cohen (#216550); síndrome de Wolfram (#222300, #604928); y Trastornos de la Biogénesis Peroxisomal (#214100, #234580, #601539, #614866, #614867, #214110, #614863, #616617, #614879, #266510, #614885, #614877).

5. Estrategias para el diagnóstico molecular de casos sindrómicos

Debido al alto grado de variabilidad y superposición fenotípica que existe en las ERS, el diagnóstico basado solo en las características clínicas puede ser difícil y, a veces, engañoso. Por lo que la identificación de las variantes genéticas causales es de gran relevancia por varias razones: a) complementa los hallazgos clínicos y confirma el diagnóstico clínico; b) permite comprender el curso de la historia natural y establecer un pronóstico adecuado para el paciente; c) da la posibilidad de asesoramiento genético para prevenir la transmisión de la enfermedad; d) es esencial para la inclusión en ensayos clínicos y en futuras terapias dirigidas a genes; y e) permite la identificación de nuevos genes y mecanismos de enfermedad [27,148,149].

5.1. *Human Phenotype Ontology*

La ontología fenotípica humana (*Human Phenotype Ontology*, HPO por sus siglas en inglés) es un recurso que define sistemáticamente y organiza los fenotipos humanos de forma jerárquica asociados a patología fenotípica humana (Figura 9) [150–152]. Fue desarrollada en 2008 con más de 8000 términos HPO que representaban tanto anomalías fenotípicas individuales como las distintas entradas clínicas del catálogo en línea de genes humanos y trastornos genéticos, *Online Mendelian Inheritance in Man* (OMIM, por sus siglas en inglés) [153]. Actualmente, HPO ya contiene más de 15 000 términos y ha sido adoptado como el estándar mundial para la descripción de fenotipos [152].

El uso de términos HPO tiene amplias aplicaciones clínicas y de investigación que ayudan a realizar una medicina de precisión, como son el análisis combinado de genotipos y fenotipos, descubrimiento de enfermedades genéticas y mecanismos o análisis de cohortes [154–158]. Por lo tanto, estos términos proporcionan la herramienta más completa para el fenotipado computacional profundo de los casos, pudiendo ayudar en la definición de la enfermedad, la descripción de las anomalías clínicas o el diagnóstico genómico [152,159].

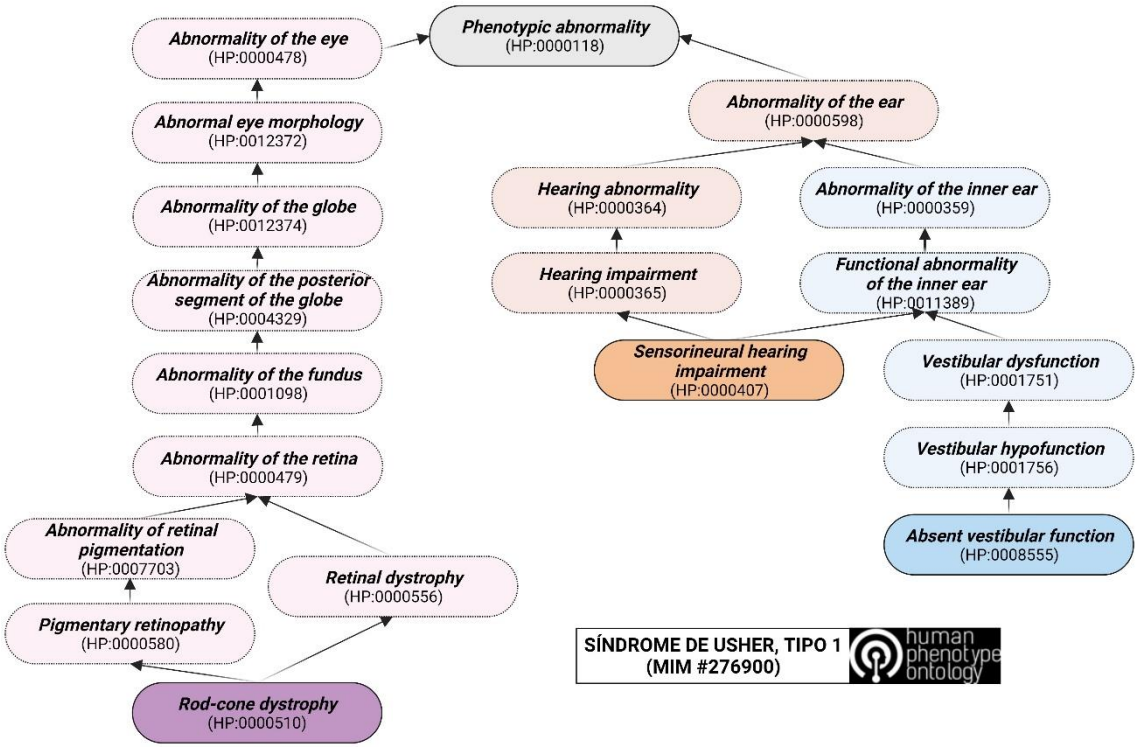


Figura 9. *Ejemplo de estructura jerárquica de datos usando Human Phenotype Ontology (HPO) para los signos clínicos del Síndrome de Usher de tipo 1. Imagen creada con BioRender.*

5.2. Secuenciación masiva

En las dos últimas décadas, la importancia de la secuenciación masiva (en inglés, *Next Generation Sequencing*, NGS) ha aumentado significativamente a medida que se iba disponiendo de un mayor abanico de técnicas y reduciendo su tiempo, coste, complejidad de protocolos y análisis bioinformático de los datos [160,161]. Actualmente, la NGS es el enfoque más rentable para el análisis genético de las formas sindrómicas complejas [162], habiéndose demostrado que su aplicación mejora el diagnóstico molecular en comparación con otros métodos de cribado tradicionales [163,164], como son la secuenciación Sanger, *microarrays* comerciales de genotipado basados en la tecnología *APEX* (en inglés, *Arrayed Primer Extension*) o mapeos de homocigosidad usando *arrays* de SNPs (polimorfismo de un solo nucleótido, por sus siglas en inglés) de todo el genoma.

La NGS permite la secuenciación rápida y fiable de áreas más amplias del genoma mediante la generación paralela y de forma masiva de un gran número de fragmentos de ADN [165]. Además del ADN nuclear también se puede aplicar esta tecnología para secuenciar el genoma mitocondrial [166].

Existen distintas aproximaciones de la NGS en función de la aplicación o la región del genoma a estudiar (Figura 10), entre las que destacan: a) la secuenciación dirigida (en inglés, *Targeted Sequencing*, TS), que permite capturar paneles de genes y/o determinadas regiones de interés del genoma [167]; b) secuenciación del exoma clínico (en inglés, *Clinical Exome Sequencing*, CES), en el que se capturan y secuencian todas las regiones codificantes de los genes ya asociados a patología en OMIM; c) WES, es el método para secuenciar todas las regiones codificantes del genoma, lo que da como resultado aproximadamente al 1% del genoma completo [168,169]; y d) secuenciación del genoma completo (en inglés, *Whole Genome*

Sequencing, WGS), secuencia todo el genoma, incluidos los intrones y las regiones intergénicas [170,171].

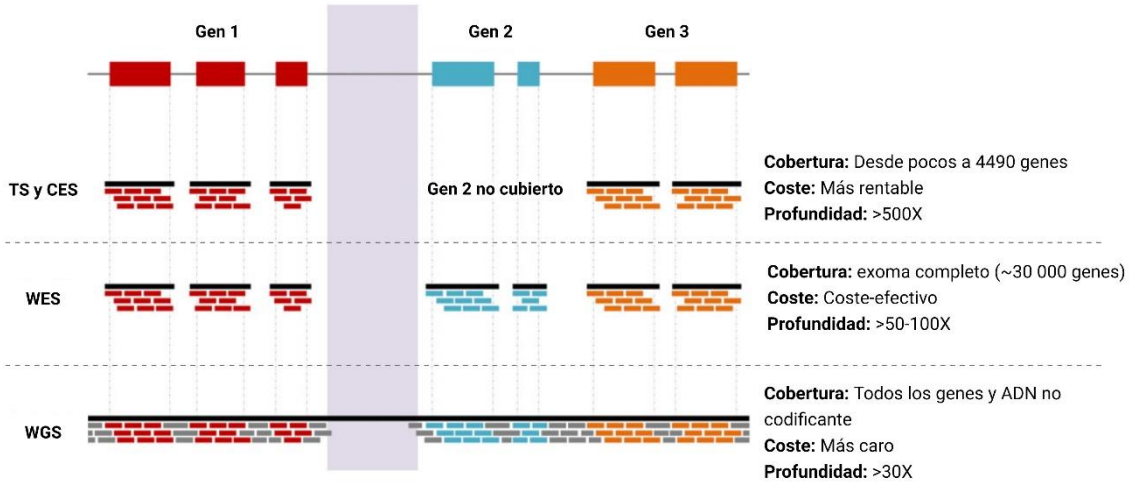


Figura 10. *Tipos de secuenciación masiva. Esquema de las distintas aproximaciones de NGS, entre las que se incluyen la secuenciación dirigida (TS), secuenciación del exoma clínico (CES), secuenciación del exoma completo (WES) y secuenciación del genoma completo (WGS). Los pequeños "ladrillos" corresponden a las lecturas de las distintas regiones, que al alinearse con un genoma de referencia, permite la identificación de variantes de un solo nucleótido y del número de copia. La región lila corresponde a una zona genómica difícil de secuenciar y no cubierta por ninguno de los métodos de NGS. Imagen modificada de Méjécase et al. (2020) [172] con BioRender.*

Con la NGS se crean grandes cantidades de datos que deben analizarse e interpretarse de manera eficiente, lo cual es crucial para encontrar la causa de una enfermedad, diferentes mecanismos genéticos o incluso comprender el efecto patogénico de una variante específica. Para el procesamiento de los datos generados (Figura 11), se necesitan distintas herramientas bioinformáticas que permitan el control de calidad de la secuenciación y alinear las secuencias con un genoma de referencia, ensamblarlas y anotarlas [173]. Para la priorización de las variantes, hay que considerar una serie de parámetros que faciliten su filtrado, como son la calidad de cada variante en la secuenciación (p. ej. cobertura y profundidad de lectura), su frecuencia en bases de datos poblacionales, efecto que tiene en la proteína o presencia en bases de datos clínicas o en la literatura [174]. Finalmente, las variantes priorizadas se interpretan y clasifican según las guías internacionales, en benigna (clase 1), probablemente benigna (clase 2), variante de significado incierto (VUS, clase 3), probablemente patogénica (clase 4) y patogénica (clase 5) [175,176].

Además del estudio de las variantes de un solo nucleótido (SNV, por sus siglas en inglés), también hay que tener en cuenta la presencia de deleciones o duplicaciones en el genoma [177]. Para el análisis de estas CNVs se pueden usar diversas estrategias, donde la amplificación de la sonda dependiente de la ligadura multiplex (en inglés, *Multiplex Ligation-dependent Probe Amplification*, MLPA) y los *arrays* de CGH (Hibridación Genómica Comparada) son las opciones más tradicionales. En los últimos años se han desarrollado algoritmos bioinformáticos para detectar CNVs a partir de los datos de NGS [178,179].

Finalmente, la variante causal candidata detectada por la NGS es validada mediante secuenciación Sanger o, en el caso de las CNVs, mediante MLPA o *array* de CGH. Cuando sea posible se realiza un estudio de co-segregación en la familia [28].

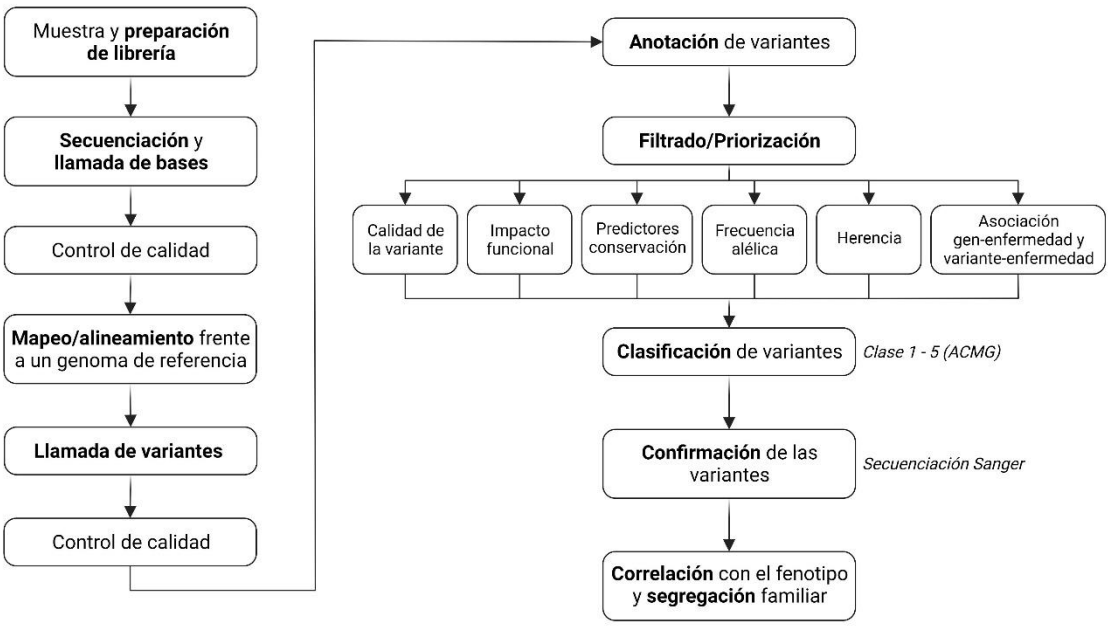


Figura 11. *Flujo de trabajo para la identificación, anotación y priorización de variantes a partir de datos de secuenciación masiva. Abreviaturas: ACMG, Colegio Americano de Genética y Genómica Médica (por sus siglas en inglés). Imagen modificada de Brett et al. (2014) y Marshall et al. (2020) [180,181] con BioRender.*

La CES y WES son herramientas poderosas cuando se trata de determinar la causa genética de una enfermedad heterogénea, como es el caso de las ERS [182,183]. Además, estas tecnologías permiten el reanálisis y la revisión de los casos estudiados anteriormente usando paneles virtuales actualizados de genes o nuevas herramientas bioinformáticas [184]. Sin embargo, existe un porcentaje de casos aún no caracterizados tras usar CES/WES, el cual podría deberse a problemas técnicos (p. ej. falta de cobertura en ciertas zonas exónicas), mutaciones no detectadas debido a limitaciones en el análisis e interpretación de los datos, variantes no codificantes que pueden afectar a la expresión génica o estabilidad del ARNm, o la incapacidad de la WES para detectas variantes estructurales (grandes CNVs, translocaciones o inversiones) [182]. Los dos últimos problemas podrían ser resueltos con el uso de WGS [185].

5.3. Herramientas *in silico*

La NGS ha revolucionado la forma en la que se investigan y diagnostican las enfermedades genéticas humanas al permitir la identificación paralela de miles de variantes en la secuencia de ADN. Sin embargo, esa gran cantidad de datos de secuenciación también es un factor de confusión, debido a la dificultad de identificar con precisión la o las variantes patogénicas causales del fenotipo entre las miles o millones de variantes genómicas. Este reto requiere de nuevas herramientas *in silico* para la priorización de variantes [186,187], como son herramientas para la predicción de regiones de homocigosidad (p. ej. AutoMap [188]) o para evaluar el efecto de combinaciones de variantes o genes como causa de enfermedad (p. ej. ORVAL [189] o DiGePred [190]).

AutoMap (https://automap.iob.ch/) es una herramienta usada para la predicción de regiones de homocigosidad (ROH, por sus siglas en inglés) en el genoma a partir de datos de NGS con una alta especificidad y sensibilidad [188]. En casos con sospecha de un trastorno hereditario AR raro y consanguinidad, las suposiciones iniciales son que la enfermedad pueda estar causada por una variante homocigota heredada de ambos padres y que se sitúe dentro de una ROH extensa [191], reduciendo así considerablemente la cantidad de variantes a analizar. El uso de este tipo

de herramientas ahorra tiempo y recursos, ya que antes se utilizaban dos técnicas separadas, por un lado mapeos de homocigosidad usando *arrays* de SNPs y, por el otro, NGS para detectar las mutaciones puntuales [192,193].

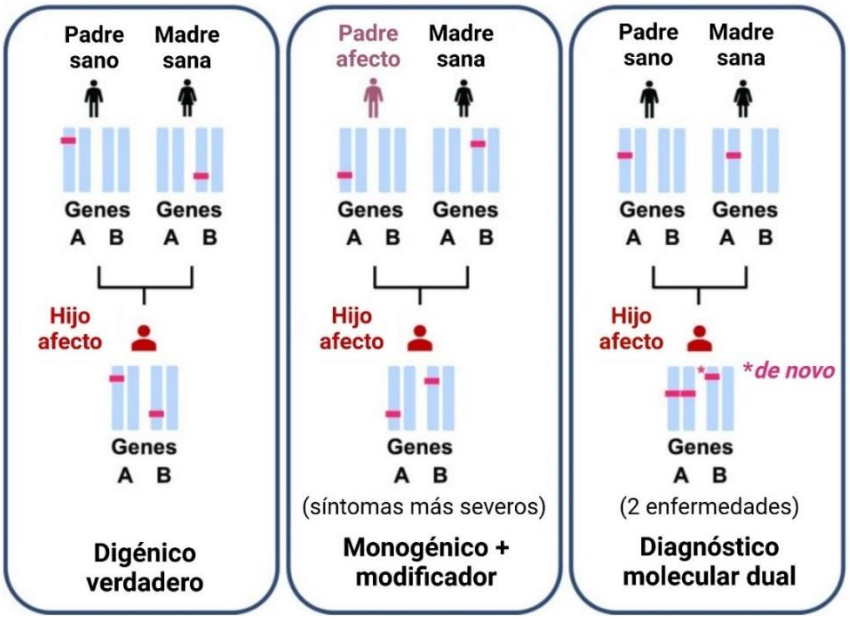


Figura 12. *Ejemplos de la clasificación de las combinaciones de variantes con la plataforma ORVAL. Imagen modificada de Papadimitriou et al. (2019) [194] con BioRender.*

ORVAL (*Oligogenic Resource for Variant Analysis*; https://orval.ibsquare.be/) es una plataforma para la predicción y exploración de combinaciones de variantes oligogénicas candidatas. La herramienta se basa en la predicción utilizando distintas variantes, genes y características biológicas de los pares de genes para clasificar las combinaciones [189,194] (Figura 12). Mientras que el predictor DiGePred (http://www.meilerlab.org/index.php/servers) sirve para clasificar pares de genes candidatos a producir una enfermedad digénica, mediante información genómica, evolutiva, funcional y redes biológicas [190]. Este predictor digénico fue desarrollado y entrenado usando DIDA (*DIgenic Disease DAtabase*; http://dida.ibsquare.be/), la mayor base de datos conocida sobre variantes y genes asociados con herencia oligogénica [195].

Ambas plataforma pueden ser herramientas extremadamente útiles en el estudio y comprensión de resultados moleculares en casos asociados a enfermedades con alta heterogeneidad genética y fenotípica y que muestren indicios de oligogenicidad [190,196,197].

OBJETIVOS

Objetivo general

El objetivo principal de esta Tesis Doctoral ha sido refinar y caracterizar subcohortes de pacientes con enfermedades hereditarias de la retina para su posible inclusión en estudios observacionales o de intervención, utilizando una combinación de nuevas técnicas de secuenciación masiva y herramientas *in silico* para ampliar el conocimiento de las bases moleculares y posibles mecanismos hereditarios de estas enfermedades.

Objetivos específicos

1. Presentar una visión global fenotípica, epidemiológica y molecular de la cohorte de pacientes con enfermedades hereditarias de la retina del Servicio de Genética del Hospital Universitario Fundación Jiménez Díaz.

2. Mejorar el diagnóstico clínico y molecular de los pacientes con enfermedades hereditarias de la retina sindrómicas, mediante el uso de una ontología fenotípica estructurada, basada en términos de la *Human Phenotype Ontology*, y secuenciación masiva.

3. Analizar y establecer las posibles combinaciones oligogénicas de variantes en familias afectadas por el síndrome de Bardet-Biedl, así como detectar posibles alelos modificadores del fenotipo.

4. Describir y detectar posibles correlaciones genotipo-fenotipo en una cohorte de pacientes con síndrome de Usher y diagnosticados genéticamente con mutaciones en el gen *MYO7A*, con el fin de realizar un estudio transversal retrospectivo de la enfermedad.

RESULTADOS

CAPÍTULO 1

Epidemiología de las enfermedades hereditarias de la retina en España

Artículo 1: *Genetic landscape of 6089 inherited retinal dystrophies affected cases in Spain and their therapeutic and extended epidemiological implications*

Perea-Romero I, Gordo G, Iancu IF, *et al.*

Publicado en *Scientific Reports*, 2021

Resumen

En este trabajo se realizó un estudio transversal retrospectivo en los 6089 casos afectos de EHR (de 4403 familias no emparentadas) derivados al Servicio de Genética de la Fundación Jiménez Díaz de todas las comunidades autónomas desde el año 1991 a agosto de 2019. Se recogieron datos clínicos, familiares y demográficos de cada paciente, incluyéndose los síntomas visuales y su edad de aparición, la presencia de cualquier manifestación sistémica, el árbol genealógico y el origen geográfico. Se realizaron estudios genéticos a las 3951 familias con ADN disponible mediante diferentes técnicas moleculares, las cuales fueron cambiando y evolucionando a lo largo del tiempo.

Clínicamente, las familias con EHR fueron clasificadas en EHR sindrómicas y no sindrómicas. Estas últimas fueron clasificadas en 2 subgrupos: "NON-RP" (fenotipos centrales o dominados por afectación de los conos) y "RP" (fenotipos periféricos o dominados por afectación de los bastones), siendo el fenotipo más común el "RP", en el 55,6% de las familias (2447/4403). Adicionalmente, las formas sindrómicas fueron las minoritarias (621/4403 familias).

Desde el punto de vista genético, se caracterizó molecularmente al 53,2% (2100/3951) de las familias estudiadas y se identificaron 1549 posibles variantes causales distintas en 142 genes. Los genes mutados con mayor frecuencia fueron *PRPH2*, *ABCA4* y *RS1* en los casos "NON-RP" autosómicos dominantes (AD), autosómicos recesivos (AR) y ligados al cromosoma X (XL), respectivamente; *RHO*, *USH2A* y *RPGR* en los casos "RP" no sindrómicos AD, AR y XL; y *USH2A* y *MYO7A* en las ERS. Las variantes más frecuentemente identificadas fueron las variantes patogénicas c.3386G>T (p.Arg1129Leu) en *ABCA4* y c.2276G>T (p.Cys759Phe) en *USH2A*.

Gracias a la identificación del gen causal, se pudo reclasificar el patrón hereditario (distinto modo de herencia *a priori* versus final) en el 8,2% de las familias y la forma clínica (de un subgrupo fenotípico a otro) en el 2,5% de ellas.

Con este trabajo se ha estudiado el panorama genético de las EHRs en España, describiendo la cohorte de pacientes más grande reportada hasta el momento. Nuestros resultados tienen importantes implicaciones para el diagnóstico genético, asesoramiento y nuevas estrategias terapéuticas tanto para la población española como para otras poblaciones afines.

Contribución de la autora

La autora trabajó en la reestructuración y curación de la base de datos del Servicio de Genética de la Fundación Jiménez Díaz, la cual englobaba en ese momento todos los casos con EHR

derivados para su estudio al Servicio de Genética durante 28 años (n = 6089). Además, participó en la recogida, estructuración y revisión de los datos clínicos, genéticos, demográficos y familiares de los 6089 pacientes de EHR que componen la cohorte.

Participó también en el análisis formal de los distintos datos recogidos, p. ej. prevalencia de los distintos subtipos clínicos, frecuencia y distribución de las variantes en el territorio español, modos de herencia y reclasificación clínica y genética de los casos.

Asimismo, la autora intervino en la escritura del manuscrito, en la revisión crítica de las distintas versiones y en la aprobación de la versión final.

El artículo y el material suplementario se encuentran publicados en:

https://www.nature.com/articles/s41598-021-81093-y

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Genetic landscape of 6089 inherited retinal dystrophies affected cases in Spain and their therapeutic and extended epidemiological implications

Irene Perea-Romero[1,2,59], Gema Gordo[1,59], Ionut F. Iancu[1,2,59], Marta Del Pozo-Valero[1,2], Berta Almoguera[1,2], Fiona Blanco-Kelly[1,2], Ester Carreño[3], Belen Jimenez-Rolando[3], Rosario Lopez-Rodriguez[1], Isabel Lorda-Sanchez[1,2], Inmaculada Martin-Merida[1,2], Lucia Pérez de Ayala[1], Rosa Riveiro-Alvarez[1,2], Elvira Rodriguez-Pinilla[1], Saoud Tahsin-Swafiri[1,2], Maria J. Trujillo-Tiebas[1,2], The ESRETNET Study Group*, The ERDC Study Group*, The Associated Clinical Study Group*, Blanca Garcia-Sandoval[2,3], Pablo Minguez[1,2], Almudena Avila-Fernandez[1,2], Marta Corton[1,2,59]✉ & Carmen Ayuso[1,2,59]✉

Inherited retinal diseases (IRDs), defined by dysfunction or progressive loss of photoreceptors, are disorders characterized by elevated heterogeneity, both at the clinical and genetic levels. Our main goal was to address the genetic landscape of IRD in the largest cohort of Spanish patients reported to date. A retrospective hospital-based cross-sectional study was carried out on 6089 IRD affected individuals (from 4403 unrelated families), referred for genetic testing from all the Spanish autonomous communities. Clinical, demographic and familiar data were collected from each patient, including family pedigree, age of appearance of visual symptoms, presence of any systemic findings and geographical origin. Genetic studies were performed to the 3951 families with available DNA using different molecular techniques. Overall, 53.2% (2100/3951) of the studied families were genetically characterized, and 1549 different likely causative variants in 142 genes were identified. The most common phenotype encountered is retinitis pigmentosa (RP) (55.6% of families, 2447/4403). The most recurrently mutated genes were *PRPH2*, *ABCA4* and *RS1* in autosomal dominant (AD), autosomal recessive (AR) and X-linked (XL) NON-RP cases, respectively; *RHO*, *USH2A* and *RPGR* in AD, AR and XL for non-syndromic RP; and *USH2A* and *MYO7A* in syndromic IRD. Pathogenic variants c.3386G>T (p.Arg1129Leu) in *ABCA4* and c.2276G>T (p.Cys759Phe) in *USH2A* were the most frequent variants identified. Our study provides the general landscape for IRD in Spain, reporting the largest cohort ever presented. Our results have important implications for genetic diagnosis, counselling and new therapeutic strategies to both the Spanish population and other related populations.

Inherited retinal diseases (IRDs) are one of the most heterogeneous clinical and genetical disorders known among all human medical conditions, characterized by the progressive loss of photoreceptor cells, resulting in severe visual impairment[1]. IRDs are classified as rare diseases, and their estimated prevalence is about 1 in 1000[2]–4000[1]. IRDs can be classified according to different clinical or genetic criteria, based upon the primary retinal cell

[1]Department of Genetics, Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [2]Center for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain. [3]Department of Ophthalmology, Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [59]These authors contributed equally: Irene Perea-Romero, Gema Gordo, Ionut F. Iancu, Marta Corton and Carmen Ayuso. *A list of authors and their affiliations appears at the end of the paper. ✉email: mcorton@quironsalud.es; cayuso@fjd.es

affected (rods, cones, retinal pigment epithelium (RPE), bipolar cells or ganglion cells), the ophthalmological findings and/or the affected gene found after the genetic testing.

All modes of inheritance can be observed (autosomal dominant (AD), autosomal recessive (AR), X-linked (XL), including rare non-Mendelian forms such as mitochondrial or digenic inheritance patterns). Age of onset of first symptoms (from early childhood to adulthood), rate of progression, association with extra-ocular symptoms (non-syndromic versus syndromic forms) or causative gene can also help to subclassify the different phenotypes[3]. The most prevailing form of IRDs is Retinitis Pigmentosa (RP [MIM: 268000]), which is estimated to affect approximately 1.5 million people worldwide[4]. RP begins with the degeneration of rod photoreceptors, resulting in night blindness and characteristic pigmentary changes in the peripheral retina. This is also considered a rod-cone dystrophy due to the subsequent cone photoreceptor death in later stages. Other forms of IRD, including cone-dominated diseases, are characterized by photophobia, reduced visual acuity and impaired colour vision (i.e. cone dystrophy (CD), achromatopsia, blue cone monochromatism), as well as generalized retinal degeneration involving simultaneously both cones and rods such as in rod-cone or cone-rod dystrophies (CRD). The most severe form of non-syndromic IRDs is Leber congenital amaurosis (LCA) characterized by congenital or early childhood blindness. Other IRD forms are characterized by central vision loss affecting primarily the macula and are therefore acknowledged as Macular Dystrophies (MD), such as Stargardt disease (STGD1 [MIM: 248200]) and Best Vitelliform Macular Dystrophy (VMD2 [MIM: 153700])[1].

Conversely, syndromic IRDs are subclassified according to the type of syndrome. The most prevalent is Usher syndrome (sensorineural hearing loss and RP) which can be further subclassified to Usher type I (USH1 [MIM: 276900]), type II (USH2 [MIM: 276901]) and type III (USH3 [MIM: 276902]), and other syndromes such as Bardet-Biedl (BBS [MIM: 209900]) or Alström (ALMS [MIM: 203800])[3].

Since the identification of rhodopsin (*RHO* [MIM: 180380]) in 1990 as the first gene involved in the development of AD-RP[5], 270 genes have been additionally described as causative of IRDs (RetNet, Retinal Information Network; https://sph.uth.edu/retnet/; accessed on March 2020). Some of these genes have been reported in only few families worldwide; therefore, their individual contribution to IRD prevalence is relatively small. The most prevalent IRD-causing genes across all populations are *ABCA4* (MIM: 601691), *RHO*, *USH2A* (MIM: 608400) and *RPGR* (MIM: 312610), which account for high percentages of some of the IRD subtypes, i.e. 70–71% in STGD1/AR-MD/AR-CRD[6], 19–25% of AD-RP, 10% of AR-RP, and 70% of XL-RP, respectively[1]. In addition, although the majority of the causative variants are private, some are more frequent, especially in Spanish families, such as *USH2A* (GenBank: NM_206933.3) c.2299delG (p.Glu767SerfsTer21) and c.2276G > T (p.Cys759Phe)[7] or *ABCA4* (GenBank: NM_000350.3) c.3386G > T (p.Arg1129Leu) and c.5882G > A (p.Gly1961Glu)[6].

The aim of this study is to present a comprehensive overview of the largest cohort of IRD patients ever reported worldwide and related to IRD in the Spanish population. The presented data includes the presumed inheritance pattern for the different phenotypic subtypes, mutational spectrum, prevalence of genes carrying likely pathogenic variants and the recurrence of disease related variants.

Results

Prevalence of IRD in Spain. The number of cases diagnosed as having IRD in our hospital (until August 2019) was 6089, and the last Spanish population registry accounted for 46,722,980 habitants giving us a minimal prevalence of 1:7673 (confidence interval (CI):1:7485–1:7871). Regional distribution of cases and prevalence can be seen in Fig. 1A,B. Considering a worldwide IRD prevalence of 1:1000[2]–4000[1], our cohort would represent 20–53% of the total patients with IRD in Spain as shown in Supplementary Table S1.

For non-syndromic IRD, our cohort was grouped in 2 categories: 1703 affected individuals (1335 families), in cone-dominated phenotypes—hereafter "NON-RP"—and 3561 affected cases from 2447 unrelated families within primarily rod affection—hereafter "RP". This resulted in a minimal prevalence of 1:27,436 (CI:1:26,192–1:28,804) and 1:13,121 (CI:12,704–13,566) for NON-RP and RP, respectively. Additionally, syndromic IRD forms represented the smallest fraction accounting for 13.6% of the patients with 825 affected individuals (621 families), resulting in a minimal prevalence of 1:56,634 (CI:1:53,016–1:60,781).

In our cohort, we have a higher proportion of cases from Madrid area (26.7%; 1625/6089).

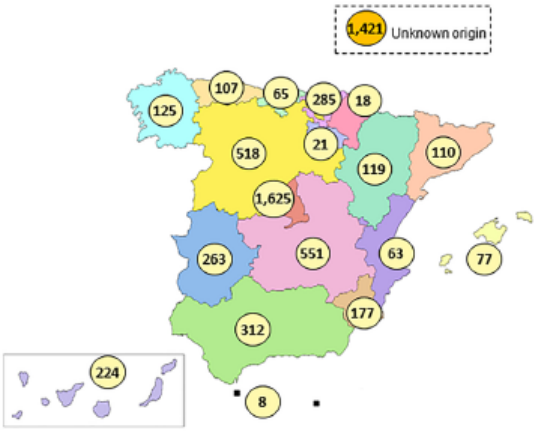
Initial classification of IRD families by clinical type and suspected mode of inheritance prior to genetic testing. Non-syndromic NON-RP and RP cases were categorized by the mode of inheritance (Fig. 2A-I and A-II). Syndromic IRD were categorized by the specific type of suspected syndrome (Fig. 2A-III), instead of inheritance type, given that most of them were sporadic (53.6%) or had recessive inheritance (40.2%). The remaining 6.2% corresponded to dominant (0.5%), X-linked (0.7%), mitochondrial inherited disease (0.2%) or non-classificable cases (4.8%).

According to this "a priori" diagnosis based on the clinical and familial history of the patients, the main inheritance pattern in non-syndromic NON-RP and RP was recessive or sporadic, representing the 68% and 75% of cases, respectively. Autosomal dominant and X-linked forms accounted for 21% and 8% for NON-RP and 15% and 8% for RP, respectively (Fig. 2A-I). Families with no familiar data were annotated as unclassified. Non-syndromic RP represents the most common phenotype, representing 55.6% of families in our cohort.

In the present cohort, 47% of the syndromic IRD index cases (270/577) suffered from USH2, followed by 17% USH1 (98/577), as well as other very rare syndromes like some atypical forms of Usher syndrome (3%; 16/577) and ciliopathies such as BBS or ALMS (16%; 90/577). A miscellanea of non-ciliopathic syndromes or unclassified symptoms were presented in 103 index cases.

Molecular studies. *Diagnostic yield.* Genetic testing was performed in a total of 3951 index cases with available DNA[6–11] (89.7% of the total cohort), including 1291 NON-RP, 2083 RP, and 577 syndromic IRD pa-

A



B

Spanish regions (autonomous communities)	N° of cases	Population	Prevalence	Confidence interval (95% confidence)
Andalusia	312	8,384,408	*	*
Aragon	119	1,308,728	*	*
Asturias	107	1,028,244	*	*
Balearic Islands	77	1,128,908	*	*
Canary Islands	224	2,127,685	*	*
Basque Country	285	2,199,088	*	*
Cantabria	65	580,229	*	*
Castile and Leon	518	2,409,164	1:4,651	(1:4,282 - 1:5,089)
Castile-La Mancha	551	2,026,807	1:3,678	(1:3,395 - 1:4,014)
Catalonia	110	7,600,065	*	*
Ceuta and Melilla	8	171,528	*	*
Extremadura	263	1,072,863	1:4,079	(1:3,640 - 1:4,640)
Galicia	125	2,701,743	*	*
La Rioja	21	315,675	*	*
Madrid	1,625	6,578,079	1:4,048	(1:3,860 - 1:4,255)
Murcia	177	1,478,509	*	*
Navarre	18	647,554	*	*
Valencian Community	63	4,963,703	*	*
Total cases of known Spanish origin	4,668	-	-	-
TOTAL cases	6,089	46,722,980	1:7,673	(1:485 - 1:7,871)

Figure 1. IRD affected cases distribution and estimated prevalence in Spain. (**A**) Distribution of RP/NON-RP affected cases across Spain. Total cases: 6089 (known Spanish origin: 4668; unknown origin: 1421). Spanish map modified using image editor from https://www.veomapas.com/mapa-mudo-de-las-comunidades-autonomas-de-espana-m103.html. (**B**) IRD estimated prevalence in Spanish regions. Data was obtained from the number of cases we had in our Hospital Service and the recorded population in the different regions. *Inconclusive data.

tients as shown in Supplementary Fig. S1. The genetic analysis procedures evolved over time since novel genetic approaches were implemented in our laboratory. A definite genetic diagnosis was established for 53.2% (2100/3951) of cases. For NON-RP families, 754 out of 1291 (58.4%) obtained a genetic diagnosis; in RP the molecular cause was identified in 1038 out of 2083 (49.8%). Similarly, 53.4% (308/577) of syndromic IRD families were genetically solved (Fig. 2B).

Final inheritance pattern and reclassification of characterized families. The identification of the causative gene allowed us to reclassify the inheritance mode in 8.2% (146/1792) of the NON-RP and RP families, thus establishing the final inheritance type (Supplementary Table S2). A comparison between the "a priori" suspected inheritance based on the pedigree and the final inheritance suggested by the molecular diagnosis was performed in characterized NON-RP and RP families (Fig. 2C). As expected, most sporadic NON-RP (n = 378) and RP cases (n = 379) were confirmed as having AR inheritance after the genetic testing. The rest of the S cases were reclassified to AD (n = 43) and XL (n = 36) (Supplementary Table S2). Twenty-four cases (11 NON-RP and 13 RP) with an initial unknown mode of inheritance were classified as: AD (n = 5), AR (n = 16), and XL (n = 3) after the molecular testing.

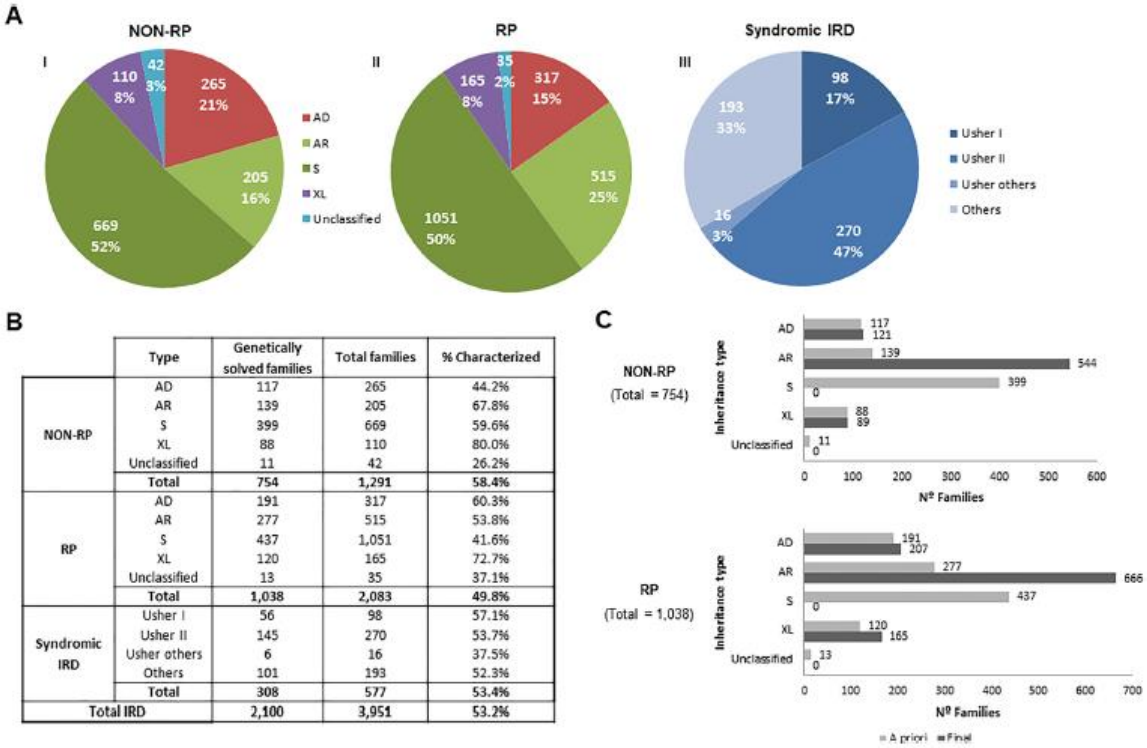
Gene landscape. In total, 1549 different pathogenic and likely pathogenic variants were identified in 142 different genes. These included SNVs (Single Nucleotide Variants) and CNVs (Copy Number Variants). As showed in Figs. 3, 4, 5, there was a wide spectrum of genes implicated in IRD, 121 of them represented in 1% or less of the cohort. The 5 most frequent mutated genes were *ABCA4, USH2A, RS1* (MIM: 300839), *CRB1* (MIM: 604210) and *RHO*.

In NON-RP families, *PRPH2* (MIM: 179605), *ABCA4* and *RS1* were the most commonly mutated genes, explaining 42.2%, 82.7% and 93.3% of the AD, AR and XL forms, respectively as shown in Supplementary Table S3.

For non-syndromic RP, 207 AD-RP families were genetically characterized with heterozygous variants identified in one of 23 genes found. The most frequent mutated genes were *RHO* (65/207; 31.4%) and *PRPF31* (MIM: 606419) (34/207; 16.4%). In AR-RP families, the most common gene mutated was *USH2A* (127/666; 19.1%). However, the number of other disease genes detected was very high (N = 70) in the 666 families characterized. For XL-RP families, both the number of cases and variety of genes were low (N = 9) as shown in Supplementary Table S4. For these patients, 41.2% (68/165) of the index cases (all males) carried a hemizygous pathogenic variant in *RPGR* (44 *RPGR_ORF15* and 24 in the rest of *RPGR* regions), and 32.7% (54/165) in *CHM* (MIM: 300390).

A total of 53 cases out of the total 2100 (2.5%) were clinically reconsidered and reclassified after genetic testing: 12 NON-RP were reclassified as RP (9/754; 1.2%; characterized with *RHO, FSCN2, PRPF8, AHI1, CNGB1, TRPM1* and *CHM*) or as syndromic IRD (3/754; 0.4%; *COL11A1, CDH3* and *MYO7A*) and 37 RP as NON-RP

www.nature.com/scientificreports/



B

	Type	Genetically solved families	Total families	% Characterized
NON-RP	AD	117	265	44.2%
	AR	139	205	67.8%
	S	399	669	59.6%
	XL	88	110	80.0%
	Unclassified	11	42	26.2%
	Total	**754**	**1,291**	**58.4%**
RP	AD	191	317	60.3%
	AR	277	515	53.8%
	S	437	1,051	41.6%
	XL	120	165	72.7%
	Unclassified	13	35	37.1%
	Total	**1,038**	**2,083**	**49.8%**
Syndromic IRD	Usher I	56	98	57.1%
	Usher II	145	270	53.7%
	Usher others	6	16	37.5%
	Others	101	193	52.3%
	Total	**308**	**577**	**53.4%**
Total IRD		**2,100**	**3,951**	**53.2%**

Figure 2. "A priori" and final classification of IRD affected cases. (**A**) "A priori" classification of IRD families with data obtained from the clinical and family history of the patients, before performing molecular tests: NON-RP (I), RP (II), and syndromic IRD (III), and subclassification according to the inheritance type in case of RP and NON-RP, and type of syndrome in case of syndromic IRD. Data obtained with the clinical and familiar history of the patients, before performing molecular tests. *AD* Autosomal Dominant, *AR* autosomal recessive, *S* sporadic, *XL* X-linked. (**B**) Proportion of genetically solved NON-RP, RP, syndromic IRD and total IRD. The diagnostic ratio in the different group of NON-RP and RP by the type of "a priori" inheritance and in the different group of syndromic IRD by type of syndrome is indicated. (**C**) Genetically solved families. Comparison of inheritance classification before (light gray) and after (dark gray) the molecular study was performed.

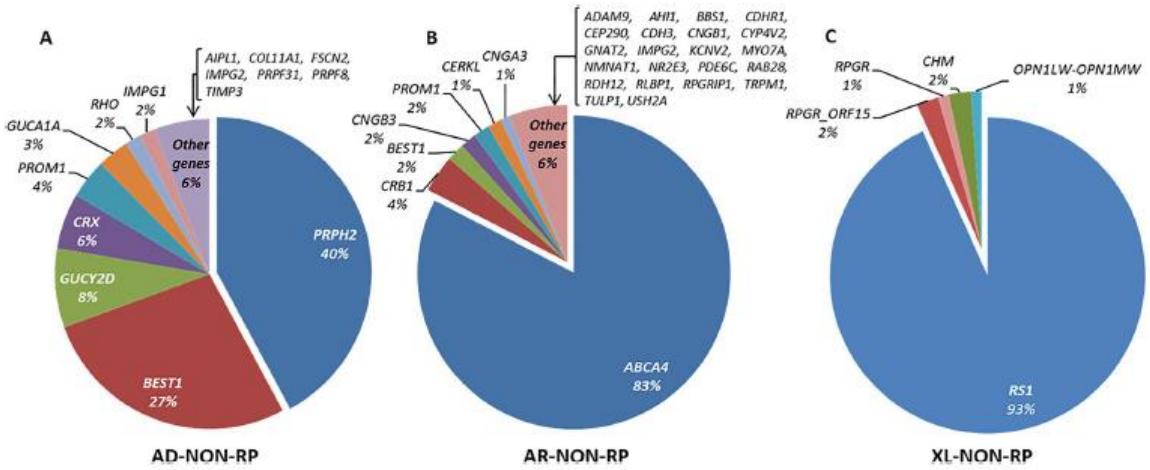


Figure 3. Classification of mutated genes in the genetically solved NON-RP affected cases. Below each gene is given the percentage that each gene was mutated in the cohort. Total characterized families: AD-NON-RP: 121; AR-NON-RP: 544; XL-NON-RP: 89.

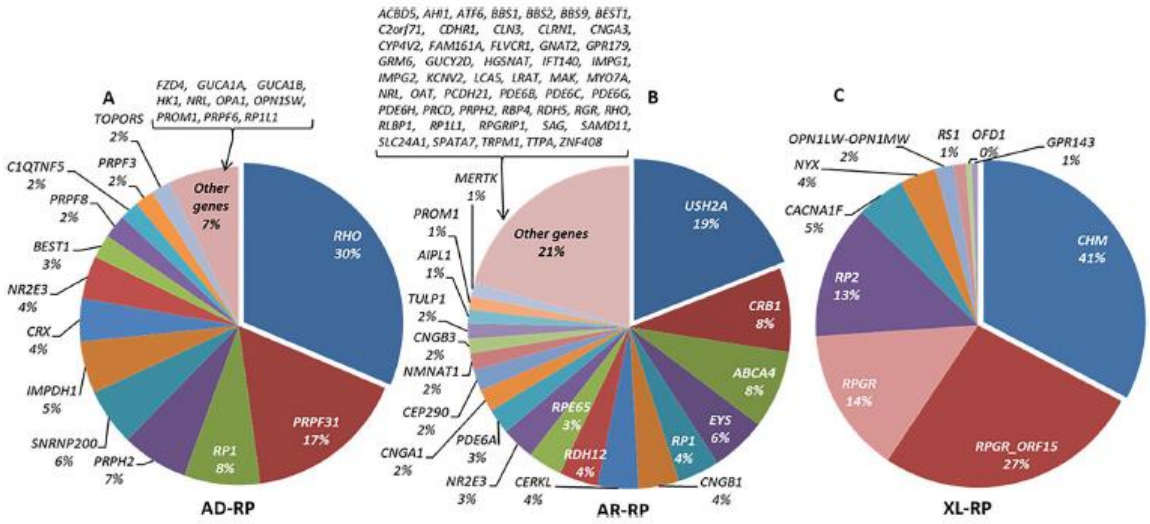


Figure 4. Classification of mutated genes in the genetically solved RP affected cases. Below each gene is given the percentage that each gene was mutated in the cohort. Total characterized families: AD-RP: 207; AR-RP: 666; XL-RP: 165.

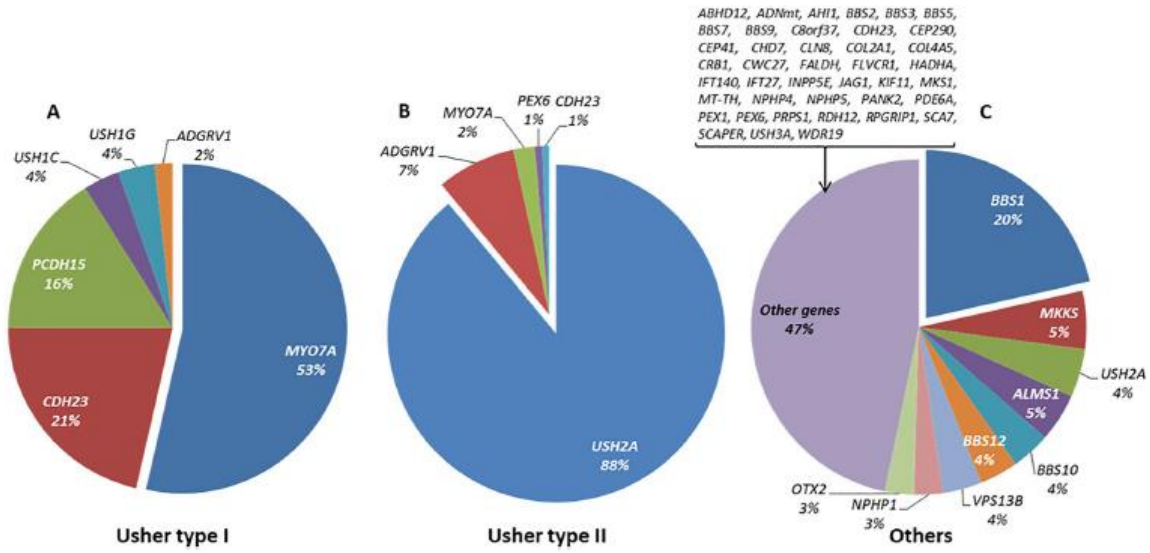


Figure 5. Classification of mutated genes in the genetically solved syndromic IRD affected cases. Below each gene is given the percentage that each gene was mutated in the cohort. Total characterized families: Usher I: 56; Usher II: 145; Others (including atypical Usher): 107.

(32/1038; 3.1%; *C1QTNF5, GUCA1A, CNGB3, CNGA3, ACBD5, GNAT2, PDE6C, ATF6, PDE6H, RS1* and *OPN1LW-OPN1MW*), syndromic IRD (1/1038; 0.1%; *MYO7A*) and other visual diseases, such as exudative familiar vitreoretinopathy, optic atrophy, albinism and gyrate atrophy (4/1038; 0.4%; *FZD4, OPA1, GPR143* and *OAT*). Moreover, 4 syndromic cases were reclassified to RP group (4/308; 1.3%; *RDH12, PDE6A* and *RPGRIP1*).

Among syndromic IRD families, the causative gene was identified in 56 families with a diagnosis of USH1, 145 of USH2, 6 of atypical Usher, and 101 of other syndromes including Bardet-Biedl or Alström syndrome, respectively. In USH1, biallelic variants in *MYO7A* (MIM: 276903) were identified in 30 out of 56 patients (53.5%). *USH2A* defects were the main cause of USH2 in 90% (129/145) of the patients. The group "others" was a clinically heterogeneous group of non-Usher cases with a total of 48 involved genes, with *BBS1* (MIM: 209901) being the most frequent one (N = 23), as shown in Supplementary Table S5.

In addition to clinical and genetic heterogeneity, the group of "others" included in syndromic IRD also presented unusual modes of inheritance, such as triallelism. In our cohort, 4 possible triallelic cases have been

	Spanish region	Gene	Nucleotide change	Amino acid change	Nº of alleles	Nº of total alleles of this gene found in the region	Frequency in the region
NON-RP	Basque Country	*ABCA4*	c.3386G>T	p.Arg1129Leu	18	93	19.40%
		ABCA4	c.5882G>A	p.Gly1961Glu	5		5.40%
	Castile-La Mancha	*ABCA4*	c.3386G>T	p.Arg1129Leu	8	60	13.30%
	Madrid	*ABCA4*	c.3386G>T	p.Arg1129Leu	47	373	12.60%
	Murcia	*ABCA4*	c.3386G>T	p.Arg1129Leu	8	71	11.30%
		ABCA4	c.5882G>A	p.Gly1961Glu	7		9.90%
Non-syndromic RP and syndromic IRD	Murcia	*USH2A*	c.2276G>T	p.Cys759Phe	11	83	13.30%
		PRCD	c.64C>T	p.Arg22Ter	6		7.20%
	Canary Islands	*NR2E3*	c.932G>A	p.Arg311Gln	8	83	9.60%
	Castile-La Mancha	*USH2A*	c.2276G>T	p.Cys759Phe	18	202	8.90%
	Madrid	*USH2A*	c.2276G>T	p.Cys759Phe	50	714	7.00%
	Extremadura	*USH2A*	c.2276G>T	p.Cys759Phe	5	87	5.70%

Table 1. Variants detected in > 5% of NON-RP, RP and syndromic IRD by Spanish regions. Regions with variants detected below this percentage are not represented in the table, and neither are regions with less than 50 mutated alleles in total or variants that were presented less than 5 times. In bold the variant which not appeared as one of the most frequent variants in Supplementary Table S6.

identified, all of them diagnosed with BBS, 3 patients carrying biallelic *BBS1* variants together with one allele in *MKKS* (MIM: 604896)[9,12] and one additional case carrying biallelic *MKKS* variants and one allele in *BBS5* (MIM: 603650). The phenotypic modifier effect of the triallelism could only be stablished in two of the 4 families. One has two affected siblings with different phenotypic severity that correlates with the presence of the third allele; and in the other both affected showed triallelism and have the same clinical manifestation. Although, it could not be stablished in the rest of the families, as they were sporadic cases.

Most frequent variants. Our findings reflect the high allelic heterogeneity in IRD. We identified 458 different disease-causing variants in 45 genes in cases "a priori" classified as NON-RP, as well as 836 in 94 genes in the "a priori" RP cases and 295 in 55 genes in the "a priori" syndromic IRD. The most common pathogenic variant detected in our NON-RP cohort was the previously known missense change *ABCA4* c.3386G>T (p.Arg1129Leu) (180 mutated alleles of 3,618; 5% of the total pathogenic alleles), presented in 21.5% (162/754) of the characterized families, in homozygous or compound heterozygous state in 18 and 144 families, respectively (Supplementary Table S6). Among the RP families, the most prevalent pathogenic variant was the missense change *USH2A* c.2276G>T (p.Cys759Phe), identified in 106 alleles in 8.4% of the solved families (87/1038); in 19 cases in homozygous and in 68 cases in compound heterozygous state. In addition, there were 15 other variants present in more than 10 genes (Supplementary Table S6). Some of the mutated genes overlap in NON-RP (Fig. 3), RP (Fig. 4) and syndromic IRD (Fig. 5).

Disease-causing variant distribution in Spain. Analysis of causing variants by the different Spanish regions resulted in a wide variety of disease-causing variants. Table 1 shows variants detected in more than 5% of characterized families, by Spanish regions. All these variants are depicted in the Supplementary Table S6 of NON-RP and RP most frequent causing variants, except for the nonsense variant *PRCD* (OMIM: 610598) (GenBank: NM_001077620.3) c.64C>T (p.Arg22Ter), that was found in homozygosity in 3 families, representing 7.2% (6/83) of the identified alleles from Murcia.

Discussion

This is the first and largest comprehensive study addressing the prevalence and epidemiology of IRD in the Spanish population. The cohort here described, comprising 6089 cases from 4403 unrelated families, is not based on a national registry of IRD patients, but it is the outcome of a very wide recruiting effort of a single center over the last 28 years. An increasing number of centers are currently performing clinical and/or genetic diagnosis of IRD in Spain, therefore our cohort did not reflect all of IRD patients in our country. Hence, to date, no accurate data about the IRD prevalence in the Spanish population is available.

In terms of representation of patients from the different Spanish regions, our cohort reflects a biased recruitment, being enriched with patients from Madrid and the surrounding regions (i.e. Castile and Leon, Castile-La Mancha, and Extremadura) probably due to the fact that our hospital has been their referral center during most of the time of the study. Other areas like Andalusia, Catalonia, Navarre or the Valencian Community had different referral centers and genetic testing is performed locally. In spite of these limitations, the large sample size of our cohort and the exhaustive molecular analysis performed over the years, together with an overall low genetic heterogeneity in the Spanish population[13], have allowed a straightforward extrapolation of prevalent genes and/or variants in IRD. Considering a worldwide prevalence of 1:1000[2]–4000[1] and an estimated Spanish population of 46.7 million, our cohort would represent 20–53% of the total patients with IRD in Spain. Despite numerous studies about the characteristics of the different IRDs in Spain, such as NON-RP and RP have been

partially published, still no global overview of NON-RP and RP diseases using a representative cohort has been addressed yet before in our country.

Several studies on IRD have been performed globally (Supplementary Table S7) and, in the two last years, some including big cohorts[14–16] or meta-analysis[17] have been published, reporting more than 125 genes explaining 55–62% of the families using several molecular techniques to achieve that[14–16] as it could be also seen in this study.

Other studies focused on stablishing the prevalence of IRD in certain regions has been performed in Western countries and in cohorts of non-syndromic RP, including Western Australia (1:6000)[18] or Maine (1:4756)[19], as well as in cohorts with general IRD and an estimated prevalence of 1:3454 in Denmark[20] or 1:3856 in Norway[21]. However, this prevalence has been reported in areas and populations with low rates of consanguinity and could be higher when consanguinity rate increases[2], which is not the common scenario in Spain nowadays.

Our study identified AR inheritance as the most common mode of inheritance for non-syndromic IRD, explaining up to 70–75% NON-RP and RP subcohorts (Fig. 2A-I and A-II). By contrast, only 7% of our NON-RP and RP families are explained by X-linked genes. These results were consistent with previous studies published[18–22]. Besides, some cases could be explained by different molecular mechanisms as the pseudodominance, incomplete penetrance or the presence of two variants in an AR gene in AD a priori families[8], so extended segregation analysis within these families are needed. Additional non-Mendelian transmission patterns were only found in exceptionally rare cases with syndromic IRD, including 3 families carrying variants affecting the mitochondrial DNA and 4 cases with apparent triallelism in BBS-associated genes. Within syndromic IRD group, most of the cases were explained by AR biallelic monogenic inheritance. Similar to previous published studies from other countries[3,23], Usher syndrome was the most prevalent form of syndromic IRD in our cohort, and more specifically, USH2, representing almost half of the total syndromic IRD families.

The overall diagnostic rate of 53.2% obtained here is similar to other studies previously reported (50–70%)[24–26]. Molecular studies allowed the identification of the genes responsible for the disease and the reclassification of the inheritance type. In our work, 8.2% of the patients were reclassified after the detection of the disease-causing variants in genes with a specific inheritance pattern. All of those were or have been previously validated. Moreover, in all the characterized sporadic cases a more accurate genetic classification and counselling could be done[8]. Additionally, a 2.5% were clinically reclassified after the genetic testing, due to a poor clinical data acquisition at the origin center. So, identification of the genetic cause of the disease represents a hallmark for the patients, firstly, regarding genetic counselling and the risk of affectation for other relatives; and secondly, given the possibility of future recruitments for clinical trials targeting specific genes and variants.

A total of 142 different genes were identified as the cause of IRD in our study, but it is important to notice that each subgroup of the cohort (AD, AR and XL NON-RP and RP) has an enrichment of characterized cases in specific genes.

For instance, *PRPH2* was mutated in more than a third of AD-NON-RP families, followed by *BEST1* (MIM: 607854). As expected, *ABCA4* was the most prevalent gene in AR-NON-RP families. Recent studies in Norway[21] and Korea[22] also identified this gene as one of the most prevalent mutated genes. A study published by Birtel et al.[27] in patients with MD and cone/cone-rod dystrophy showed a similar distribution of mutated genes, with *ABCA4*, *PRPH2* and *BEST1* responsible for 74% of their solved cases. For the XL-NON-RP subcohort, *RS1* was the most frequently mutated gene.

Non-syndromic RP presented a wider spectrum of causative genes, with *RHO*, *USH2A* and *RPGR* (*RPGR_ORF15* and the rest of *RPGR* regions) being the most prevalent ones in AD-RP, AR-RP and XL-RP subcohorts, respectively. Our findings are in line with those published in other studies[8,28]. For instance, Hartong et al.[3], showed as well *MYO7A*, *USH2A* and *BBS1* to be the most frequently mutated genes in USH1, USH2 and BBS, respectively. Other studies in different populations highlighted different genes as the most representative in their IRD cohorts. For example, Eisenberger et al.[24] described *RP1* (MIM: 603937) (11.3%) and *EYS* (MIM: 612424) (9.4%) as the most frequent genes in German patients with AR-RP, and Kim et al.[22] detected that *EYS* (22%) and *PDE6B* (MIM: 180072) (17%) are most frequently involved in AR-RP in Korean patients. *EYS* was also the most prevalent causative gene in the Japanese population studied by Maeda et al.[29], implicated in 21 out of 33 AR-RP patients (63.6%), whereas in our population *EYS* was mutated in 5.5% of the families with "a priori" AR-RP diagnosis, being the fourth most frequent gene, after *USH2A*, *CRB1* and *ABCA4*. However, the order of the causative genes in AR-RP changes after reviewing the clinical data of *ABCA4* related IRD patients, since they were mostly reclassified as NON-RP, downgrading *EYS* as the third most common gene in AR-RP in our population. This result supports an eastward gradient in the frequency of *EYS* variants in patients throughout the world and within Europe, being more frequent in Germany than in Spain.

The most frequent causing variants detected in our study appeared, as expected, in *ABCA4* and *USH2A*, the most prevalent mutated genes in the Spanish population[6–8,30]. *ABCA4* c.3386G > T (p.Arg1129Leu) is a variant almost exclusively found in Spanish NON-RP patients[6,30], being probably a Spanish founder mutation[31,32]. However, *USH2A* c.2276G > T (p.Cys759Phe) is not exclusive from the Spanish population and has been reported in other populations[33].

According to the geographical distribution of the variants within the country, no differences between regions were observed. In NON-RP, the two most common *ABCA4* variants were also the most represented in regions with variant frequencies above 5%. Meanwhile, in RP we found a higher representation of the most common *USH2A* variant, which appeared above 5% of the total alleles in four regions. Finally, two variants appeared to be more frequent in some regions, i.e. *PRCD* c.64C > T (p.Arg22Ter) in Murcia and *NR2E3* (MIM: 604485) (GenBank: NM_014249.4) c.932G > A (p.Arg311Gln) in the Canary Islands, where a founder effect could be happening.

Our results delineate the genetic background of the Spanish IRD patients, indicating a wide range of causative genes involved in the disease. Some of the causing variants identified are also frequent in Europe. Some examples include the *ABCA4* c.5882G > A (p.Gly1961Glu), reported with high prevalence in the Italian, German

and Spanish populations[30,34,35]; the c.2276G>T (p.Cys759Phe) as one of the most frequent variants in *USH2A*, especially in European countries[36], *USH2A* c.2299delG (p.Glu767SerfsTer21), which is possibly an ancestral European pathogenic variant[37]; *BBS1* (GenBank: NM_024649.5) c.1169T>G (p.Met390Arg), identified previously by Mykytyn et al.[38] in 22 North American BBS probands with North European ancestry; and *CRB1* (GenBank: NM_201253.3) c.2843G>A (p.Cys948Tyr), identified repeatedly in different European countries[39,40]. Finally, *RHO* (GenBank: NM_000539.3) c.1040C>T (p.Pro347Leu), described in the Italian and French populations[41,42] and also in non-European cohorts[5,43,44].

Variants found in individuals from East Mediterranean and Middle-Eastern countries also appeared in our Spanish cohort: *PRCD* c.64C>T (p.Arg22Ter) found by Sharon et al.[14] in homozygosis in 15 Israeli Muslim Arab families, and by Beheshtian et al.[45] in a Persian family; and *FAM161A* (MIM: 613596) (GenBank: NM_001201543.2) c.1355_1356delCA (p.Thr452SerfsTer3), which was identified in Jewish families mainly originating from North African countries[46]. As mentioned above, the variant in *PRCD* was found with a higher frequency in the region of Murcia, and this could be due to the settlement of Muslim populations during several centuries during the Middle Ages[13]. *FAM161A* does not have a significant specific geographical distribution in Spain.

Remarkably, we identified three pathogenic variants with high frequency in Spain: *ABCA4* c.3386G>T (p.Arg1129Leu), previously mentioned; *CERKL* (MIM: 608381) (GenBank: NM_201548.5) c.847C>T (p.Leu283Phe), first described by Tuson et al.[47], and *RP1* c.1625C>G (p.Ser542Ter) previously described originally as a Spanish founder pathogenic variant. These three variants had been scarcely reported outside the Spanish population. In the case of *RP1* c.1625C>G (p.Ser542Ter) variant[48], because of its presence in 11 out of 244 unrelated families, we can extrapolate that it may very well account for approximately 4.5% of all AR-RP cases in the Spanish population. Other groups also identified this variant in Swiss patients[26].

In conclusion, this study shows the general landscape of the genetic underpinnings of IRD in Spain and will help design clinical and preventive healthcare approaches to this disorder in our country.

Materials and methods

Cohort description. A retrospective analysis was performed including all IRD patients from our Spanish registry at the Fundación Jiménez Díaz University Hospital (FJD, Madrid, Spain) from 1991 until August 2019. This patient registry includes: all patients referred to the Genetic Service at the FJD for genetic diagnostic testing and/or counselling due to a previous clinical suspicion of IRD, and patients without genetic analysis in our unit but identified in the shared electronic clinical history of our same-company hospitals using ICD (International Classification of Diseases) terms. The complete cohort contains 6089 IRD affected cases (including index cases and affected relatives) belonging to 4403 unrelated families as shown in Supplementary Fig. S1.

This study was approved by the Ethics Committee of the FJD under approval number 134/2016_FJD and fulfilled all the tenets of the Declaration of Helsinki and its further reviews. A written informed consent form was obtained from all the patients or their legal guardians.

IRD classification and diagnostic criteria. During this study, different clinical, demographic and familiar data were collected, including (i) family pedigree; (ii) age at onset of visual acuity loss, extent of visual field loss, night blindness and/or other early symptoms of retinal dystrophy; (iii) presence of any systemic findings suggestive of syndromic forms of IRD; (iv) geographical origin of the patients.

Clinical diagnosis was based on ophthalmic examination, including measurement of best-corrected visual acuity, visual field testing, fundus examination and, if possible, full-field electroretinography, fundus autofluorescence and spectral domain optical coherence tomography scan. NON-RP and RP include non-syndromic IRD, and their clinical classification was done according to previously described criteria[6,8]. NON-RP group include most patients with CD, CRD and achromatopsia, although some of them were included in the RP group due to incomplete phenotyping at the moment of the diagnosis. Non-syndromic LCA cases were also included in RP.

For NON-RP and RP families, an "a priori" inheritance pattern (AD/AR/XL/sporadic (S)) was established according to previously described criteria[1]. The subgroup of XL-RP also included choroideremia cases.

For cases not extensively described in the first referral, a generic classification was made as NON-RP or RP. Criteria for syndromic IRD diagnosis were previously described[7,9].

Information about the geographic origin of all the IRD cases from Spain was available in 4668. They are distributed throughout the 17 different Spanish communities (Fig. 1A).

Inheritance reclassification of IRD cases. After molecular diagnosis, inherited patterns were reviewed and compared with "a priori" data of each family. Statistical analysis between these data sets to assess the global association in the NON-RP group was made using the Fisher's exact test with a p equal to 0.497. Whereas for the RP group, Chi-square test was used with a p below 0.001. Comparisons for each type of inheritance have also been made with the Fisher's exact test in the NON-RP Unclassified subgroup and with the Chi-square test in the rest. Fisher's exact test was used in those cases in which more than 20% of expected values were below than 5, or at least one of the expected frequencies was below 1. Regarding the significance levels chosen, we have a global comparison for which the significance level is the usual threshold of 0.05 and p-value is not corrected, and several post-hoc comparisons for which Bonferroni's multiple comparisons adjustment is applied, multiplying the p-values by the number of comparisons.

Prevalence of IRD. In this study, we performed a retrospective analysis of the largest cohort of patients with IRD from Spain, whom were recruited during a period of 28 years by a single center, the FJD. The FJD is a center of reference for molecular diagnosis of IRD from all over the country, especially in some specific autonomous

communities, like Castile and Leon, Castile-La Mancha, Extremadura or Madrid. On the other hand, as we take into account other Spanish regions, there are other centers of reference and the prevalence data obtained is inconclusive.

Prevalence was calculated for each clinical type of the disease by dividing the total number of diagnosed IRD cases by the total population in Spain, published by the Spanish Statistical Office (INE; http://www.ine.es) in January 2019.

Genomic screening. Genomic DNA samples were obtained from the FJD Biobank from a total of 3951 families (89.7%), including 1291 NON-RP, 2083 non-syndromic RP and 577 syndromic IRD families. Molecular studies were performed using different molecular techniques as shown in Supplementary Table S8. According to the technology available and the knowledge on the genetic determinants of IRD at the time of the diagnosis, a maximum of 291 different genes involved in IRD were processed for the molecular characterization (Supplementary S1 Appendix). In these studies, index cases were initially screened, analysed following the American College of Medical Genetics and Genomics (ACMG; https://www.acmg.net/docs/standards_guidelines_for_the_interpretation_of_sequence_variants.pdf) variants classification guidelines. If potentially disease-causing variants were found, segregation analysis was performed when DNA samples from relatives were available.

In the general description of mutated genes and frequent pathogenic variants, only fully molecularly characterized index cases were considered. Patients with a heterozygote allele in a recessive gene were counted as uncharacterized.

The frequency of recurrent IRD causing variants was established considering not only the total Spanish population, but also the different geographical regions of Spain (Fig. 1A), in order to assess the possibility of identifying any endemic or founder effects. Pathogenic variants with a prevalence above 5% in a particular region were recorded, and only those with a higher prevalence were considered for further analysis.

Data availability

Part of the NGS data are available in public, open access repositories such as the European Genome-Phenome Archive (EGA; https://www.ebi.ac.uk/ega/home; EGAD00001005746 and EGAD00001005498), RD-Connect (https://rd-connect.eu/) and the Collaborative Spanish Variant Server (CSVS; http://csvs.babelomics.org/) as aggregated data. The rest of the data are available upon reasonable request.

Received: 28 October 2020; Accepted: 31 December 2020
Published online: 15 January 2021

References

1. Ayuso, C. & Millan, J. M. Retinitis pigmentosa and allied conditions today: A paradigm of translational research. *Genome Med.* **2**, 34. https://doi.org/10.1186/gm155 (2010).
2. Hanany, M., Rivolta, C. & Sharon, D. Worldwide carrier frequency and genetic prevalence of autosomal recessive inherited retinal diseases. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 2710–2716. https://doi.org/10.1073/pnas.1913179117 (2020).
3. Hartong, D. T., Berson, E. L. & Dryja, T. P. Retinitis pigmentosa. *Lancet* **368**, 1795–1809. https://doi.org/10.1016/S0140-6736(06)69740-7 (2006).
4. Vaidya, P. & Vaidya, A. Retinitis pigmentosa: Disease encumbrance in the eurozone. *Int. J. Ophthalmol. Clin. Res.* **2**, 030. https://doi.org/10.23937/2378-346x/1410030 (2015).
5. Dryja, T. P. *et al.* Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N. Engl. J. Med.* **323**, 1302–1307. https://doi.org/10.1056/NEJM199011083231903 (1990).
6. Riveiro-Alvarez, R. *et al.* Outcome of ABCA4 disease-associated alleles in autosomal recessive retinal dystrophies: Retrospective analysis in 420 Spanish families. *Ophthalmology* **120**, 2332–2337. https://doi.org/10.1016/j.ophtha.2013.04.002 (2013).
7. Blanco-Kelly, F. *et al.* Clinical aspects of Usher syndrome and the USH2A gene in a cohort of 433 patients. *JAMA Ophthalmol.* **133**, 157–164. https://doi.org/10.1001/jamaophthalmol.2014.4498 (2015).
8. Martin-Merida, I. *et al.* Genomic landscape of sporadic retinitis pigmentosa: Findings from 877 Spanish Cases. *Ophthalmology* **126**, 1181–1188. https://doi.org/10.1016/j.ophtha.2019.03.018 (2019).
9. Sanchez-Navarro, I. *et al.* Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. *Sci. Rep.* **8**, 5285. https://doi.org/10.1038/s41598-018-23520-1 (2018).
10. Martin-Merida, I. *et al.* Toward the mutational landscape of autosomal dominant retinitis pigmentosa: A comprehensive analysis of 258 Spanish families. *Investig. Ophthalmol. Vis. Sci.* **59**, 2345–2354. https://doi.org/10.1167/iovs.18-23854 (2018).
11. Avila-Fernandez, A. *et al.* Mutation analysis of 272 Spanish families affected by autosomal recessive retinitis pigmentosa using a genotyping microarray. *Mol. Vis.* **16**, 2550–2558 (2010).
12. Castro-Sanchez, S. *et al.* Exploring genotype-phenotype relationships in Bardet–Biedl syndrome families. *J. Med. Genet.* **52**, 503–513. https://doi.org/10.1136/jmedgenet-2015-103099 (2015).
13. Gayan, J. *et al.* Genetic structure of the Spanish population. *BMC Genom.* **11**, 326. https://doi.org/10.1186/1471-2164-11-326 (2010).
14. Sharon, D. *et al.* A nationwide genetic analysis of inherited retinal diseases in Israel as assessed by the Israeli inherited retinal disease consortium (IIRDC). *Hum. Mutat.* https://doi.org/10.1002/humu.23903 (2019).
15. Pontikos, N. *et al.* Genetic basis of inherited retinal disease in a molecularly characterized cohort of more than 3000 families from the United Kingdom. *Ophthalmology* **127**, 1384–1394. https://doi.org/10.1016/j.ophtha.2020.04.008 (2020).
16. Goetz, K. E. *et al.* Genetic testing for inherited eye conditions in over 6000 individuals through the eyeGENE network. *Am. J. Med. Genet. C Semin. Med. Genet.* **184**, 828–837. https://doi.org/10.1002/ajmg.c.31843 (2020).
17. Jaffal, L. *et al.* The genetics of rod-cone dystrophy in Arab countries: A systematic review. *Eur. J. Hum. Genet.* https://doi.org/10.1038/s41431-020-00754-0 (2020).
18. Chelva, E. *et al.* A retinitis pigmentosa register for western Australia. *Aust. N. Z. J. Ophthalmol.* **20**, 311–317 (1992).
19. Bunker, C. H., Berson, E. L., Bromley, W. C., Hayes, R. P. & Roderick, T. H. Prevalence of retinitis pigmentosa in Maine. *Am. J. Ophthalmol.* **97**, 357–365. https://doi.org/10.1016/0002-9394(84)90636-6 (1984).
20. Bertelsen, M., Jensen, H., Bregnhoj, J. F. & Rosenberg, T. Prevalence of generalized retinal dystrophy in Denmark. *Ophthalmic Epidemiol.* **21**, 217–223. https://doi.org/10.3109/09286586.2014.929710 (2014).

21. Holtan, J. P., Selmer, K. K., Heimdal, K. R. & Bragadottir, R. Inherited retinal disease in Norway—A characterization of current clinical and genetic knowledge. *Acta Ophthalmol.* https://doi.org/10.1111/aos.14218 (2019).
22. Kim, M. S. *et al.* Genetic mutation profiles in Korean patients with inherited retinal diseases. *J. Korean Med. Sci.* **34**, e161. https://doi.org/10.3346/jkms.2019.34.e161 (2019).
23. Motta, F. L., Martin, R. P., Filippelli-Silva, R., Salles, M. V. & Sallum, J. M. F. Relative frequency of inherited retinal dystrophies in Brazil. *Sci. Rep.* **8**, 15939. https://doi.org/10.1038/s41598-018-34380-0 (2018).
24. Eisenberger, T. *et al.* Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: The example of retinal dystrophies. *PLoS ONE* **8**, e78496. https://doi.org/10.1371/journal.pone.0078496 (2013).
25. Huang, X. F. *et al.* Genotype–phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet. Med.* **17**, 271–278. https://doi.org/10.1038/gim.2014.138 (2015).
26. Tiwari, A. *et al.* Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. *Sci. Rep.* **6**, 28755. https://doi.org/10.1038/srep28755 (2016).
27. Birtel, J. *et al.* Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. *Sci. Rep.* **8**, 4824. https://doi.org/10.1038/s41598-018-22096-0 (2018).
28. Bravo-Gil, N. *et al.* Unravelling the genetic basis of simplex Retinitis Pigmentosa cases. *Sci. Rep.* **7**, 41937. https://doi.org/10.1038/srep41937 (2017).
29. Maeda, A. *et al.* Development of a molecular diagnostic test for Retinitis Pigmentosa in the Japanese population. *Jpn. J. Ophthalmol.* **62**, 451–457. https://doi.org/10.1007/s10384-018-0601-x (2018).
30. Riveiro-Alvarez, R. *et al.* Frequency of ABCA4 mutations in 278 Spanish controls: An insight into the prevalence of autosomal recessive Stargardt disease. *Br. J. Ophthalmol.* **93**, 1359–1364. https://doi.org/10.1136/bjo.2008.148155 (2009).
31. Del Pozo-Valero, M. *et al.* Genotype–phenotype correlations in a Spanish cohort of 506 families with biallelic ABCA4 pathogenic variants. *Am. J. Ophthalmol.* **219**, 195–204. https://doi.org/10.1016/j.ajo.2020.06.027 (2020).
32. Cremers, F. P. M., Lee, W., Collin, R. W. J. & Allikmets, R. Clinical spectrum, genetic complexity and therapeutic approaches for retinal disease caused by ABCA4 mutations. *Prog. Rettn. Eye Res.* **79**, 100861. https://doi.org/10.1016/j.preteyeres.2020.100861 (2020).
33. Seyedahmadi, B. J., Rivolta, C., Keene, J. A., Berson, E. L. & Dryja, T. P. Comprehensive screening of the USH2A gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. *Exp. Eye Res.* **79**, 167–173. https://doi.org/10.1016/j.exer.2004.03.005 (2004).
34. Rivera, A. *et al.* A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. *Am. J. Hum. Genet.* **67**, 800–813. https://doi.org/10.1086/303090 (2000).
35. Passerini, I. *et al.* Novel mutations in of the ABCR gene in Italian patients with Stargardt disease. *Eye (Lond)* **24**, 158–164. https://doi.org/10.1038/eye.2009.35 (2010).
36. Dreyer, B. *et al.* Identification of novel USH2A mutations: Implications for the structure of USH2A protein. *Eur. J. Hum. Genet0* **8**, 500–506. https://doi.org/10.1038/sj.ejhg.5200491 (2000).
37. Aller, E. *et al.* The USH2A c.2299delG mutation: dating its common origin in a Southern European population. *Eur. J. Hum. Genet.* **18**, 788–793. https://doi.org/10.1038/ejhg.2010.14 (2010).
38. Mykytyn, K. *et al.* Identification of the gene (BBS1) most commonly involved in Bardet–Biedl syndrome, a complex human obesity syndrome. *Nat. Genet.* **31**, 435–438. https://doi.org/10.1038/ng935 (2002).
39. Kousal, B. *et al.* Phenotypic features of CRB1-associated early-onset severe retinal dystrophy and the different molecular approaches to identifying the disease-causing variants. *Graefes Arch. Clin. Exp. Ophthalmol.* **254**, 1833–1839. https://doi.org/10.1007/s00417-016-3358-2 (2016).
40. Bujakowska, K. *et al.* CRB1 mutations in inherited retinal dystrophies. *Hum. Mutat.* **33**, 306–315. https://doi.org/10.1002/humu.21653 (2012).
41. Ziviello, C. *et al.* Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): A comprehensive study of 43 Italian families. *J. Med. Genet.* **42**, e47. https://doi.org/10.1136/jmg.2005.031682 (2005).
42. Audo, I. *et al.* Spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophy patients. *Investig. Ophthalmol. Vis. Sci.* **51**, 3687–3700. https://doi.org/10.1167/iovs.09-4766 (2010).
43. Roshandel, D. *et al.* Rhodopsin gene mutation analysis in Iranian patients with autosomal dominant retinitis pigmentosa. *Int. Ophthalmol.* https://doi.org/10.1007/s10792-019-01099-4 (2019).
44. Yang, G. *et al.* Spectrum of rhodopsin gene mutations in Chinese patients with retinitis pigmentosa. *Mol. Vis.* **20**, 1132–1136 (2014).
45. Beheshtian, M. *et al.* Impact of whole exome sequencing among Iranian patients with autosomal recessive retinitis pigmentosa. *Arch. Iran. Med.* **18**, 776–785 (2015).
46. Bandah-Rozenfeld, D. *et al.* Homozygosity mapping reveals null mutations in FAM161A as a cause of autosomal-recessive retinitis pigmentosa. *Am. J. Hum. Genet.* **87**, 382–391. https://doi.org/10.1016/j.ajhg.2010.07.022 (2010).
47. Tuson, M., Marfany, G. & Gonzalez-Duarte, R. Mutation of CERKL, a novel human ceramide kinase gene, causes autosomal recessive retinitis pigmentosa (RP26). *Am. J. Hum. Genet.* **74**, 128–138. https://doi.org/10.1086/381055 (2004).
48. Avila-Fernandez, A. *et al.* Identification of an RP1 prevalent founder mutation and related phenotype in Spanish patients with early-onset autosomal recessive retinitis. *Ophthalmology* **119**, 2616–2621. https://doi.org/10.1016/j.ophtha.2012.06.033 (2012).

Acknowledgements

The authors would like to thank Ignacio Mahillo for the statistical support, and all patients who participated in the study.

Author contributions

Conceptualization, I.P.R., G.G., I.F.I., M.P.V., B.A., F.B.K., E.C., B.J.R., I.M.M., R.R.A., B.G.S., P.M., A.A.F., M.C. and C.A.; Data curation, I.P.R., G.G., I.F.I., M.P.V., L.O., L.P.A., C.L. and C.A.; Formal analysis, I.P.R., G.G., I.F.I., M.P.V., B.A., F.B.K., A.B.A., R.C.M., E.C., R.F.S., J.G.M., I.G.V., A.G.P., L.H.B., F.I.B., B.J.R., E.L., M.A.L.M., R.L.R., I.L.S., I.M.M., A.M.R., L.O., L.P.A., R.R.A., M.R.A., E.R.P., C.S.J., S.T.S., M.J.T.T., C.V.M., C.V., O.Z., D.A.G., A.A., R.P.C., I.S.N., R.A., S.B., F.P.M.C., R.W.J.C., E.D.B., H.H., S.K., C.R., D.S., G.A., M.B., M.C., J.M.M., D.V., B.G.S., P.M., A.A.F., M.C. and C.A.; Funding acquisition, C.A.; Investigation, I.P.R., G.G., I.F.I., M.P.V., B.A., F.B.K., A.B.A., R.C.M., E.C., R.F.S., J.G.M., I.G.V., A.G.P., L.H.B., F.I.B., B.J.R., E.L., M.A.L.M., R.L.R., I.L.S., I.M.M., A.M.R., L.O., L.P.A., R.R.A., M.R.A., E.R.P., C.S.J., S.T.S., M.J.T.T., C.V.M., C.V., O.Z., D.A.G., J.A.L., A.A., D.C., P.F.S.J., L.G.M., M.G.H., C.L., M.I.L.M., R.P.C., L.R.J.D.S., C.R., R.S.A., I.S.N., S.D.T., E.V., R.A., S.B., F.P.M.C., R.W.J.C., E.D.B., H.H., S.K., C.R., D.S., G.A., M.B., M.C., J.M.M., D.V., B.G.S., P.M., A.A.F., M.C. and C.A.; Methodology, I.P.R., G.G., I.F.I., M.P.V., D.A.G., J.A.L., A.A., D.C., P.F.S.J., L.G.M., M.G.H., C.L., M.I.L.M., R.P.C., L.R.J.D.S., C.R., R.S.A., I.S.N., S.D.T., E.V., E.A., M.J.G., G.G.G., I.H., T.J., R.A., S.B., F.P.M.C., R.W.J.C., E.D.B.,

H.H., S.K., C.R., D.S., G.A., M.B., M.C., J.M.M., D.V., B.G.S., P.M., A.A.F., M.C. and C.A.; Project administration, C.A.; Resources, F.B.K., E.C., B.J.R., I.L.S., E.R.P., S.T.S., E.A., M.C.A.C., M.J.B.M., S.B., C.B.L., S.B., J.C.M., C.C., C.C.P, M.F.B., A.F.R., E.G.G., M.J.G., M.G.B., L.M.G.C., G.G.G., B.G., B.G.F., N.G., E.G.N., I.H., I.H.A., C.I., S.I.A., T.J., I.L.R., M.A.L.A., V.L.G., F.L.G., L.M., P.M.P., M.M.I., R.O.I., F.P.M., G.P.N., F.J.R.F., R.R.L., M.R.P., L.R.P., B.R.S., J.R., N.R., R.S.V., A.S., I.V.P., E.V.D., R.A., S.B., F.P.M.C., R.W.J.C., E.D.B., H.H., S.K., C.R., D.S., G.A., M.B., M.C., J.M.M., D.V., B.G.S., P.M. and C.A.; Software, I.F.I., B.A., R.A., S.A., F.P.M.C., R.W.J.C., E.D.B., H.H., S.K., C.R., D.S. and P.M.; Supervision, M.C. and C.A.; Validation, I.P.R., G.G., I.F.I., M.P.V., B.A., F.B.K., A.B.A., R.C.M., E.C., R.F.S., J.G.M., I.G.V., A.G.P., L.H.B., F.I.B., B.J.R., E.L., M.A.L.M., R.L.R., I.L.S., I.M.M., A.M.R., L.O., L.P.A., R.R.A., M.R.A., E.R.P., C.S.J., S.T.S., M.J.T.T., C.V.M., C.V., O.Z., D.A.G., J.A.L., A.A., D.C., P.F.S.J., L.G.M., M.G.H., C.L., M.I.L.M., R.P.C., L.R.J.D.S., C.R., R.S.A., I.S.N., S.D.T., E.V., E.A., M.C.A.C., M.J.B.M., S.B., C.B.L., S.B., J.C.M., C.C., C.C.P., M.F.B., A.F.R., E.G.G., M.J.G., M.G.B., L.M.G.C., G.G.G., B.G., B.G.F., N.G., E.G.N., I.H., I.H.A., C.I., S.I.A., T.J., I.L.R., M.A.L.A., V.L.G., F.L.G., L.M., P.M.P., M.M.I., R.O.I., F.P.M., G.P.N., F.J.R.F., R.R.L., M.R.P., L.R.P., B.R.S., J.R., N.R., R.S.V., A.S., I.V.P., E.V.D., G.A., M.B., M.C., J.M.M., D.V., B.G.S., P.M., A.A.F., M.C. and C.A.; Visualization, I.P.R., G.G., I.F.I., M.C. and C.A.; Writing—original draft, I.P.R., G.G., I.F.I., M.P.V., M.G.H., M.C. and C.A.; Writing—review & editing, all authors.

Funding

This work was supported by the Instituto de Salud Carlos III (ISCIII) of the Spanish Ministry of Health (FIS; PI16/00425 and PI19/00321), Centro de Investigación Biomédica en Red Enfermedades Raras (CIBERER, 06/07/0036), IIS-FJD BioBank (PT13/0010/0012), Comunidad de Madrid (CAM, RAREGenomics Project, B2017/BMD-3721), European Regional Development Fund (FEDER), the Organización Nacional de Ciegos Españoles (ONCE), Fundación Ramón Areces, Fundación Conchita Rábago and the University Chair UAM-IIS-FJD of Genomic Medicine. Irene Perea-Romero is supported by a PhD fellowship from the predoctoral Program from ISCIII (FI17/00192). Ionut F. Iancu is supported by a grant from the Comunidad de Madrid (CAM, PEJ-2017-AI/BMD7256). Marta del Pozo-Valero is supported by a PhD grant from the Fundación Conchita Rábago. Berta Almoguera is supported by a Juan Rodes program from ISCIII (JR17/00020). Pablo Minguez is supported by a Miguel Servet program from ISCIII (CP16/00116). Marta Corton is supported by a Miguel Servet program from ISCIII (CPII17/00006). The funders played no role in study design, data collection, data analysis, manuscript preparation and/or publication decisions.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-81093-y.

Correspondence and requests for materials should be addressed to M.C. or C.A.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

The ESRETNET Study Group

Ana Bustamante-Aragones[1,2], Rocio Cardero-Merlo[1,2], Ruth Fernandez-Sanchez[1], Jesus Gallego-Merlo[1,2], Ines Garcia-Vara[1], Ascension Gimenez-Pardo[1,2], Laura Horcajada-Burgos[1], Fernando Infantes-Barbero[1,2], Esther Lantero[1], Miguel A. Lopez-Martinez[1], Andrea Martinez-Ramas[1], Lorena Ondo[1], Marta Rodriguez de Alba[1,2], Carolina Sanchez-Jimeno[1,2], Camilo Velez-Monsalve[1], Cristina Villaverde[1,2], Olga Zurita[1,2], Domingo Aguilera-Garcia[1], Jana Aguirre-Lamban[1], Ana Arteche[1], Diego Cantalapiedra[1], Patricia Fernandez-San Jose[1], Liliana Galbis-Martinez[1], Maria Garcia-Hoyos[1], Carlos Lombardia[1], Maria I. Lopez-Molina[1], Raquel Perez-Carro[1], Luciana R. J. Da Silva[1], Carmen Ramos[1], Rocio Sanchez-Alcudia[1], Iker Sanchez-Navarro[1], Sorina D. Tatu[1], Elena Vallespin[1], Elena Aller[2,4], Sara Bernal[2,9],

Maria J. Gamundi[16], Gema Garcia-Garcia[2,4], Inmaculada Hernan[16], Teresa Jaijo[2,4], Guillermo Antiñolo[2,55], Montserrat Baiget[2,9], Miguel Carballo[16], Jose M. Millan[2,4] & Diana Valverde[56,57,58]

[4]Department of Genetics, Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. [9]Department of Genetics, Hospital de la Santa Creu I Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain. [16]Molecular Genetics Unit, Hospital de Terrassa, Consorci Sanitari de Terrassa, Terrassa, Catalonia, Spain. [55]Department of Maternofoetal Medicine, Genetics and Reproduction, Institute of Biomedicine of Seville (IBIS), University Hospital Virgen del Rocío-CSIC-University of Seville, Seville, Spain. [56]Department of Biochemistry, Genetics and Immunology, Facultad de Biología, Universidad de Vigo, Pontevedra, Galicia, Spain. [57]Research Group on Rare Diseases and Pediatric Medicine, Instituto de Investigacion Sanitaria Galicia Sur (IISGS), Vigo, Galicia, Spain. [58]Centro de Investigaciones Biomédicas (CINBIO), Universidad de Vigo, Vigo, Galicia, Spain.

The ERDC Study Group

Rando Allikmets[40,41], Sandro Banfi[42,43], Frans P. M. Cremers[44], Rob W. J. Collin[45], Elfride De Baere[46], Hakon Hakonarson[47,48,49], Susanne Kohl[50], Carlo Rivolta[51,52,53] & Dror Sharon[54]

[40]Department of Ophthalmology, Columbia University, New York, NY, USA. [41]Department of Pathology and Cell Biology, Columbia University, New York, NY, USA. [42]Medical Genetics, Department of Precision Medicine, Università degli Studi della Campania 'Luigi Vanvitelli', Naples, Italy. [43]Telethon Institute of Genetics and Medicine, Pozzuoli, Italy. [44]Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands. [45]Department of Human Genetics and Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands. [46]Center for Medical Genetics Ghent (CMGG), Ghent University and Ghent University Hospital, Ghent, Belgium. [47]Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [48]Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [49]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [50]Institute for Ophthalmic Research, Center for Ophthalmology, University of Tübingen, Tübingen, Germany. [51]Clinical Research Center, Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland. [52]Department of Ophthalmology, University Hospital Basel, Basel, Switzerland. [53]Department of Genetics and Genome Biology, University of Leicester, Leicester, UK. [54]Department of Ophthalmology, Hadassah Medical Center, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.

The Associated Clinical Study Group

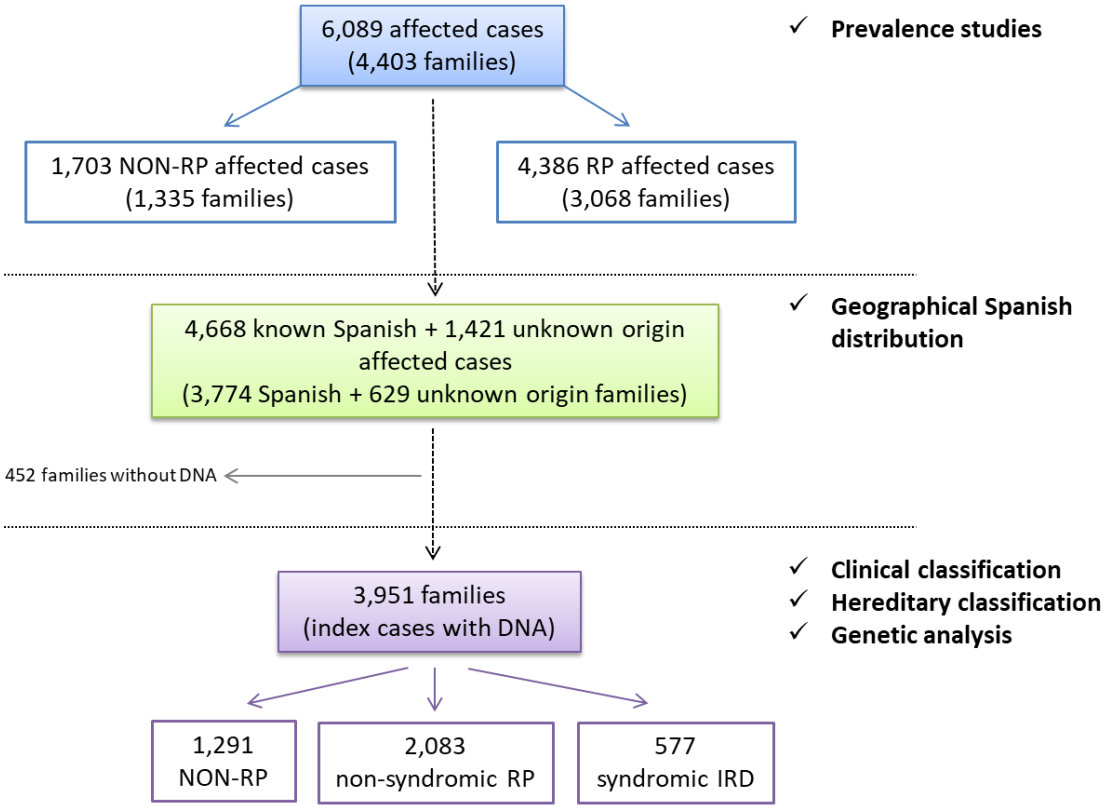
Maria C. Alonso-Cerezo[5], Maria J. Ballesta-Martinez[2,6], Sergi Beltran[7], Carmen Benito Lopez[8], Jaume Català-Mora[10], Claudio Catalli[11], Carmen Cotarelo-Perez[12], Miguel Fernandez-Burriel[13], Ana Fontalba-Romero[14], Enrique Galán-Gómez[15], Maria Garcia-Barcina[17], Loida M. Garcia-Cruz[18], Blanca Gener[11], Belen Gil-Fournier[19], Nancy Govea[20], Encarna Guillen-Navarro[2,6], Ines Hernando Acero[21], Cristina Irigoyen[22,23,24], Silvia Izquierdo-Álvarez[25], Isabel Llano-Rivas[11], Maria A. López-Ariztegui[11], Vanesa Lopez-Gonzalez[2,6], Fermina Lopez-Grondona[1], Loreto Martorell[26,27], Pilar Mendez-Perez[15], Maria Moreno-Igoa[28,29], Raluca Oancea-Ionescu[12], Francesc Palau-Martinez[30], Guiomar Perez de Nanclares[31], Feliciano J. Ramos-Fuentes[2,32], Raquel Rodriguez-Lopez[33], Montserrat Rodriguez-Pedreira[34], Lydia Rodriguez-Peña[2,6], Berta Rodriguez-Sanchez[34], Jordi Rosell[2,20,35], Noemi Rosello[36], Raquel Saez-Villaverde[37], Alfredo Santana[18], Irene Valenzuela-Palafoll[38] & Eva Villota-Deleu[39]

[5]Center for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain. [6]Medical Genetics Unit, Pediatrics Service, Hospital Clínico Universitario Virgen de la Arrixaca, IMIB-Arrixaca, Murcia, Murcia, Spain. [7]CNAG-CRG, Center for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Catalonia, Spain. [8]Genetics Section, Hospital Universitario Carlos Haya, Málaga, Andalusia, Spain. [10]Ophthalmology Service, Hospital Sant Joan de Déu, Barcelona, Catalonia, Spain. [11]Department of Medical Genetics, Hospital Universitario Cruces, Bilbao, Basque Country, Spain. [12]Clinical Genetics Unit, Hospital Universitario Clínico San Carlos, Madrid, Madrid, Spain. [13]Genetics Unit, Hospital de Mérida, Mérida, Badajoz, Extremadura, Spain. [14]Genetics Service, Hospital Universitario Marqués de Valdecilla (HUMV), Santander, Cantabria, Spain. [15]Clinical Genetics Unit, Pediatrics Service, Hospital Universitario de Badajoz, Badajoz, Extremadura, Spain. [17]Genetics Unit, Hospital Universitario de Basurto, Osakidetza Basque Health Service, Bilbao, Basque Country, Spain. [18]Clinical Genetics Unit, Complejo Hospitalario Insular-Materno Infantil, Las Palmas de Gran Canaria, Canary Islands, Spain. [19]Department of Genetics, Hospital Universitario de Getafe, Madrid, Madrid, Spain. [20]Genetics Unit, Hospital Son Espases, Palma de Mallorca, Balearic Islands, Spain. [21]Genetics Deparment, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain. [22]Department of Ophthalmology, Hospital Universitario de Donostia, San Sebastián, Basque Country, Spain. [23]Division of Neurosciences, Biodonostia Health Research Institute, San Sebastián, Basque Country, Spain. [24]Department of Ophthalmology, University of the Basque Country-UPV/EHU, Vizcaya, Basque Country, Spain. [25]Clinical Genetics Department, Hospital Universitario

Miguel Servet (HUMS), Zaragoza, Aragon, Spain. [26]Cellular Therapy Service, Blood and Tissue Bank, Building Dr. Frederic Duran I Jordà, Barcelona, Catalonia, Spain. [27]Musculoskeletal Tissue Engineering Group, Vall D'Hebron Research Institute (VHIR), Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain. [28]Department of Medical Genetics, Complejo Hospitalario de Navarra (CHN), Pamplona, Navarre, Spain. [29]Navarra Institute for Health Research (IdiSNA), Pamplona, Navarre, Spain. [30]Molecular Genetics Unit, Hospital Sant Joan de Deu, Barcelona, Catalonia, Spain. [31]Molecular (Epi)Genetic Lab, Bioaraba Health Research Institute, Araba University Hospital, Vitoria-Gasteiz, Alava, Basque Country, Spain. [32]Clinical Genetics and Functional Genomics Unit, Pediatrics Service, Hospital Clinico Universitario "Lozano Blesa", Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Aragon, Spain. [33]Department of Clinical Analysis, Hospital Universitario General de Valencia, Valencia, Valencian Community, Spain. [34]Genetics Unit, Hospital Materno Infantil Teresa Herrera, Hospital Universitario de A Coruña, A Coruña, Galicia, Spain. [35]Instituto Investigación Illes Balears (IDISBA), Palma de Mallorca, Balearic Islands, Spain. [36]Department of Ophthalmology, Hospital de la Santa Creu I Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain. [37]Department of Genetics, Hospital Universitario Donostia, San Sebastian, Basque Country, Spain. [38]Genetics Unit, Hospital Universitari Vall D'Hebron, Barcelona, Catalonia, Spain. [39]Instituto Oftalmológico Fernández-Vega, Oviedo, Asturias, Spain.

Supplementary files

Supplementary Figure S1. Workflow diagram followed along the study.



Supplementary Table S1. Estimated prevalence in the 20%, 53% and 100% of the population of Spain.

	20%	53%	100%
NON-RP (N=1.703)	1:5 487 (1:5 238 - 1:5 761)	1:14 541 (13 882 - 1:15 266)	1:27 436 (1:26 192 - 1:28 804)
RP (N=3.561)	1:2 624 (1:2 541 - 1:2 713)	1:6 954 (1:6 733 - 1:7 190)	1:13 121 (1:12 704 - 1:13 566)
Syndromic IRD (N=825)	1:11 327 (1:10 603 - 1:12 156)	1:30 016 (1:28 099 - 1:32 214)	1:56 634 (1:53 016 - 1:60 781)
Total RD (N=6.089)	1:1 535 (1:1 497 - 1:1 574)	1:4.067 (1:3 967 - 1:4 172)	1:7.673 (1:7 485 - 1:7 871)

Considering a worldwide prevalence of 1:1,000-4,000, our cohort would represent 20-53% of the total patients with IRD in Spain. Confidence intervals between brackets. RD: retinal dystrophies; IRD: inherited retinal dystrophies; RP: retinitis pigmentosa.

Supplementary Table S2. Comparative table of a priori vs final inheritance pattern and reclassification.

	NON-RP	RP	Total
Inheritance classification without changes	310 (41.1%)	555 (53.5%)	865 (48.3%)
***A priori* S – final AR**	378 (50.1%)	379 (36.5%)	757 (42.2%)
***A priori* unclassified to AD, AR or XL**	11 (1.5%)	13 (1.2%)	24 (1.3%)
Reclassification (other types)	55 (7.3%)	91 (8.8%)	146 (8.2%)
Total	754	1,038	1,792

Regarding their a priori and final inheritance patterns in the NON-RP and RP characterized families, 4 categories have been made: i) families without changes in the inheritance classification (the a priori inheritance pattern is the same as the final one), ii) patients with no family history characterized with an AR gene, iii) unclassified families due to the lack of a priori family information and iv) properly reclassified families (other types) with a change in the a priori inheritance pattern due to the causative gene found after the genetic testing. AD: autosomal dominant; AR: autosomal recessive; S: sporadic.

Supplementary Table S3. Genes identified in NON-RP group subdivided by mode of inheritance.

Nº of families	Gene	Inheritance
51	*PRPH2*	AD
33	*BEST1*	AD
10	*GUCY2D*	AD
7	*CRX*	AD
5	*PROM1*	AD
4	*GUCA1A*	AD
2	*RHO**	AD
2	*IMPG1*	AD
1	*AIPL1*	AD
1	*COL11A1**	AD
1	*FSCN2**	AD
1	*IMPG2*	AD
1	*PRPF31*	AD
1	*PRPF8**	AD
1	*TIMP3*	AD
450	*ABCA4*	AR
21	*CRB1*	AR
9	*BEST1*	AR
9	*CNGB3*	AR
9	*PROM1*	AR
8	*CERKL*	AR
5	*CNGA3*	AR
3	*USH2A*	AR
3	*BBS1*	AR
3	*RDH12*	AR
3	*RLBP1*	AR
2	*CDHR1*	AR
2	*NR2E3*	AR
2	*RAB28*	AR
1	*ADAM9*	AR
1	*AHI1**	AR
1	*CEP290*	AR
1	*CDH3**	AR
1	*CNGB1**	AR
1	*GNAT2*	AR
1	*IMPG2*	AR
1	*KCNV2*	AR
1	*MYO7A**	AR
1	*NMNAT1*	AR
1	*PDE6C*	AR
1	*RPGRIP1*	AR
1	*TRPM1**	AR
1	*CYP4V2*	AR
1	*TULP1*	AR
83	*RS1*	XL
2	*CHM**	XL

Nº of families	Gene	Inheritance
2	*RPGR_ORF15*	XL
1	*RPGR*	XL
1	*OPN1LW-OPN1MW*	XL
754	**Total families**	

There is a total of 15 genes found in the AD-NON-RP group, 29 in the AR-NON-RP and 4 in the XL-NON-RP. AD: autosomal dominant; AR: autosomal recessive; RP: retinitis pigmentosa; XL: X-linked.

* Clinically reclassified to RP (*RHO, FSCN2, PRPF8, AHI1, CNGB1, TRPM1 and CHM) or syndromic IRD (COL11A1, CDH3* and *MYO7A*) groups.

Supplementary Table S4. Genes identified in RP group subdivided by mode of inheritance.

Nº of families	Gene	Inheritance
65	*RHO*	AD
34	*PRPF31*	AD
16	*RP1*	AD
14	*PRPH2*	AD
12	*SNRNP200*	AD
11	*IMPDH1*	AD
9	*CRX*	AD
9	*NR2E3*	AD
5	*PRPF8*	AD
5	*BEST1*	AD
4	*C1QTNF5**	AD
4	*PRPF3*	AD
4	*TOPORS*	AD
3	*NRL*	AD
3	*PRPF6*	AD
2	*RP1L1*	AD
1	*FZD4**	AD
1	*GUCA1A**	AD
1	*GUCA1B*	AD
1	*HK1*	AD
1	*OPA1**	AD
1	*OPN1SW*	AD
1	*PROM1*	AD
127	*USH2A*	AR
56	*CRB1*	AR
52	*ABCA4*	AR
37	*EYS*	AR
28	*RP1*	AR
27	*CNGB1*	AR
27	*CERKL*	AR
26	*RDH12*	AR
23	*RPE65*	AR

Nº of families	Gene	Inheritance
22	*NR2E3*	AR
17	*PDE6A*	AR
15	*CNGA1*	AR
14	*CEP290*	AR
11	*NMNAT1*	AR
10	*CNGB3**	AR
10	*TULP1*	AR
9	*AIPL1*	AR
9	*MERTK*	AR
9	*PROM1*	AR
7	*FAM161A*	AR
7	*RPGRIP1*	AR
7	*SPATA7*	AR
6	*C2orf71*	AR
6	*LCA5*	AR
6	*PDE6B*	AR
5	*PRCD*	AR
5	*SAG*	AR
4	*HGSNAT*	AR
4	*CDHR1*	AR
4	*CLN3*	AR
4	*CNGA3**	AR
4	*GUCY2D*	AR
4	*LRAT*	AR
4	*RLBP1*	AR
4	*RDH5*	AR
3	*BBS1*	AR
3	*BBS2*	AR
3	*IMPG2*	AR
3	*MAK*	AR
3	*RP1L1*	AR
2	*ACBD5**	AR
2	*GNAT2**	AR
2	*GRM6*	AR
2	*IMPG1*	AR
2	*NRL*	AR
2	*PDE6C**	AR
2	*SAMD11*	AR
2	*SLC24A1*	AR
2	*TRPM1*	AR
2	*ZNF408*	AR
2	*RHO*	AR
1	*AHI1*	AR
1	*BEST1*	AR
1	*ATF6**	AR
1	*BBS9*	AR
1	*CLRN1*	AR
1	*CYP4V2*	AR
1	*FLVCR1*	AR

Nº of families	Gene	Inheritance
1	*GPR179*	AR
1	*IFT140*	AR
1	*KCNV2*	AR
1	*MYO7A**	AR
1	*OAT**	AR
1	*PCDH21*	AR
1	*PDE6G*	AR
1	*PDE6H**	AR
1	*RBP4*	AR
1	*RGR*	AR
1	*PRPH2*	AR
1	*TTPA*	AR
54	*CHM*	XL
44	*RPGR_ORF15*	XL
24	*RPGR*	XL
22	*RP2*	XL
8	*CACNA1F*	XL
6	*NYX*	XL
3	*OPN1LW-OPN1MW**	XL
2	*RS1**	XL
1	*OFD1*	XL
1	*GPR143**	XL
1,038	**Total families**	

There is a total of 23 genes found in the AD-RP group, 70 in the AR-RP and 9 in the XL-RP. AD: autosomal dominant; AR: autosomal recessive; RP: retinitis pigmentosa; XL: X-linked.

* Clinically reclassified to RP (*C1QTNF5, GUCA1A, CNGB3, CNGA3, ACBD5, GNAT2, PDE6C, ATF6, PDE6H, OPN1LW-OPN1MW* and *RS1*) or syndromic IRD (*MYO7A*) groups. Moreover, 4 genes were related to other visual diseases (*FZD4, OPA1, GPR143 and OAT*).

Supplementary Table S5. Genes identified in syndromic IRD (USH1, USH2, atypical Usher syndrome and other syndromes).

Nº of families	Gene	Disease
30	*MYO7A*	USH1
12	*CDH23*	USH1
9	*PCDH15*	USH1
2	*USH1C*	USH1
2	*USH1G*	USH1
1	*ADGRV1*	USH1
129	*USH2A*	USH2
11	*ADGRV1*	USH2
3	*MYO7A*	USH2
1	*CDH23*	USH2
1	*PEX6*	USH2
5	*USH2A*	Usher others
1	*USH3A*	Usher others
23	*BBS1*	others
6	*MKKS*	others
5	*ALMS1*	others
4	*BBS10*	others
4	*BBS12*	others
4	*VPS13B*	others
3	*NPHP1*	others
3	*OTX2*	others
2	*ADNmt*	others
2	*AHI1*	others
2	*BBS2*	others
2	*CEP290*	others
2	*CWC27*	others
2	*NPHP5*	others
2	*PEX1*	others
2	*RDH12**	others
2	*SCAPER*	others
1	*ABHD12*	others
1	*BBS3*	others
1	*BBS5*	others
1	*BBS7*	others
1	*BBS9*	others
1	*C8orf37*	others
1	*CDH23*	others
1	*CEP41*	others
1	*CHD7*	others
1	*CLN8*	others
1	*COL4A5*	others
1	*CRB1*	others
1	*FALDH*	others
1	*FLVCR1*	others
1	*HADHA*	others
1	*IFT140*	others

Nº of families	Gene	Disease
1	*IFT27*	others
1	*INPP5E*	others
1	*JAG1*	others
1	*KIF11*	others
1	*MKS1*	others
1	*MT-TH*	others
1	*NPHP4*	others
1	*PANK2*	others
1	*PDE6A**	others
1	*PEX6*	others
1	*PRPS1*	others
1	*RPGRIP1**	others
1	*COL2A1*	others
1	*SCA7*	others
1	*WDR19*	others
308	**Total families**	

There is a total of 6 genes found in USH1, 5 in USH2, 2 in atypical Usher syndrome and 48 in other syndromes. IRD: inherited retinal dystrophies; USH1: Usher syndrome type 1; USH2: Usher syndrome type 2.

* Clinically reclassified to RP (*RDH12, PDE6A* and *RPGRIP1*).

Supplementary Table S6. Most frequent causing-variants in NON-RP, RP and syndromic IRD in the Spanish population.

	Nº of alleles	Gene	Nucleotide change	Amino acid change	Reported phenotype	Allele frequency: Spanish cohort	GnomAD allele frequency: ALL	GnomAD allele frequency: Southern European	PMID
NON-RP	180	*ABCA4*	c.3386G>T	p.Arg1129Leu	STGD1	4.98e-2	2.97e-4	8.61e-4	9295268
	62	*ABCA4*	c.5882G>A	p.Gly1961Glu	STGD1	1.71e-2	4.56e-3	8.18e-3	9295268
	26	*ABCA4*	c.5819T>C	p.Leu1940Pro	FF, arMD	7.19e-3	2.12e-5	0.000	11385708
	25	*ABCA4*	c.3210_3211dupGT	p.Ser1071CysfsTer14	STGD1	6.91e-3	1.99e-5	1.74e-4	9295268
	25	*ABCA4*	c.1804C>T	p.Arg602Trp	STGD1	6.91e-3	4.39e-5	0.000	9973280
	24	*ABCA4*	c.4457C>T	p.Pro1486Leu	STGD1	6.63e-3	1.22e-4	1.82e-4	9973280
	23	*ABCA4*	c.2888delG	p.Gly963AlafsTer14	STGD1	6.36e-3	3.98e-6	0.000	11385708
	20	*ABCA4*	c.6179T>G	p.Leu2060Arg	CRD	5.53e-3	Not found	Not found	11385708
	18	*ABCA4*	c.5929G>A	p.Gly1977Ser	STGD1	4.98e-3	7.98e-6	0.000	9781034
	15	*ABCA4*	c.3322C>T	p.Arg1108Cys	STGD1	4.15e-3	1.27e-4	1.72e-4	9973280
	14	*ABCA4*	c.5714+5G>A	p.?	STGD1	3.87e-3	3.06e-4	1.13e-3	9466990
	14	*ABCA4*	c.5044_5058del15	p.Val1682_Val1686del	STGD1	3.87e-3	2.80e-5	0.000	9295268
	13	*ABCA4*	c.4918C>T	p.Arg1640Trp	STGD1	3.59e-3	3.18e-5	0.000	9973280
	13	*ABCA4*	c.634C>T	p.Arg212Cys	STGD1	3.59e-3	1.07e-4	1.73e-4	9503029
	12	*ABCA4*	c.5461-10T>C	p.?	CRD	3.32e-3	2.27e-4	0.000	15614537
	10	*PRPH2*	c.584G>T	p.Arg195Leu	CACD	2.76e-3	3.98e-6	0.000	14557183
	10	*ABCA4*	c.3056C>T	p.Thr1019Met	STGD1	2.76e-3	3.18e-5	0.000	9973280
RP	106 (138)[a]	*USH2A*	c.2276G>T	p.Cys759Phe	arRP; Usher II	3.81e-2[c]	9.68e-4	2.25e-3	10775529 / 26806561
	48	*CERKL*	c.847C>T	p.Arg283Ter	arRP	1.33e-2	3.32e-4	8.71e-4	14681825
	27	*CNGB1*	c.2957A>T	p.Asn986Ile	adRP	7.46e-3	1.18e-3	4.34e-4	17564971

	Nº of alleles	Gene	Nucleotide change	Amino acid change	Reported phenotype	Allele frequency: Spanish cohort	GnomAD allele frequency: ALL	GnomAD allele frequency: Southern European	PMID
	24	*RP1*	c.1625C>G	p.Ser542Ter	arRP, early-onset	6.63e-3	4.25e-5	8.65e-5	22917891
	21	*RDH12*	c.295C>A	p.Leu99Ile	LCA	5.8e-3	6.01e-5	1.72e-4	15322982
	21	*CRB1*	c.2843G>A	p.Cys948Tyr	arRP, early-onset	5.8e-3	2.03e-4	8.66e-5	18055821
	18	*CNGA1*	c.94C>T	p.Arg32Ter	arRP	4.98e-3	7.23e-5	2.63e-4	12362048
	17	*CNGB3*	c.1148delC	p.Thr383IlefsTer13	Achr.; CD	4.7e-3	1.75e-3	1.47e-3	10888875
	15	*NR2E3*	c.932G>A	p.Arg311Gln	ESCS	4.15e-3	3.95e-4	4.17e-4	10655056
	13	*CRB1*	c.613_619del	p.Ile205AspfsTer13	LCA	3.59e-3	3.19e-5	1.74e-4	11231775
	12	*RHO*	c.1040C>T	p.Pro347Leu	adRP	3.32e-3	3.54e-6	0.000	2215617 / 29847639
	10	*NR2E3*	c.119-2A>C	p.?	ESCS, arRP	2.76e-3	5.26e-4	0.000	10655056
	10	*FAM161A*	c.1355_1356delCA	p.Thr452SerfsTer3	arRP	2.76e-3	9.97e-5	1.74e-4	20705279
Syndromic IRD	51 (60)[a,b]	*USH2A*	c.2299delG	p.Glu767SerfsTer21	arRP; Usher II	1.66e-2[c]	7.02e-4	5.19-e4	9624053
	33 (42)[a,b]	*BBS1*	c.1169T>G	p.Met390Arg	arRP; BBS	1.16e-2[c]	1.57e-3	2.50e-3	12118255
	32 (138)[a]	*USH2A*	c.2276G>T	p.Cys759Phe	arRP; Usher II	3.81e-2[c]	9.68e-4	2.25e-3	10775529 / 26806561
	11 (20)[a,b]	*USH2A*	c.9799T>C	p.Cys3267Arg	arRP; Usher II	5.53e-3[c]	7.08e-6	0.000	17085681

AR-MD: autosomal recessive macular dystrophy; CACD: central areolar choroidal dystrophy; STGD1: Stargardt disease 1; FF: fundus flavimaculatus; ACHM: achromatopsia; AD-RP: autosomal dominant retinitis pigmentosa; AR-RP: autosomal recessive retinitis pigmentosa; BBS: Bardet-Biedl syndrome; CD: cone dystrophy; ESCS: enhanced S-cone syndrome; LCA: Leber congenital amaurosis. Total identified alleles: 3,618. Total identified NON-RP alleles: 1,300. Total different NON-RP variants: 458. Total identified non-syndromic RP alleles: 1,716. Total different non-syndromic RP variants: 836 (appearing 40 of them also in syndromic IRD). Total identified syndromic IRD alleles: 602. Total identified syndromic IRD variants: 295 (appearing 40 of them also in non-syndromic RP).

The gnomAD ALL and Southern European frequencies for each allele were extracted from the gnomAD Exomes v2.1.1 dataset.

a These variants can be found both in RP and syndromic IRD and depending on the second variant identified in compound heterozygosis we could observe one of these two phenotypes. The number of alleles in parentheses corresponds to the total of alleles of RP and syndromic IRD together.

b These variants can be found also in non-syndromic RP, but with a lower frequency than 10 alleles.

c These frequencies correspond to the total of alleles independently of the phenotype (non-syndromic or syndromic) of the cases: *USH2A* p.Cys759Phe (n=138 alleles); *USH2A* p.Glu767SerfsTer21 (n=60); *BBS1* p.Met390Arg (n=42); and *USH2A* p.Cys3267Arg (n=20).

Supplementary Table S7. Comparative table with the different prevalence studies from other countries.

Study	Year	Country	Number of included cases	Diseases	Screened genes	Characterized cases	Number of genes found	Techniques
Bunker *et al.*[1]	1984	Maine (USA)	226 cases	BBS, RP, USH	N/A	N/A	N/A	N/A
Chelva *et al.*[2]	1992	Western Australia	266 patients + 125 relatives	BBS, LCA, RP, USH	N/A	N/A	N/A	N/A
Ziviello *et al.*[3]	2005	Italy	43 families	adRP	12	28% (12/43)	5	Sanger sequencing
Eisenberg *et al.*[4]	2013	Miscellanea	126 patients	CCRD, LCA, RP	55	70% (88/126)	28	RD gene panel
Bertelsen *et al.*[5]	2014	Denmark	3,076 cases	IRD	N/A	N/A	N/A	N/A
Huang *et al.*[6]	2014	China	179 families	IRD	252	55.3% (99/179)	35	Customized targeted gene panel
Beheshtian *et al.*[7]	2015	Iran	13 families	arRP	245	77% (10/13)	9	WES
Tiwari *et al.*[8]	2016	Switzerland	58 patients	IRD	Unknown	64% (37/58)	18	WES
Bravo-Gil *et al.*[9]	2017	Spain	106 cases	sRP	68	62.26% (66/106)	26	Customized targeted gene panel
Birtel *et al.*[10]	2018	Germany	251 patients	CCRD, MD	Unknown	74% (185/251)	22	Sanger, MLPA, NGS-panel
Maeda *et al.*[11]	2018	Japan	94 probands	CCRD, RP, BCR, USH, STGD	39	47.9% (45/94)	19	Customized targeted gene panel
Motta *et al.*[12]	2018	Brazil	1,246 patients (1,159 families)	IRD	Unknown	71.6% (400/559)	66	aCGH, APEX, WES, NGS-panel, Sanger
Kim *et al.*[13]	2019	South Korea	86 cases	IRD	204	44.2% (38/86)	22	Customized targeted gene panel
Holtan *et al.*[14]	2020	Norway	866 patients	IRD	Unknown	32% (207/650)	56	APEX. HTS panel
Sharon *et al.*[15]	2019	Israel	3,413 cases (2,420 families)	IRD	Unknown	56% (1,369/2,420)	129	Several

Study	Year	Country	Number of included cases	Diseases	Screened genes	Characterized cases	Number of genes found	Techniques
Pontikos *et al.*[16]	2020	United Kingdom	4,236 patients (3,195 families)	IRD	Unknown	N/A	135	Single-gene and gene-panel testing, WES, WGS
Goetz *et al.*[17]	2020	USA and Canada	6,403 cases (5,385 families)	IRD, eye diseases	>200	62.1% (3,448/5,552)	Unknown	Several
Jaffal *et al.*[18]	2020	11 Arab countries	407 individuals from 30 studies (meta-analysis)	sRCD, arRDC	63	N/A	33	NGS, homozygosity mapping, Sanger
This work	-	Spain	6,089 cases (4,403 families)	IRD	291	53.2% (2,100/3,951)	142	Several[a]

adRP: autosomal dominant retinitis pigmentosa; APEX: Arrayer Primer Extension; arRCD: autosomal recessive rod-cone dystrophy; arRP: autosomal recessive retinitis pigmentosa; BBS: Bardet-Biedl syndrome; BCR: Bietti crystallin retinopathy; CCRD: cone/cone-rod dystrophies; HTS: high-throughput sequencing; IRD: inherited retinal dystrophies; LCA: Leber congenital amaurosis; MD: macular dystrophy; MLPA: Multiplex Ligation-dependent Probe Amplification; NGS: Next Generation Sequencing; RD: retinal dystrophies; RP: retinitis pigmentosa; sRCD: sporadic rod-cone dystrophy; sRP: sporadic retinitis pigmentosa; STGD: Stargardt disease; USH: Usher syndrome; WES: Whole Exome Sequencing; WGS: Whole Genome Sequencing.

a All the different techniques used are described in the Supplementary Table S8.

Supplementary Table S8. Summary table with all the different molecular approaches that have been used during the 28 years of styudy, divided by NON-RP, RP and syndromic IRD and their a priori mode of inheritance.

	Type	Technologies
NON-RP	AD	- Denaturing HPLC - High resolution melting scanning - Sanger sequencing - Haplotype analysis - Customized targeted gene panel - Commercial clinical exome - WES
	AR[19-23]	- Commercial APEX-based genotyping microarrays (ABCR400 microarray) - Denaturing HPLC - High resolution melting scanning - Sanger sequencing - Customized targeted gene panel - Commercial clinical exome - WES - smMIPS - MLPA - aCGH - Haplotype analysis
	XL	- Sanger sequencing - Haplotype analysis - Commercial APEX-based genotyping microarrays (xlRP microarray including RPGR-ORF15 sequencing) - Customized targeted gene panel - Commercial clinical exome
	Unclassified[22]	- Commercial clinical exome - smMIPS
RP	AD[24-28]	- SSCP - DGGE - Denaturing HPLC - Sanger sequencing

Type	Technologies
	- Commercial APEX-based genotyping microarrays (adRP, xlRP microarray including RPGR-ORF15 sequencing) - Customized targeted gene panel (74 IRD-associated genes) - Commercial clinical exome - WES - MLPA - aCGH - Haplotype analysis
AR[20,23,29-33]	- Commercial APEX-based genotyping microarrays (arRP, LCA, ABCR400, xlRP microarray including RPGR-ORF15 sequencing) - Denaturing HPLC - Haplotype analysis - Customized targeted gene panel (75 IRD-associated genes) - Commercial clinical exome - WES - WGS - MLPA
S[30,34]	- DGGE - Denaturing HPLC - Commercial APEX-based genotyping microarrays (arRP, LCA, ABCR400 microarray) - Sanger sequencing - Customized targeted gene panels (75 IRD-associated genes and 82 RP-associated genes) - Commercial clinical exome - WES - WGS - MLPA - aCGH
XL	- Sanger sequencing - DGGE - Commercial APEX-based genotyping microarrays (arRP, adRP, LCA, CSNB, xlRP microarrays including RPGR-ORF15 sequencing) - Haplotype analysis

	Type	Technologies
		- Customized targeted gene panel - Commercial clinical exome - WES - WGS - MLPA
	Unclassified	- Sanger sequencing - Commercial APEX-based genotyping microarrays (arRP, Usher microarray) - Denaturing HPLC - Haplotype analysis - Customized targeted gene panel - Commercial clinical exome
Syndromic IRD	Usher I[31,35]	- Commercial APEX-based genotyping microarrays (Usher syndrome microarray) - Sanger sequencing - Customized targeted gene panel - Commercial clinical exome - MLPA
	Usher II[23,31,35]	- Commercial APEX-based genotyping microarrays (Usher syndrome microarray) - Sanger sequencing - Customized targeted gene panel - Commercial clinical exome - MLPA
	Usher others	- SSCP - Sanger sequencing - Commercial APEX-based genotyping microarrays (arRP, LCA, Usher syndrome, BBS microarrays) - Customized targeted gene panel - Commercial clinical exome
	Others[36-38]	- Commercial APEX-based genotyping microarrays (BBS, ALMS, LCA, arRP microarrays) - Sanger sequencing - Customized targeted gene panels - Commercial clinical exome - WES

Type	Technologies
	- aCGH - MLPA - Haplotype analysis - Mitochondrial DNA sequencing

aCGH: Microarray-based Comparative Genomic Hybridization; adRP: autosomal dominant retinitis pigmentosa; ALMS: Alström syndrome; APEX: Arrayed Primer Extension; arRP: autosomal recessive retinitis pigmentosa; BBS: Bardet-Biedl syndrome; CSNB: congenital stationary night blindness; DGGE: CG-clamped denaturing gradient gel electrophoresis; HPLC: High-Performance Liquid Cromatography; IRD: inherited retinal dystrophies; LCA: Leber congenital amaurosis; MLPA: Multiplex Ligation-dependent Probe Amplification; RP: retinitis pigmentosa; smMIPS: single-molecule molecular inversion probes; SSCP: Single-strand conformation polymorphism (SSCP); WES: Whole Exome Sequencing; WGS: Whole Genome Sequencing; xlRP: X-linked retinitis pigmentosa.

Supplementary Appendix S1. Lists of studied genes

Different diagnostic technologies were used to study the cases. A maximum of 291 genes were analysed.

In cases of non-syndromic inherited retinal dystrophies (IRD) (NON-RP and RP), the following 190 genes were studied: *ABCA4, ACO2, ADAM9, ADIPOR1, AFG3L2, AGBL5, AHR, AIPL1, ARHGEF18, ARL2BP, ARL3, ARL6, ASRGL1, ATF6, ATL3, BBS1, BBS2, BEST1, C12orf65, C1QTNF5, C21orf2, C2orf71, C8orf37, CA4, CABP4, CACNA1F, CACNA2D4, CAPN5, CCT2, CDH3, CDH3, CDHR1, CEP290, CEP78, CERKL, CFH, CHM, CLCC1, CLRN1, CLUAP1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, CRB1, CRX, CTNNA1, CYP4V2, DHDDS, DHX38, DMD, DRAM2, DTHD1, EFEMP1, ELOVL1, ELOVL4, EMC1, EYS, FAM161A, FSCN2, FZD4, GDF6, GNAT1, GNAT2, GNB3, GPR125, GPR143, GPR179, GRK1, GRM6, GUCA1A, GUCA1B, GUCY2D, HGSNAT, HK1, HMCN1, IDH3B, IFT140, IFT172, IFT81, IMPDH1, IMPG1, IMPG2, IQCB1, ITM2B, KCNJ13, KCNV2, KIAA1549, KIZ, KLHL7, LCA5, LRAT, LRIT3, LRP5, MAK, MAPKAPK3, MERTK, MFN2, MFRP, MFSD8, MIR204, MVK, NBAS, NDP, NEK2, NEUROD1, NMNAT1, NR2E3, NR2F1, NRL, NYX, OAT, OFD1, OPA1, OPN1LW, OPN1MW, OPN1SW, OTX2, PCDH21, PCDHB14, PDE6A, PDE6B, PDE6C, PDE6G, PDE6H, PGK1, PITPNM3, PLA2G5, POC1B, POMGNT1, PRCD, PRDM13, PROM1, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, PRPH2, RAB28, RAX2, RB1, RBP3, RBP4, RCBTB1, RD3, RDH12, RDH5, REEP6, RGR, RGS9, RGS9BP, RHO, RIMS1, RLBP1, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPGRIP1, RS1, RTN4IP1, SAG, SAMD11, SEMA4A, SLC24A1, SLC7A14, SNRNP200, SPATA7, SPP2, TEAD1, TIMM8A, TIMP3, TMEM126A, TOPORS, TRNT1, TRPM1, TSPAN12, TTC8, TTLL5, TULP1, UNC119, USH2A, VCAN, ZNF408, ZNF513.*

In patients with syndromic IRD, the following 127 genes were studied: *ABCC6, ABHD12, ACBD5, ACO2, ADAMTS18, ADGRV1, ADIPOR1, AFG3L2, AHI1, ALMS1, ARL6, ARSG, ATXN7, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS3, BBS4, BBS5, BBS7, BBS9, C8orf37, CC2D2A, CDH23, CEP164, CEP19, CEP250, CEP290, CEP41, CEP78, CHD7, CIB2, CLN3, CLN8, CLRN1, COL11A1, COL2A1, COL4A5, COL9A1, CSPP1, CWC27, DFNB31, ELOVL4, ESPN, EXOSC2, FALDH, FLVCR1, GNPTG, HADHA, HARS, HGSNAT, HMX1, IFT140, IFT172, IFT27, IFT81, INPP5E, INVS, IQCB1, JAG1, KCNJ13, KIF11, KSS, LAMA1, LRP5, LZTFL1, MFN2, MKKS, MKS1, MT-ATP6, MT-TH, MT-TL1, MT-TP, MT-TS2, MYO7A, NPHP1, NPHP3, NPHP4, NPHP5, OFD1, OPA3, PANK2, PAX2, PCDH15, PCYT1A, PDZD7, PEX1, PEX2, PEX6, PEX7, PHYH, PLK4, PNPLA6, POC1B, POC5, PRPS1, RDH11, RPGRIP1L, SCA7, SCAPER, SDCCAG8, SLC25A46, TIMM8A, TMEM216, TMEM237, TREX1, TRIM32, TRNT1, TTC8, TTPA, TUB, TUBGCP4, TUBGCP6, USH1C, USH1G, USH2A, USH3A, VCAN, VPS13B, WDPCP, WDR19, WFS1, ZNF423.*

Some genes were included in both groups because they have been reported in syndromic and non-syndromic forms of IRD.

References of the Supplementary Material

1. Bunker, C.H., Berson, E.L., Bromley, W.C., Hayes, R.P. & Roderick, T.H. Prevalence of retinitis pigmentosa in Maine. Am J Ophthalmol 97, 357-65, doi: 10.1016/0002-9394(84)90636-6 (1984).
2. Chelva, E., McLaren, T.L., Kay, S.M., Collins, D.W., Black, J.L. & Candy, D.R. A retinitis pigmentosa register for western Australia. Aust N Z J Ophthalmol 20, 311-7, doi: 10.1111/j.1442-9071.1992.tb00743.x (1992).
3. Ziviello, C. et al. Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): a comprehensive study of 43 Italian families. J Med Genet 42, e47, doi: 10.1136/jmg.2005.031682 (2005).
4. Eisenberger, T. et al. Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example od retinal dystrophies. PLoS One 8, e78496, doi: 10.1371/journal.pone.0078496 (2013).
5. Bertelsen, M., Jensen, H., Bregnhøj, J.F. & Rosenberg, T. Prevalence of generalized retinal dystrophy in Denmark. Ophthalmic Epidemiol 21, 217-23, doi: 10.3109/09286586.2014.929710 (2014).
6. Huang, X.F. et al. Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. Genet Med 17, doi: 10.1038/gim.2014.138 (2015).
7. Beheshtian, M. et al. Impact of whole exome sequencing among Iranian patients with autosomal recessive retinitis pigmentosa. Arch Iran Med 18, 776-85 (2015).
8. Tiwari, A. et al. Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. Sci Rep 6, 28755, doi: 10.1038/srep28755 (2016).
9. Bravo-Gil, N. et al. Unravelling the genetic basis of simplex Retinitis Pigmentosa cases. Sci Rep 7, 41937, doi: 10.1038/srep41937 (2017).
10. Birtel, J. et al. Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. Sci Rep 8, 4824, doi: 10.1038/s41598-018-22096-0 (2018).
11. Maeda, A. et al. Development of a molecular diagnostic test for Retinitis Pigmentosa in the Japanese population. Jpn J Ophthalmol 62, 451-457, doi: 10.1007/s10384-018-0601-x (2018).
12. Motta, F.L., Martin, R.P., Filipelli-Silva, R., Vallim Salles, M. & Ferraz Sallum, J.M. Relative frequency of inherited retinal dystrophies in Brazil. Sci Rep 8, 15939, doi: 10.1038/s41598-018-34380-0 (2018).
13. Kim, M.S. et al. Genetic Mutation Profiles in Korean Patients with Inherited Retinal Diseases. J Korean Med Sci 34, e161, doi: 10.3346/jkms.2019.34.e161 (2019).
14. Holtan, J.P., Selmer, K.K., Heimdal, K.R. & Bragadóttir, R. Inherited retinal disease in Norway – a characterization of current clinical and genetic knowledge. Acta Ophthalmol 98, 286-295, doi: 10.1111/aos.14218 (2020).
15. Sharon, D. et al. A nationwide genetic analysis of inherited retinal diseases in Israel as assessed by the Israeli inherited retinal disease consortium (IIRDC). Hum Mutat 41, 140-149, doi: 10.1002/humu.23903 (2020).
16. Pontikos, N. et al. Genetic Basis of Inherited Retinal Disease in a Molecularly Characterized Cohort of More Than 3000 Families from the United Kingdom. Ophthalmology 127, 1384-1394, doi: 10.1016/j.ophtha.2020.04.008 (2020).

17. Goetz, K.E. et al. Genetic testing for inherited eye conditions in over 6,000 individuals through the eyeGENE network. Am J Med Genet C Semin Med Genet 184, 828-837, doi: 10.1002/ajmg.c.31843 (2020).
18. Jaffal, L., Joumaa, H., Mrad, Z., Zeitz, C., Audo, I. & El Shamieh, S. The genetics of rod-cone dystrophy in Arab countries: a systematic review. Eur J Hum Genet, doi: 10.1038/s41431-020-00754-0 (2020).
19. Riveiro-Alvarez, R. et al. Frequency of ABCA4 mutations in 278 Spanish controls: an insight into the prevalence of autosomal recessive Stargardt disease. Br J Ophthalmol 93, 1359-64, doi: 10.1136/bjo.2008.148155 (2008).
20. Riveiro-Alvarez, R. et al. Outcome of ABCA4 disease-associated alleles in autosomal recessive retinal dystrophies: retrospective analysis in 420 Spanish families. Ophthalmology 120, 1332-7, doi: 10.1016/j.ophtha.2013.04.002 (2013).
21. Del Pozo-Valero, M. et al. Expanded Phenotypic Spectrum of Retinopathies Associated with Autosomal Recessive and Dominant Mutations in PROM1. Am J Ophthalmol 207, 204-214, doi: 10.1016/j.ajo.2019.05.014 (2019).
22. Del Pozo-Valero, M. et al. Genotype-Phenotype Correlations in a Spanish Cohort of 506 Families With Biallelic ABCA4 Pathogenic Variants. Am J Ophthalmol 219, 195-204, doi: 10.1016/j.ajo.2020.06.027 (2020).
23. Corton, M. et al. Exome sequencing of index patients with retinal dystrophies as a tool for molecular diagnosis. PLoS One 8, e65574, doi: 10.1371/journal.pone.0065574 (2013).
24. Martin-Merida, I. et al. Toward the Mutational Landscape of Autosomal Dominant Retinitis Pigmentosa: A Comprehensive Analysis of 258 Spanish Families. Invest Ophthalmol Vis Sci 59, 2345-2354, doi:10.1167/iovs.18-23854 (2018).
25. Fernandez-San Jose, P. et al. Targeted Next-Generation Sequencing Improves the Diagnosis of Autosomal Dominant Retinitis Pigmentosa in Spanish Patients. Invest Opthalmol Vis Sci 56, 2173-82, doi: 10.1167/iovs.14-16178 (2015).
26. Martin-Merida, I. et al. Analysis of the PRPF31 Gene in Spanish Autosomal Dominant Retinitis Pigmentosa Patients: A Novel Genomic Rearrangement. Invest Ophthalmol Vis Sci 58, 1045-1053, doi: 10.1167/iovs.16-20515 (2017).
27. Blanco-Kelly, F. et al. Dominant Retinitis Pigmentosa, p.Gly56Arg Mutation in NR2E3: Phenotype in a Large Cohort of 24 Cases. PLoS One 11, e0149473, doi: 10.1371/journal.pone.0149473 (2016).
28. Almoguera, B. et al. Application of Whole Exome Sequencing in Six Families with an Initial Diagnosis of Autosomal Dominant Retinitis Pigmentosa: Lessons Learned. PLoS One 10, e0133624, doi: 10.1371/journal.pone.0133624 (2015).
29. Vallespin, E. et al. Mutation screening of 299 Spanish families with retinal dystrophies by Leber congenital amaurosis genotyping microarray. Invest Ophthalmol Vis Sci 48, 5653-61, doi: 10.1167/iovs.07-0007 (2007).
30. Perez-Carro, R. et al. Panel-based NGS Reveals Novel Pathogenic Mutations in Autosomal Recessive Retinitis Pigmentosa. Sci Rep 6, 19531, doi: 10.1038/srep19531 (2016).
31. Perez-Carro, R. et al. Unravelling the pathogenic role and genotype-phenotype correlation of the USH2A p.(Cys759Phe) variant among Spanish families. PLoS One 13, e0199048, doi: 10.1371/journal.pone.0199048 (2018).
32. Avila-Fernandez, A. et al. Mutation analysis of 272 Spanish families affected by autosomal recessive retinitis pigmentosa using a genotyping microarray. Mol Vis 16, 2550-8 (2010).
33. Avila-Fernandez, A. et al. Whole-exome sequencing reveals ZNF408 as a new gene associated with autosomal recessive retinitis pigmentosa with vitreal alterations. Hum Mol Genet 24, 4037-48, doi: 10.1093/hmg/ddv140 (2015).

34. Martin-Merida, I. et al. Genomic Landscape of Sporadic Retinitis Pigmentosa: Findings from 877 Spanish Cases. Ophthalmology 126, 1181-1188, doi: 10.1016/j.ophtha.2019.03.018 (2019).
35. Blanco-Kelly, F. et al. Clinical aspects of Usher syndrome and the USH2A gene in a cohort of 433 patients. JAMA Ophthalmol 133, 157-64, doi: 10.1001/jamaophthalmol.2014.4498 (2015).
36. Sanchez-Navarro, I. et al. Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. Sci Rep 8, 5285, doi: 10.1038/s41598-018-23520-1 (2018).
37. Almoguera, B. et al. Expanding the phenotype of PRPS1 syndromes in females: neuropathy, hearing loss and retinopathy. Orphanet J Rare Dis 9, 190, doi: 10.1186/s13023-014-0190-9 (2014).
38. Tatour, Y. et al. Mutations in SCAPER cause autosomal recessive retinitis pigmentosa with intellectual disability. J Med Genet 54, 698-704, doi: 10.1136/jmedgenet-2017-104632 (2017).

CAPÍTULO 2

Caracterización de pacientes sindrómicos no resueltos

Artículo 2: *NGS and phenotypic ontology-based approaches increase the diagnostic yield in syndromic retinal diseases*

Perea-Romero I, Blanco-Kelly F, Sanchez-Navarro I, *et al.*

Publicado en *Human Genetics*, 2021

Resumen

En este trabajo se realizó un estudio de cohortes prospectivo y retrospectivo en 100 casos índice, seleccionados por los siguientes criterios de inclusión: i) sospecha de ERS; ii) prueba genética negativa o no informativa (casos retrospectivos hasta final de 2017 (n=82)); o iii) sin prueba genética previa (casos prospectivos nuevos entre 2018 y 2020 (n=18)). Los pacientes con sospecha de USH fueron excluidos.

Los datos clínicos disponibles se anotaron usando términos HPO y se clasificaron en 7 categorías clínicas, según si había sospecha de un síndrome reconocible (ciliopatías o síndromes específicos no ciliares) o no (casos restantes agrupados según los principales síntomas extraoculares en 5 categorías).

Los estudios genéticos retrospectivos se realizaron usando diferentes métodos moleculares y bioinformáticos según la disponibilidad en cada momento. Posteriormente, los casos no caracterizados se seleccionaron prospectivamente para utilizar otros enfoques de NGS para ampliar el número de genes analizados.

Después de la clasificación fenotípica, las ciliopatías fueron las ERS más comunes (35%), siendo el BBS la condición más frecuente.

Se obtuvo una tasa de caracterización global del 52%, incluyendo 6 casos caracterizados de forma incompleta por un gen que explicaba parcialmente el fenotipo. Se lograron mejores tasas de caracterización en los subgrupos de casos prospectivos (83%) y de síndromes bien reconocibles (62%). El 27% de los casos completamente caracterizados fueron reclasificados a una categoría clínica diferente después de la identificación del gen causante de la enfermedad. Además, tres de los 11 pacientes con trastornos de la retina acompañados de un síntoma extraocular aislado ("RD+OTHER"), la enfermedad se explica por un gen de EHR no sindrómica. Se encontró un diagnóstico genético dual en el 4% de los casos completamente caracterizados. Adicionalmente, se encontraron asociaciones fenotípicas nuevas o extremadamente raras en el 17% de los casos con diagnóstico genético completo.

En resumen, la CES sería el enfoque más apropiado para el estudio de los casos prospectivos, mientras que la WES y el reanálisis bioinformático aumentarían el diagnóstico de los casos retrospectivos no caracterizados al 45%, principalmente de aquellos con síntomas inespecíficos.

Con este trabajo se ha descrito un enfoque integral para las ERS en la práctica clínica, basado en el uso de ontología fenotípica estructurada y secuenciación masiva. Asimismo, se ha puesto de manifiesto la importancia de una evaluación clínica exhaustiva, así como de la selección de la

prueba molecular más adecuada para resolver estos casos complejos y dilucidar nuevas asociaciones.

Contribución de la autora

La autora recopiló, estructuró y curó los datos clínicos, demográficos y familiares de los casos afectos de ERS no caracterizados (n = 100). Anotó todos los datos clínicos con términos HPO. Clasificó clínicamente los casos en subgrupos clínicos ya existentes y elaboró nuevas categorías clínicas de clasificación y sus criterios. Analizó todos los nuevos datos de NGS y reanalizó los ya existentes. Durante la estancia predoctoral en el *Institute of Molecular and Clinical Ophthalmology Basel*, la autora reanalizó parte de los datos de NGS con un *pipeline* bioinformático propio desarrollado por el grupo del Dr. C Rivolta.

Participó en el análisis estadístico i) de la distribución de los términos HPO entre los casos caracterizados completamente y los no caracterizados, ii) de la tasa de caracterización en función de distintos parámetros (tipo de NGS o recurrencia de la enfermedad en la familia), iii) de la comparación de las tasas de caracterización de distintas cohortes de la literatura en comparación a la nuestra. Participó en el establecimiento de nuevas asociaciones genotipo-fenotipo en algunos de los casos caracterizados.

Finalmente, escribió y revisó de forma crítica las sucesivas versiones del manuscrito.

El artículo y el material suplementario se encuentran publicados en:

https://link.springer.com/article/10.1007/s00439-021-02343-7

Human Genetics (2021) 140:1665–1678
https://doi.org/10.1007/s00439-021-02343-7

ORIGINAL INVESTIGATION

NGS and phenotypic ontology-based approaches increase the diagnostic yield in syndromic retinal diseases

I. Perea-Romero[1,2] · F. Blanco-Kelly[1,2] · I. Sanchez-Navarro[1] · I. Lorda-Sanchez[1,2] · S. Tahsin-Swafiri[1,2] · A. Avila-Fernandez[1,2] · I. Martin-Merida[1,2] · M. J. Trujillo-Tiebas[1,2] · R. Lopez-Rodriguez[1,2] · M. Rodriguez de Alba[1] · I. F. Iancu[1,2] · R. Romero[1,2] · M. Quinodoz[3,4,5] · H. Hakonarson[6,7,8] · Blanca Garcia-Sandova[2,9] · P. Minguez[1,2] · M. Corton[1,2] · C. Rivolta[3,4,5] · C. Ayuso[1,2]

Received: 23 April 2021 / Accepted: 10 August 2021 / Published online: 26 August 2021

Abstract

Syndromic retinal diseases (SRDs) are a group of complex inherited systemic disorders, with challenging molecular underpinnings and clinical management. Our main goal is to improve clinical and molecular SRDs diagnosis, by applying a structured phenotypic ontology and next-generation sequencing (NGS)-based pipelines. A prospective and retrospective cohort study was performed on 100 probands with an a priori diagnosis of non-Usher SRDs, using available clinical data, including Human Phenotype Ontology annotation, and further classification into seven clinical categories (ciliopathies, specific syndromes and five others). Retrospective molecular diagnosis was assessed using different molecular and bioinformatic methods depending on availability. Subsequently, uncharacterized probands were prospectively screened using other NGS approaches to extend the number of analyzed genes. After phenotypic classification, ciliopathies were the most common SRD (35%). A global characterization rate of 52% was obtained, with six cases incompletely characterized for a gene that partially explained the phenotype. An improved characterization rate was achieved addressing prospective cases (83%) and well-recognizable syndrome (62%) subgroups. The 27% of the fully characterized cases were reclassified into a different clinical category after identification of the disease-causing gene. Clinical-exome sequencing is the most appropriate first-tier approach for prospective cases, whereas whole-exome sequencing and bioinformatic reanalysis increases the diagnosis of uncharacterized retrospective cases to 45%, mostly those with unspecific symptoms. Our study describes a comprehensive approach to SRDs in daily clinical practice and the importance of thorough clinical assessment and selection of the most appropriate molecular test to be used to solve these complex cases and elucidate novel associations.

✉ C. Ayuso
cayuso@fjd.es

1 Department of Genetics, Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain

2 Center for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain

3 Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland

4 Department of Ophthalmology, University of Basel, Basel, Switzerland

5 Department of Genetics and Genome Biology, University of Leicester, Leicester, UK

6 Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, USA

7 Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA, USA

8 Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

9 Department of Ophthalmology, Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain

Introduction

Inherited retinal diseases (IRDs) are a clinically and genetically heterogeneous group of ocular rare diseases which, due to the dysfunction of the retina, lead to blindness (Ayuso and Millan 2010; Sundaram et al. 2012). IRDs have an estimated worldwide prevalence of between 1 in 1000 (Hanany et al. 2020) and 1 in 4000 (Ayuso and Millan 2010).

In IRDs, visual impairment can be the sole symptom or can appear together with other features (syndromic forms) such as auditory, nervous, renal, endocrine, musculoskeletal, hepatic, or cardiac anomalies (Wright et al. 2018a). Syndromic retinal diseases (SRDs) are a group of rare and complex inherited systemic diseases representing 20–30% of all IRDs. Some SRDs are well-known and recognizable syndromes, the most common being Usher syndrome (USH, MIM #276901) and other ciliopathies, such as Bardet-Biedl (BBS, MIM #209900), Alström (ALMS, MIM #203800), Joubert (JBTS, MIM #213300), and Senior–Løken (SLSN, MIM #266900) syndromes. However, SRDs also include other rare non-ciliary syndromes related to different cellular components (Werdich et al. 2014) such as Golgi apparatus-related disorders (Cohen syndrome, COH, MIM #216550), endoplasmic reticulum-associated disorders (Wolfram syndrome, WFS, MIM #222300), lysosomal storage disorders (Platt et al. 2018), peroxisome biogenesis disorders (PBD) (Argyriou et al. 2016), and mitochondrial disorders (Kearns–Sayre syndrome, KSS, MIM #530000). In clinical practice, however, the high clinical heterogeneity that patients usually present complicates phenotypic classification into well-defined syndromes, making diagnosis extremely challenging (Chiang and Trzupek 2015). Moreover, inheritance of these disorders can follow any type of Mendelian pattern or be one of a number of very rare non-Mendelian forms such as oligogenic, digenic, or mitochondrial inheritance (Mockel et al. 2011; Werdich et al. 2014; Gazzo et al. 2016). To date, about 94 known genes have been associated with syndromic retinal dystrophies (The Retinal Information Network, RetNet: https://sph.uth.edu/retnet/; last accessed January 2021).

Next-generation sequencing (NGS) technologies are currently the gold-standard approach for cost-effective genetic analysis in extremely complex syndromic forms (Wright et al. 2015). Their previous application into SRD cases has been proven to improve the diagnostic yield when compared to other traditional, time-consuming genotyping methods (Sanchez-Navarro et al. 2018; Abu Diab et al. 2019). Moreover, whole-exome sequencing (WES) and whole-genome sequencing (WGS) allow further reanalysis and revisions of previously-investigated cases using updated virtual panels, reanalysis with new bioinformatic tools (Wright et al. 2018b) and strategies using ontologies, which are useful for conducting targeted studies (Köhler et al. 2009).

The development of a clinical and genetic pipeline for the precision study of SRDs could help to anticipate additional underlying systemic complications that require periodic surveillance for early detection, management, and treatment. Such a pipeline would have repercussions for reproductive risk assessment, genetic counseling and patient selection for clinical trials of gene-based therapies (Ayuso and Millan 2010; Lee and Garg 2015; Sadagopan 2017).

The main aim of this study is to improve clinical and molecular SRD diagnosis, applying new structured Human Phenotype Ontology (HPO)-based phenotypic and NGS-based pipelines. This research has resulted in the creation of a working protocol for the precision study of SRDs in our institution.

Materials and methods

Subjects

Study subjects were recruited through a search of the database of the Genetics Department at the Fundación Jiménez Díaz Hospital (FJD) (Madrid, Spain), which includes a cohort of 4403 families with diverse IRDs referred for genetic testing since 1991 (Perea-Romero et al. 2021). Probands selected for study were required to meet the following inclusion criteria: (1) presumed diagnosis of SRD; (2) retrospective cases with a negative or non-informative genetic testing until December 2017 ($n = 82$), or new prospective cases between January 2018 and October 2020 ($n = 18$). Patients with typical Usher syndromes were excluded from this study.

This research has been approved by the FJD Research Ethics Committee and complies with all the principles of the Declaration of Helsinki and further revisions. All patients, or their legal guardians when necessary, signed a written informed consent form before entering the study.

Phenotypic data and classification

Clinical examination was made according to previously established criteria and included ophthalmic, physical, and additional examinations as previously described in Piñeiro-Gallego et al. (2012), Sanchez-Navarro et al. (2018) and Galbis-Martínez et al. (2021), as well as self-reported health data. Clinical and family history were reviewed through clinical reports, specific questionnaires, and/or electronic health records for each participant. Presumed diagnosis at the time of referral for genetic testing was also considered.

Probands were classified into seven different major a priori phenotypic groups using a modified categorization

from Sanchez-Navarro et al. (2018). The diagnostic criteria used during phenotypic classification are described in Supplementary Table S1.

Probands with a well-recognizable syndrome were organized under two disease groups: (1) ciliopathies (BBS, ALMS, JBTS, or SLSN) or suspicion of ciliopathy (ciliopathies-like), due to the presence of most ciliopathy-associated signs such as postaxial polydactyly, molar tooth sign on magnetic resonance imaging, or polycystic kidney disease, among others; and (2) other specific SRDs, including known or presumed clinical entities (Supplementary Table S1).

The remaining unclassified cases were grouped according to the major extra-ocular symptoms using ontology terms derived from HPO annotation (Köhler et al. 2009) and classified as (3) hearing loss (HL) and/or neurodevelopmental disorders, (4) neuropathy/myopathy and/or suspicion of mitochondrial DNA (mtDNA) disorder, (5) skeletal disorders, (6) other unspecific symptoms, and (7) unclassified due to the lack of additional clinical information. These five categories are summarized in the Supplementary Table S1.

Molecular screening and bioinformatic analysis

Over the study period, different molecular strategies were used in genetic testing and, in some cases, the same proband had been studied by several methods. A summary of the molecular analysis performed in our cohort is described in Supplementary Fig. S1.

The 18 prospective probands were mostly analyzed as a first-tier analysis using commercial clinical exome sequencing (CES) approaches: TruSight One Sequencing Panel kit (Illumina, San Diego, CA, USA) or Clinical Exome Solution (Sophia Genetics, Boston, MA, USA). Libraries were prepared following the instructions of each manufacturer and sequenced on a NextSeq500 platform (Illumina) (Martin-Merida et al. 2019). Bioinformatic analysis for single nucleotide variants (SNVs) was performed using the Illumina software from BaseSpace coupled with the VariantStudio v.3.0.12 software, or SOPHiA DDM platform (Sophia Genetics), respectively. Variant filtering and prioritization were based on read depth ≥ 20, frequency of the alternative allele $> 20\%$, and minor allelic frequency (MAF) in Genome Aggregation Database (gnomAD < 0.02). Potentially pathogenic variants were prioritized for a 136-gene subpanel of non-syndromic and syndromic IRD genes; if a negative result was obtained, CES data were later prioritized with an expanded virtual panel of up to 377 genes (Supplementary Table S2). Copy number variant (CNV) detection was carried out using the CoNVading software (Johansson et al. 2016) or SOPHiA DDM platform, respectively.

The 82 probands recruited before 2018 had been unsuccessfully screened using different approaches over time including classical molecular genetics, aCGH, and/or a customized 121-gene targeted NGS approach (Castro-Sánchez et al. 2015; Sanchez-Navarro et al. 2018).

After these preliminary analyses, uncharacterized probands ($n = 79$) were additionally screened using exome sequencing to extend the number of analyzed genes, including the above described CES approach.

Afterwards, 34 probands were analyzed by means of WES, as well as three more cases directly sequenced by this NGS technique. WES was carried out mostly using the Agilent SureSelect Human All Exon V5 kit and sequenced on an Illumina HiSeq2500 (Tatour et al. 2017). First, potentially pathogenic variants were prioritized in the extended 447-gene subpanel of non-syndromic and syndromic IRD, optic atrophy, and associated genes (Supplementary Table S2) using an in-house pipeline described in Supplementary Table S3. For gene discovery purposes, the WES data of 26 probands were analyzed using a hypothesis-free approach, including homozygosity mapping in probands with family history of consanguinity using the AutoMap tool (Quinodoz et al. 2021). Variant prioritization was performed as described in Supplementary Table S3. Re-analysis of NGS data from uncharacterized probands was performed every 2 years using updated in-house pipelines (Supplementary Table S3).

Finally, in five retrospective cases with suspected mitochondrial disorder, mtDNA sequencing was carried out using the QIAseq targeted Human mitochondrial DNA kit (QIAGEN GMBH) on an Illumina MiniSeq. Bioinformatic analysis, sequence alignment, and variant annotation were performed using the GeneGlobe Data Analysis Center (QIAgen). Variant prioritization was performed by integrating data from the MITOMAP database (https://www.mitomap.org/) using a custom script. The mtDNA haplogroup was established using HaploGrep (https://haplogrep.uibk.ac.at/).

Variant classification and validation studies

Candidate variants obtained were analyzed in detail and filtered according to disease databases such as OMIM (https://www.omim.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), HGMD Professional 2020.4 and/or Leiden Open Variation Databases (LOVD, https://www.lovd.nl/). Variant classification was carried out using a 5-class system (class 1 for benign to class 5 for pathogenic variants) following the recommendations of the American College of Medical Genetics and Genomics (ACMG) (Richards et al. 2015) and the European Society of Human Genetics (ESHG) criteria (Matthijs et al. 2016).

Sanger sequencing was performed to validate all predicted variants classified as class 4 and 5 variants and the variants of uncertain significance (VUS), or class 3, in a gene relevant to the clinical phenotype. CNVs were validated by means of a custom aCGH modified from the

arrEYE platform described in Van Cauwenbergh et al. (2017), allowing for a high-resolution study of CNVs in 125 IRD-related genes. Homozygosity was confirmed in all homozygous variants using CNV detection tools in the CES or WES data. Segregation analysis for SNVs and CNVs was also performed when DNA samples were available for affected and unaffected family members.

A proband was considered to have been characterized when any of these situations occurred: (1) identification of potentially biallelic variants classified as class 3, 4, or 5 in a recessive gene in homozygosis or a class 3 accompanying a class 4 or 5 variant; (2) identification of a heterozygous allele for a class 4 or 5 variant in a dominant gene; or (3) identification of a hemizygous class 4 or 5 variant in an X-linked gene. Furthermore, variants were required to explain the phenotype totally or partially and segregate in the pedigree (when this analysis was possible).

Statistical analysis

To test the improvement of the proposed approaches in the rate of characterization achieved, a Chi-squared test was performed and *p*-values lower than 0.05 were considered significant.

Results

Cohort description and phenotypic a priori classification

Here, we analyzed a cohort of 100 uncharacterized probands (54 females and 46 males) presenting an SRD.

A total of 82 uncharacterized cases were retrospectively identified from our electronic database from over 26 years (from 1991 to 2017) of studies. These cases had been unsuccessfully screened using different approaches over time, including classical genetic tests, aCGH, and/or a customized 121-gene targeted NGS approach, and finally new technologies including CES and/or WES. In the 26 uncharacterized cases after WES analysis, reanalysis was performed by means of new bioinformatic approaches, including reannotation of old data (Supplementary Table S3).

Eighteen probands were prospectively recruited at the FJD over the last 3-year period (from 2018 to 2020) after being referred for genetic testing and were further screened by CES approaches as first-tier analysis (Supplementary Fig. S1).

After thorough revision of the clinical history, patients were classified following a priori diagnosis of SRD considering the main symptoms and HPO criteria defined in Supplementary Table S1. Probands were split into seven clinical groups (Fig. 1). The most common SRD in our cohort was ciliopathies or ciliopathy-like disorders with 35 probands. Specifically, BBS/BBS-like was the most widely represented phenotype, as half (18/35) of the probands with suspected ciliopathies, followed by ALMS and JBTS with seven probands in each of both well-recognized phenotypes. Furthermore, some specific non-ciliary conditions were also suspected in our cohort, such as COH, WFS, Alport syndrome (ATS, MIM #301050), ceroid lipofuscinosis (CLN, MIM #256730), long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD deficiency, MIM #609016), and Mulibrey nanism (MUL, MIM #253250).

Among the group of 54 probands without a well-known syndrome, HL and/or neurodevelopmental disorders (ND) were the extra-ocular symptoms most frequently associated with retinopathy in half (28/54) of these probands. In 23 out of 28 probands, HL and neurodevelopmental disorders usually presented as isolated systemic findings, while in the remaining five, both appeared together. Eleven probands presented miscellaneous isolated unspecific systemic symptoms (RD + OTHER), such as type I diabetes mellitus, obesity, dysmorphias, diverse congenital malformations, pancytopenia, or neuroendocrine alterations, which impaired classification as a more distinctive retinal syndrome.

A total of 925 HPO terms were annotated in the cohort from 377 different terms. The most common non-ocular ontology system group was the one comprising neurodevelopmental abnormalities-related terms (Supplementary Table S4 and Fig. 2a). There were no significant differences in the distribution of HPO terms between the fully characterized and uncharacterized cases, although there was slight enrichment of terms related to alterations in the central nervous (CNS), musculoskeletal, and cardiovascular systems in the subgroup of uncharacterized patients (Fig. 2b).

Outcomes and diagnostic yield of genetic testing

Of the 100 probands, 82 retrospective cases were screened in this study using new clinical and/or exome approaches. The workflow used is described in Supplementary Fig. S1. In retrospective cases analyzed by CES, the diagnostic yield was 25% (20/79) (Fig. 3a).

In 34 retrospective cases with inconclusive findings using small gene panels, WES were sequentially performed to analyze an extended 447-gene panel. In addition, three more cases were directly sequenced by WES. The overall diagnostic yield of WES analysis was 43% (16/37; 14 totally and two partially conclusive characterized cases) (Fig. 3a), representing the highest diagnostic yield for retrospective cases. Considering only the groups of well-recognizable syndromes, the diagnostic rate of WES increased to 75% (6/8) (Supplementary Table S5). After the first WES data analysis of the 37 cases, in the 26 cases that remained uncharacterized, a WES reanalysis was conducted using new bioinformatic

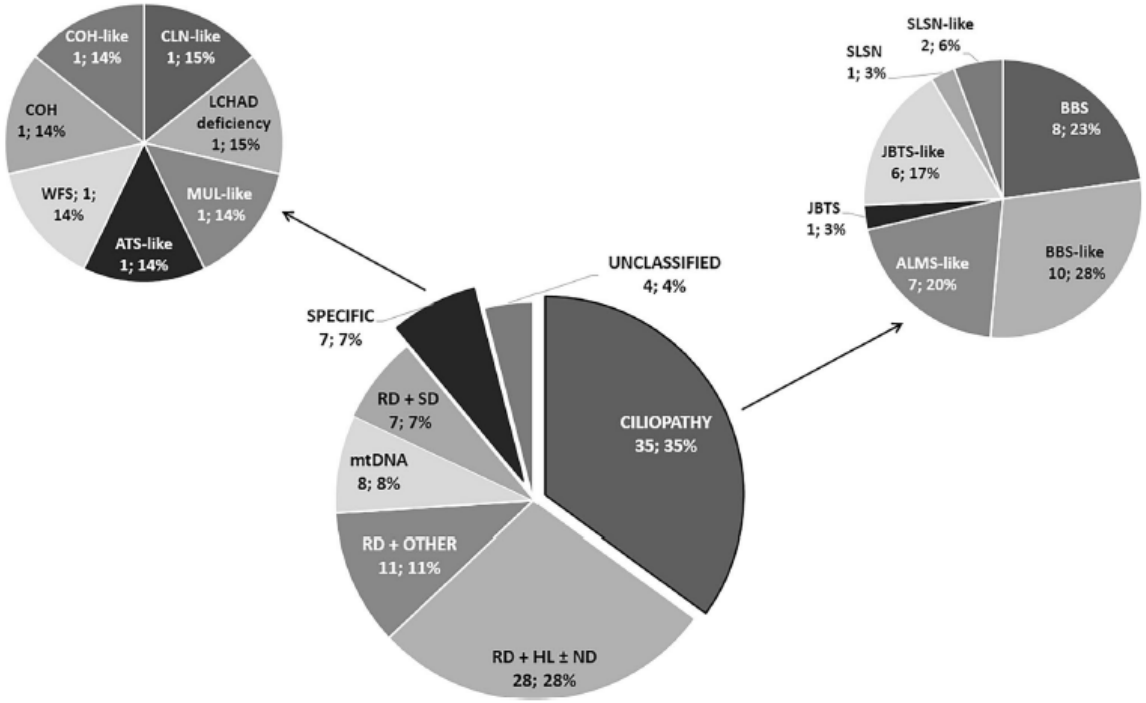


Fig. 1 Presumed a priori diagnosis and phenotypic classification. All the cases were classified according to their phenotype into seven different categories: (i) suspicion of ciliopathy or ciliopathy-like (CILIOPATHY); (ii) suspicion of specific rare disease (SPECIFIC); (iii) RD + hearing loss and/or neurodevelopmental disorder (RD + HL ± ND); (iv) RD + neuropathy or myopathy or a suspicion of mitochondrial DNA disorder (mtDNA); (v) RD + skeletal disorder (RD + SD); (vi) RD + other (RD + OTHER); and (vii) SRDs unclassified without clinical information (UNCLASSIFIED). Subsequently, the patients with suspicion of ciliopathy or ciliopathy-like and specific rare disease were divided into different clinical entities using the pipeline provided in the Supplementary Table S1. *ALMS* Alström syndrome, *ATS* Alport syndrome, *BBS* Bardet-Biedl syndrome, *CLN* ceroid lipofuscinosis, neuronal, *COH* Cohen syndrome, *JBTS* Joubert syndrome, *LCHAD deficiency* long-chain 3-hydroxiacyl-CoA dehydrogenase deficiency, *MUL* Mulibrey nanism, *RCD* rod-cone dystrophy, *RD* retinal dystrophy, *SLSN* Senior–Løken syndrome, *SRD* syndromic retinal diseases, *WFS* Wolfram syndrome

approaches, including variant reannotation with the latest updated tools and hypothesis-free variant prioritization. This reanalysis led to a characterization rate of 19% (5/26), also showing a higher yield for cases with well-recognizable syndromes (50%; 2/4) (Supplementary Table S5). The specific reasons that led to the characterization of cases during reanalysis are detailed in Supplementary Table S6.

In addition, 18 prospective cases were analyzed by means of CES as a first-tier approach, including SNVs and read-depth CNV analysis, with a significant diagnostic rate of 83% (15/18) (Fig. 3a and Supplementary Table S5).

For disease groups, the molecular diagnostic rate is described in Fig. 3b, in which 62% (26/42) of probands suspected to present well-recognizable syndromes (CILIOPATHY and SPECIFIC) were characterized, whereas, for the remaining groups of patients categorized according to the major extra-ocular symptoms, characterization decreased to 45% (26/58). Patients with an a priori suspicion of "CILIOPATHY" have been the most genetically diagnosed, with a detection rate of 66% (23/35).

The overall diagnostic yield in our cohort was 52%. Cases were classified according to their genetic outcome in four different subgroups (Supplementary Fig. S2). First, characterized cases, i.e. those with a fully conclusive molecular diagnosis, including 46 probands with likely causative variants that fully explained the phenotypic presentation and six cases presented likely causative variants that only explained part of the phenotype, but not the other concomitant symptoms. Among the uncharacterized cases, we identified six cases with monoallelic pathogenic variants in a recessive gene that fitted the phenotypic presentation and four probands with inconclusive molecular diagnosis due to the identification of a monoallelic VUS in a plausible gene to explain the phenotype presented. Finally, we did not identify any likely causal or pathogenic variants in 38 probands to date, even after the last data reanalysis performed in 2020 (Supplementary Table S5 and S6).

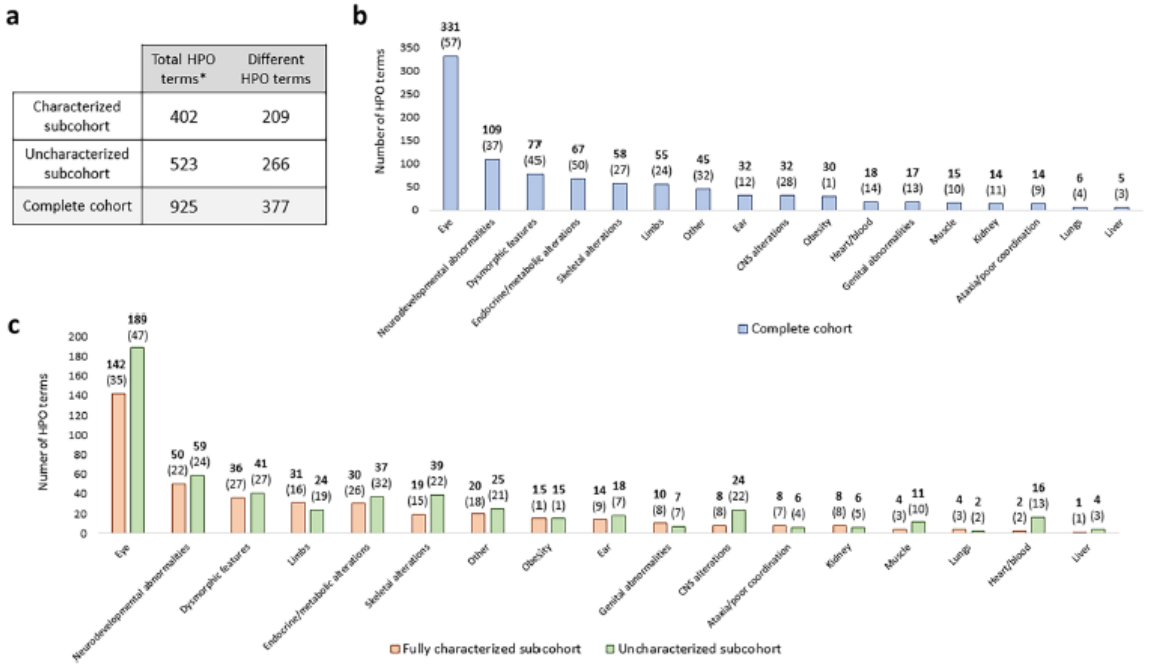


Fig. 2 Summary of the HPO terms grouped by system. **a** Total identified and different HPO terms in the complete cohort and the fully characterized and uncharacterized (partially characterized, monoallelic, and negative cases) subcohorts. *The phenotypic terms without HPO annotation ($n=19$) have been excluded. **b** All the terms identified in the complete cohort were classified. Numbers in bold represent the total HPO terms identified in each category, while the number of different HPO terms appears in brackets. **c** All the terms identified in the fully characterized and the uncharacterized subcohort were classified

Segregation analyses were conducted in 55% of the families (34/62) in which at least one variant was found.

Most of the cases were isolated cases (77%) (Supplementary Table S8). The characterization rate between the familial and sporadic cases showed a trend to be significant ($p=0.0544$).

Mutational spectrum

A total of 75 VUS/likely pathogenic SNVs and 7 CNVs were found in 47 different genes (Supplementary Table S7 and Supplementary Fig. S3). Variants in 16 genes were identified in probands suspected of ciliopathy and three with specific rare diseases, and a case with suspicion of mtDNA disorder was characterized with a pathogenic variant in the mitochondrial *MT-ATP6* gene (MIM *516060). In the remaining four groups, variants in 31 different genes were found.

Among genes in which causative variants have been found, nine have been related to both autosomal dominant and recessive patterns of inheritance. Biallelic variants associated to recessive inheritance were found in five of those genes (*ACO2, PDE6B, PEX6, RDH12*, and *WFS1*) and were confirmed by parental segregation analysis in the 83% of them (five out of six cases). Moreover, four cases showed a monoallelic variant in one of those nine genes (*GLI1, HK1, OTX2* and *RHO*), including one de novo variant (RP-2176) (Supplementary Table S8). In all the cases with autosomal dominant inheritance suspicion, the presence of a second mutated allele (SNV or CNV) in the same gene was excluded and allele frequency and type of mutations were compatible with an autosomal dominant inheritance.

The five most mutated genes were *BBS1* (MIM *209901), *AHI1* (MIM *608894), *MKKS* (MIM *604896), *C8orf37* (MIM *614477), and *VPS13B* (MIM *607817). Moreover, the most prevalent allele in our cohort was the previously described p.Met390Arg in *BBS1* (NM_024649.4), with an allelic frequency of 6% (7/113 identified alleles) in four characterized probands (RP-2634, RP-2846, RP-2996, and RP-3115), occurring in homozygosis 3 times, and once in compound heterozygosis in a case with suspected triallelism, appearing together with a monoallelic variant in *BBS5* (MIM *603650).

The most prevalent mode of inheritance was autosomal recessive (79%), followed by autosomal dominant (9%), X-linked (2%), and mitochondrial (2%) (Fig. 3c). In addition, atypical inheritance presentations were found in 4

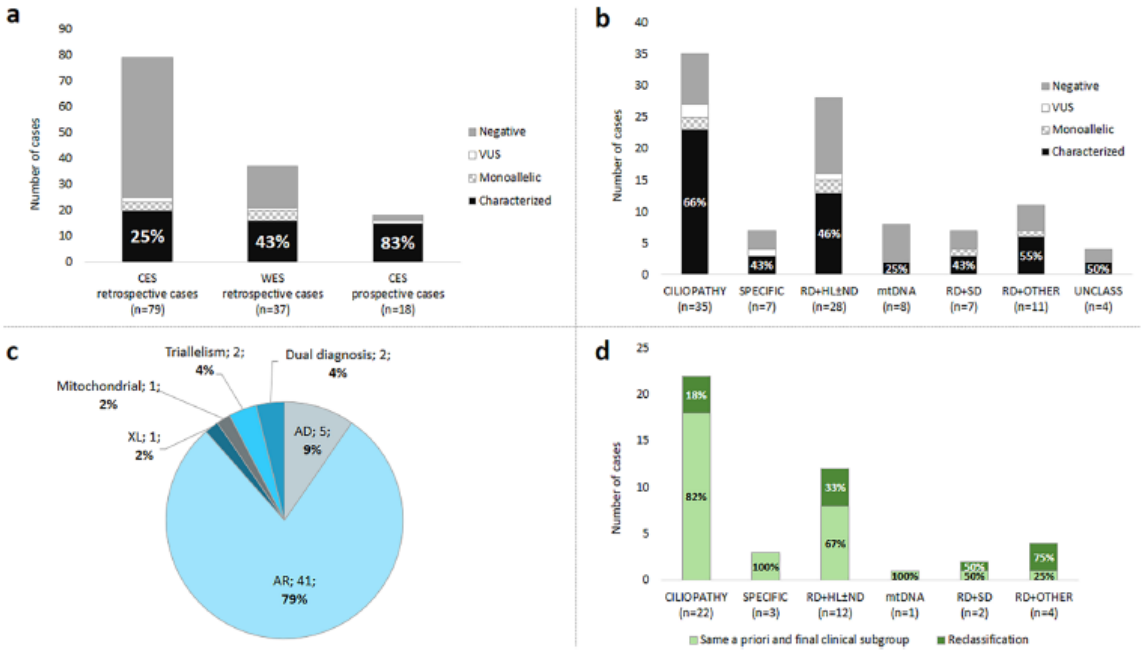


Fig. 3 Molecular results. The genetic results were divided considering the a priori clinical groups: (i) suspicion of ciliopathy or ciliopathy-like (CILIOPATHY); (ii) suspicion of specific rare disease (SPECIFIC); (iii) RD + hearing loss and/or neurodevelopmental disorder (RD + HL ± ND); (iv) RD + neuropathy or myopathy or a suspicion of mitochondrial DNA disorder (mtDNA); (v) RD + skeletal disorder (RD + SD); (vi) RD + other (RD + OTHER); and vii) SRDs unclassified without clinical information (UNCLASS). **a** Diagnostic yields of molecular testing depending on the NGS technology carried out. Cases were divided into retrospective, CES (clinical-exome sequencing) ($n = 79$), using CES after the previous preliminary analysis, and prospective ($n = 18$), CES as first-tier approach. The 34 retrospective cases with inconclusive CES results were screened using WES (whole exome sequencing) plus 3 more cases directly sequenced by WES. **b** Molecular study results by clinical subgroup. **c** Inheritance mode distribution. The fully ($n = 46$) and incompletely ($n = 6$) characterized cases were classified according to the Mendelian inheritance mode of the causative gene and grouped into autosomal dominant (AD), autosomal recessive (AR), or X-linked (XL) trait. Non-Mendelian inheritances (mitochondrial and triallelism) were also considered, as well as the cases with co-occurrence of two different genetic causes. **d** Final classification of the SRD cohort after genetic testing. Cases that were reclassified into another clinical subgroup due to the identification of the causative gene are indicated

families. Interestingly, two suspected triallelic cases (4%) were found in two families (RP-2634 and RP-2966), involving ciliopathy-related genes. We identified a proband (RP-2634) carrying biallelic pathogenic variants (p.Met390Arg and c.951+58C>T) in *BBS1* with a monoallelic *BBS5* variant (p.Arg207His). This last variant did not appear in her affected sibling, who presented a milder non-syndromic phenotype. We additionally found a patient (RP-2966) to be homozygous for a novel splicing variant (c.156-1G>T) in *C8orf37* in combination with a novel monoallelic nonsense variant (p.Cys344*) in *WDPCP* (MIM *613580). In two probands (RP-1018 and RP-1321), a confirmed dual genetic diagnosis encompassing a genomic rearrangement and a monogenic disease was present (4%). First, in an affected girl (RP-1018), the presence of a ~ 4.75 Mb de novo deletion in chromosome X was observed within Xq22.32-22.2 (hg19: chrX:5748782-10477366), involving two OMIM genes, i.e., *NLGN4* and *MID1*, which are associated with Mental retardation, X-linked (MIM #300495) and Opitz GBBB syndrome, type I (MIM #300000), respectively. Although subsequent studies in peripheral blood could not demonstrate skewed inactivation of the X chromosome, this deletion, together with the presence of biallelic *USH2A* (MIM *608400) variants, may explain the phenotype of the patient suffering from early onset retinitis pigmentosa (RP), global developmental delay, tip-toe gait, talipes equinovarus, and dysmorphic features (Supplementary Fig. S4). The proband RP-1321, who presented RP, cochlear malformation, neurodevelopmental alterations, recurrent pneumonia, hypotonia, and limb malformations and carried a likely pathogenic hemizygous missense variant in *NYX* (MIM *300278) and a 1q21.1q21.2 microduplication of 1.2 Mb (Supplementary Fig. S5). This maternally inherited CNV produces a recognized autosomal dominant duplication syndrome with incomplete penetrance and variable expressivity

(MIM #612475) of psychiatric/developmental disorders, articulation abnormalities, and hypotonia.

Final clinical and genetic classification

A total of 46 SRD probands were fully genetically and phenotypically characterized (Supplementary Table S8) following the molecular studies. Those included two previously unclassified SRD probands (RP-1007 and RP-2879), now categorized as BBS and COH. Considering only those patients with a priori diagnosis, 27% (12/44) of cases were reclassified into another clinical subgroup after NGS-based genetic testing thanks to the identification of a clearly causative gene that explained the full phenotype (Supplementary Table S8).

Remarkably, we observed several likely new or extremely rare phenotypic associations for 8 genes in 17% (8/46) of the fully characterized probands (Table 1).

Regarding disease-groups reclassification, characterized probands suspected of well-recognizable syndromes (CILIOPATHY and SPECIFIC) were reclassified less frequently (16%; 4/25) after the molecular studies than the characterized patients grouped according to their non-specific extra-ocular symptoms (42%; 8/19). Interestingly, we obtained a higher clinical accuracy in the group of ciliopathies (82%; 18/22). Reclassification rates above 33% were achieved in the clinical subgroup with RD + HL and/or neurodevelopmental alteration (Fig. 3d).

As detailed in Fig. 4, three cases without a priori suspicion of well-known SRD were found to be carrying likely causal variants in non-syndromic RD-associated genes. In the proband RP-1022, who presented RP, optic neuropathy, and mild unilateral hearing impairment, the homozygous missense variant p.(Ser2983Tyr) in *EYS* (MIM *612424) was found; therefore, this case was reclassified as non-syndromic. Patient RP-0118 had early-onset RP and type 1 diabetes mellitus and carried the heterozygous variant p.(Arg132Trp) in *RHO* (MIM *300023), the most frequent gene in autosomal dominant RP; thus, he was reclassified to non-syndromic. Finally, the proband RP-1175 with early-onset RP and obesity was solved with a homozygous missense variant p.(Leu93Pro) in *RDH12* (MIM *608830) associated with non-syndromic RP. In those three cases, the isolated extra-ocular symptom does not seem to be associated with the genetic cause of the retinal presentation and is likely explained by a non-genetic cause.

After genetic testing, six cases were partially characterized as not completely fitting the expected phenotype linked to the causative gene (Supplementary Table S8). In four cases (RP-0567, RP-1021, RP-1702, and RP-3047), visual alteration was clearly explained by causal variants in early-onset RD genes, i.e., *RPGRIP1* (MIM *605446), *PDE6B* (MIM *180072), *RPGR* (MIM *312610), and *RDH12*. However, other systemic presentations, including myopathic alterations, glomerulonephritis, preaxial polydactyly, genital abnormalities, and dysmorphic features, remained unsolved. On the other hand, the visual phenotype was apparently not related to the genetic findings in two families. In two affected siblings belonging to family RP-0897, we identified two compound heterozygous variants in *GALE*. This recessive gene is associated with mild galactose deficiency (MIM #230350), which seems to explain the fact that the family only presented cognitive delay, hepatomegaly, and splenomegaly. However, GALE deficiency has never been associated with retinal dystrophies (RD). In the proband RP-3055 suffering from a complex presentation of syndromic cone-rod dystrophy, we identified a heterozygous microdeletion of ~ 16.5 Mb on 3q11.2q13.31 (hg19: chr3:97483365-113953480), involving at least 60 OMIM genes, which included *ARL6* (Bardet-Biedl syndrome, MIM #600151), *IMPG2* (retinitis pigmentosa, MIM #613581; and macular dystrophy, vitelliform, MIM #616152), *ZBTB11* (Intellectual developmental disorder, MIM #618383), and *ATP6V1A* (developmental and epileptic encephalopathy, MIM #618012), among others. This microdeletion has been associated with chromosome 3q13.31 deletion syndrome (MIM #615433), thus, it could explain the cognitive impairment of our patient. However, visual findings and other extraocular alterations remain unexplained.

Another six probands presented a monoallelic pathogenic variant in a gene that could fully or partially explain the phenotype. On the one hand, we were able to obtain a partial genetic characterization related with their full (RP-1483 and RP-2001) or partial (RP-0620 and RP-1854) a priori diagnosis in four of them. In addition, on the other two probands carrying previously reported monoallelic *USH2A* variant (RP-0838) and monoallelic *BBS1* and *BBS5* variants (RP-1581) (Sanchez-Navarro et al. 2018), no additional findings were obtained following the reanalysis of NGS data.

Additionally, we found four carriers of monoallelic VUS in a gene related with their phenotype (RP-0249, RP-2184, RP-2394, and RP-2882) (Supplementary Table S8).

Discussion

SRDs are a highly heterogeneous group of ocular diseases characterized by a challenging molecular characterization and clinical management (Shaheen et al. 2016; Tatour and Ben-Yosef 2020). In the literature, it is rather frequent to find studies focused on specific syndromes (Knopp et al. 2015; Vilboux et al. 2017) or single panel-based studies (Shaheen et al. 2016; Jiman et al. 2020), which provide a biased landscape of SRDs. We present the study of a cohort comprising naïve and previously studied cases of well-known syndromes

Table 1 New and rare phenotype–genotype associations among completely and partially characterized cases in the cohort

Case	Initial diagnosis (clinical category)	Gene (variants)	Associated phenotypes (OMIM)	New/rare association	Post-test diagnosis (clinical category)	Observations	PMID
RP-0094	RP + OPA + cataracts + HL (RD + HL)	***ACO2*** (p.Cys592Tyr and p.Arg767Cys)	OPA; ICRD	Hearing impairment (HP: 0000365)	RP + OPA + cataracts + HL (RD + HL)	Gene associated with a wide range of phenotypes, though deafness is highly uncommon	32449285; 32519519
RP-2310	RP + Asperger syndrome (RD + ND)	***ARL13B*** (c.57_59+16dup, homozygosis)	JBTS	Asperger syndrome	RP + ND (RD + ND)	Selective loss of ARL13B reported as one of the mechanisms underlying ASD	28787594
RP-2995	ALMS-like (CILIOPATHY)	***C8orf37*** (p.Asp10Lysfs*12, homozygosis)	RP; CRD; BBS	Sensorineural hearing impairment (HP: 0000407)	Atypical BBS (CILIOPATHY)	New association, since hearing complications are usually produced by chronic otitis media	22713813
RP-1436	SLSN-like (CILIOPATHY)	***IFT140*** (p.Gly1229Val and c.3661-9T>G)	RP; SRTD with/without polydactyly	Absence of skeletal findings	Early-onset RP + PKD (CILIOPATHY)	Patient presented a mild phenotype	–
RP-0132	RP + HL + DI + PD + SD (RD + SD)	***IFT81*** (p.Gln657*, homozygosis)	SRTD with/without polydactyly	Visual features together with skeletal findings	Skeletal ciliopathy (CILIOPATHY)	Visual and skeletal findings have been independently reported in association with *IFT81*, but not both kind of symptoms in the same patient	26275418; 32233951; 32783357
RP-2032	RP + CD + HL + ID (RD + HL ± ND)	***MCOLN1*** (c.878-1G>A, homozygosis)	ML	Hearing impairment (HP: 0000365)	Atypical ML (SPECIFIC)	No previous reports of a relationship between HL and *MCOLN1*	–
RP-1691	LCA + CM (RD + OTHER)	***OTX2*** (p.Ser169*)	MCOPS; CPHD	Atresia of the external auditory canal (HP: 0000413); microtia, third degree (HP: 0011267)	LCA + CM (RD + OTHER)	Absence of the characteristic neuroendocrine alterations or ocular developmental anomalies of the *OTX2*-related disorders	20486942; 29588463

Table 1 (continued)

Case	Initial diagnosis (clinical category)	Gene (variants)	Associated phenotypes (OMIM)	New/rare association	Post-test diagnosis (clinical category)	Observations	PMID
RP-3018	RP + HL (RD + HL)	***PEX6*** (p.Glu439Glyfs*3 and p.Arg876Trp)	HMLR; PBD	Mild phenotype	RP + HL (RD + HL)	Pediatric patient; mandatory clinical reassessment in a few years to monitor the possible development of new symptoms commonly associated with *PEX6*-related disorders, as previously described	32214787

Reference transcripts: NM_001098.3 for *ACO2*, NM_001174150.1 for *ARL13B*, NM_177965.3 for *C8orf37*, NM_014714.4 for *IFT140*, NM_001143779.2 for *IFT81*, NM_020533.3 for *MCOLN1*, NM_001270525.1 for *OTX2*, and NM_000287.4 for *PEX6*

ASD autism spectrum disorders, *BBS* Bardet-Biedl syndrome, *CD* corneal dystrophy, *CM* congenital malformations, *CPHD* pituitary hormone deficiency, combined, *CRD* cone-rod dystrophy, *DFNB* deafness, *DI* diabetes insipidus, *HL* hearing loss, *HMLR* Heimler syndrome, *ICRD* infantile cerebellar-retinal degeneration, *ID* intellectual disability, *JBTS* Joubert syndrome, *MCOPS* microphthalmia, syndromic, *ML* Mucolipidosis, *ND* neurodevelopmental disorder, *OPA* optic atrophy, *PBD* Peroxisome Biogenesis Disorder, *PD* psychiatric disorder, *PKD* polycystic kidney disease, *RP* retinitis pigmentosa, *SRTD* short-rib thoracic dysplasia

and unspecific SRD patients from a single center, focusing on the challenges derived from diagnosis and molecular characterization.

Within the ~ 80 SRDs already described, the most common subgroup is ciliopathies (Tatour and Ben-Yosef 2020), BBS being the most frequent condition representing 36% of our cohort (Perea-Romero et al. 2021); this result is similar to the 25–45% frequencies related to non-Usher syndromic families reported in different populations around the world (Motta et al. 2018; Sharon et al. 2020; Holtan et al. 2020).

Apart from well-defined syndromes, SRDs can appear as the combination of visual alterations along with one or more affected systems, where the most common are those involving the CNS (68%) and the ear (33%) (Yang et al. 2020; Tatour and Ben-Yosef 2020). These data are corroborated by the case distribution in our cohort, with rates of 59% and 28%, respectively.

The overall characterization rate achieved in our study was 52% using mainly targeted gene panel testing but also WES analysis; this rate is similar to that observed in other cohorts (Manara et al. 2019; Jiman et al. 2020), ranging between 55% and 60% (Supplementary Table S9). However, a diagnostic rate of 85% was reported in a cohort formed exclusively by consanguineous families with ciliopathies (Shaheen et al. 2016). A similar yield was observed in our prospective analysis of 18 consecutive cases referred over a 3-year period using clinical-exome sequencing to perform a simultaneous analysis of SNVs and CNVs. Molecular diagnostic rates for SRD are highly variable, ranging from 25 to 66% for specific phenotypic classification. Higher yields were obtained for patients presenting well-recognized syndromes, even when smaller gene panels were used in CES. This highlighted the importance of deep phenotyping in delineating SRDs.

However, the high rates obtained in other patients with less specific systemic presentations clearly indicate the utility of our approach to identify the underlying genetic causes and for differential diagnosis. This is the case for patients with retinal disorders accompanied with an isolated extraocular symptom. In 27% of patients within this category, RD is finally explained by a non-syndromic gene. Moreover, half of the a priori unclassified cases were successfully grouped after molecular testing. Thus, this NGS-based approach helps to improve the prognostic and management of the patients.

WES has been established as the best approach for the study of SRD cases, as it improves molecular diagnosis and understanding due to its efficacy in identifying new variants, phenotype-genotype correlations, and causative genes (Vilboux et al. 2017; Gupta et al. 2017; Boczek et al. 2018; Abu Diab et al. 2019; Jaffal et al. 2019; Cogné et al. 2020).

Several studies described the importance of periodic NGS reanalysis to increase diagnostic rates up to 30% in unsolved

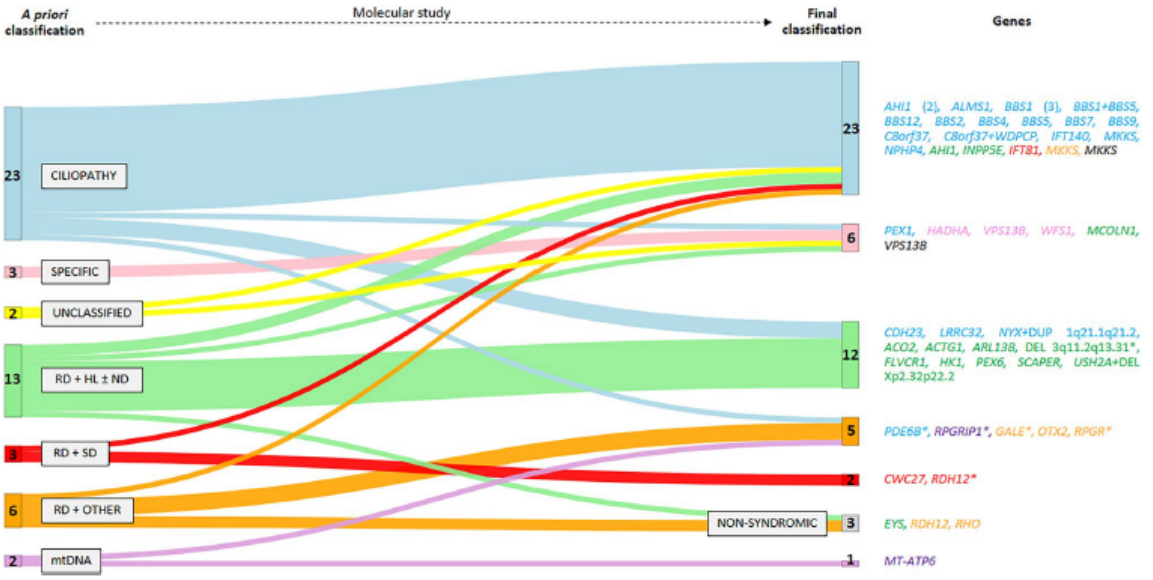


Fig. 4 Sankey chart of the clinical distribution of the characterized cases before and after molecular studies and the results of this analysis. The causative genes appear with the color corresponding to the clinical group to which each case was assigned a priori. Sankey chart was created using the Sankey Diagram Generator by Dénes Csala, based on the Sankey plugin for D3 by Mike Bostock (https://sankey.csaladen.es/; 2014). *Phenotypically partially characterized. *DEL* deletion, *DUP* duplication, *RD* retinal dystrophy, *HL* hearing loss, *mtDNA* mitochondrial DNA, *ND* neurodevelopmental disorder, *SD* skeletal disorder

patients (Wenger et al. 2017; Wright et al. 2018b; Alfares et al. 2018; Ewans et al. 2018; Jalkh et al. 2019; Li et al. 2019; Liu et al. 2019) depending on the spectrum of genetic disorders analyzed, type of NGS, mode of reanalysis, or time period since the first analysis (Supplementary Table S10). Specifically, the WES reanalysis may allow characterization of an additional 30% of patients with WES without the need for whole-genome sequencing (WGS) (Alfares et al. 2018). In our cohort, the WES-related reanalysis yield is 19% due to the application of a hypothesis-free approach in genomic data analysis, the description of a new gene (without known association on first analysis), and the compilation of new data from clinical reassessment of the patient.

According to our data and clinical routine, we suggest carrying out reanalysis of the data from RD cases previously studied by NGS in these circumstances: (1) cases with new clinical or family information; and (2) cases in which a new candidate gene related to clinical presentation is reported in the literature. However, if in the 2 years following first analysis, these circumstances are not met, a reanalysis of NGS data should be undertaken in the light of the large volume of newly published information.

One of the problems related to the use of NGS using large gene panels or hypothesis-free approaches is the identification of VUS and the underlying difficulties associated to interpretation, since they are frequently the only positive finding in molecular testing (Frebourg 2014). In our cohort, 4% of cases were carriers of monoallelic VUS in a recessive gene related with their phenotypic presentation. Similarly, another six patients showed monoallelic class 4/5 variants in recessive genes found in this or previous studies (Sanchez-Navarro et al. 2018). To solve those cases, it is highly recommended to periodically reassess the clinical significance of the VUS and also carry out further WGS, in order to search for a potential second pathogenic allele in untargeted regions such as deep-intronic or regulatory elements.

Ninety-eight percent of the unsolved cases in this cohort underwent targeted panel testing, and WES analysis was not carried out in only 42% of them. In all these patients, particularly in unsolved WES patients, WGS will be the best option to identify potential novel genes/variants or non-coding mutations that remain to be determined.

To date, many reports have highlighted the existence of co-occurrence of IRD with a rare non-ocular phenotype in SRD patients, this being an important matter in clinical management and follow-up (Ehrenberg et al. 2019). The incidence of a proven dual genetic diagnosis has been established in the range of 4.6% and 4.9% in previous reports (Yang et al. 2014; Posey et al. 2017), similar to our 4.3% value. In our cohort, dual genetic diagnosis was obtained by the combination of genomic rearrangement and a monogenic disease produced by a non-syndromic IRD gene (RP-1018

and RP-1321); this finding is in line with a previously reported case by our group of a proband solved with biallelic variants in *USH2A* (USH) and a de novo microdeletion at 17q21.31 (Koolen-de Vries syndrome, MIM #610443) (Sanchez-Navarro et al. 2018). Moreover, our dual genetic diagnostic rate could be underestimated owing to the fact that there are six probands with partial diagnosis (RP-0567, RP-0897, RP-1021, RP-1702, RP-3047, and RP-3055) that remain to be fully characterized due to the likelihood of an undiscovered second genetic disease. So, in partially characterized cases with genes associated with IRDs, genomic rearrangements might be studied, while in probands, in which only SRD genes have been analyzed, both IRD genes and large CNVs should be screened.

As new research evidence is published, new genotype–phenotype associations are discovered in SRDs, delineating its phenotypic spectrum and improving molecular diagnosis, as well as revealing the value of a robust previous clinical analysis (Abu Diab et al. 2019; Shamseldin et al. 2020). Moreover, in 8 of the characterized cases, some of the clinical features of the patient did not completely fit with the known syndrome related to the causative gene found. This may be either a fortuitous association or an expansion of the already known phenotype. For instance, a priori RP-2995 was suspected of ALMS-like, but after the molecular characterization, a homozygous frameshift variant in *C8orf37* was found. This therefore presents an atypical BBS with sensorineural hearing loss, a rare finding in this syndrome due to the fact that hearing complications are usually produced by chronic otitis media (Forsythe and Beales 2013). Sensorineural hearing impairment has been described once, in a genetically uncharacterized patient (Singh et al. 2017); our study, however, marks the first time that the reported patient has been molecularly characterized. Another example of a new association was found in the patient RP-2310, which was characterized with a homozygous splicing variant in *ARL13B* (MIM *608922). Normally, the gene *ARL13B* has been associated with JBTS and the ciliopathy spectrum; in this case, RP-2310 had rod-cone dystrophy and Asperger syndrome, which is part of the autism spectrum disorders (ASD). Selective loss of ciliary ARL13B in interneurons has been shown to alter their morphology and synaptic connectivity, leading to the disturbance of the excitatory/inhibitory excitatory balance, which is one of the mechanisms underlying neurodevelopmental disorders such as ASD (Guo et al. 2017).

In summary, this study reports findings on a heterogeneous SRD cohort and its genetic diagnostic management, expanding the phenotypical spectrum in these diseases and pointing to the importance of an extensive and accurate clinical analysis together with an adequate genetic study and choice of the NGS technology according to the specific characteristics of each case. We can conclude that HPO terms are a suitable tool for phenotypic description in SRD patients; however, based on our observations, subsequent application of established clinical criteria is highly recommendable. Furthermore, the use of well-designed and curated virtual panels leads to the best characterization rates, regardless of the NGS approach used (CES or WES). Bioinformatic analysis should include CNV evaluation and periodical reanalysis. This approach increases the diagnostic yield from 41 to 52%. Besides, it is important to note that the presence of some extra-ocular findings together with a visual disease is not always due to a single genetic cause. In our cohort there are SRD cases explained by (1) non-syndromic IRD with a non-genetic disease and (2) two different genetic diseases in the same patient. Moreover, the dual genetic diagnoses presented here were mainly due to the co-occurrence of non-syndromic IRD together with a genomic rearrangement. Thus, not only SRD genes should be considered, but also large CNVs should be screened in presumed SRD cases. Finally, inclusion of non-syndromic IRD genes in the study of SRD cases may help to uncover new phenotypic associations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00439-021-02343-7.

Acknowledgements We want to thank the patients and their families for their cooperation. We are very grateful to Olga Zurita, Lorena Ondo, Andrea Martinez and Cristina Villaverde for their invaluable technical assistance. We also thank Oliver Shaw for English editing.

Author contributions Conceptualization: IPR and CA; formal analysis: IPR, ISN, AAF, IMM, MJTT, RLR, MRdA, MC, CR and CA; funding acquisition: CA; investigation: IPR, FBK, ISN, ILS, STS, HH, BGS, PM, MC, CR and CA; methodology: IPR, FBK and CA; project administration: CA; resources: FBK, ILS, STS, RLR, CR and CA; software: IFI, RR, MQ, HH, PM and CR; supervision: CR and CA; validation: IPR, CR and CA; visualization: IPR and CA; writing—original draft: IPR and CA; and writing—review & editing: IPR, FBK, ISN, ILS, STS, AAF, IMM, MJTT, RLR, MRdA, IFI, RR, MQ, HH, BGS, PM, MC, CR and CA.

Funding This work was supported by the Instituto de Salud Carlos III (ISCIII) of the Spanish Ministry of Health (FIS; PI16/00425 and PI19/00321), Centro de Investigación Biomédica en Red Enfermedades Raras (CIBERER, 06/07/0036), IIS-FJD BioBank (PT13/0010/0012), Comunidad de Madrid (CAM, RAREGenomics Project, B2017/BMD-3721), European Regional Development Fund (FEDER), the Organización Nacional de Ciegos Españoles (ONCE), Ramón Areces Foundation (4019/012), Conchita Rábago Foundation and the University Chair UAM-IIS-FJD of Genomic Medicine. IPR is supported by a PhD fellowship from the predoctoral Program from ISCIII (FI17/00192), IFI is supported by a grant from the Comunidad de Madrid (CAM, PEJ-2017-AI/BMD7256) and PM and MC are supported by a Miguel Servet program contract from ISCIII (CP16/00116 and CPII17/00006, respectively). The funders played no role in study design, data collection, data analysis, manuscript preparation and/or publication decisions.

Availability of data and materials NGS data are available in public, open access repositories such as the European Genome-Phenome

Archive (EGA; https://www.ebi.ac.uk/ega/home; EGAD00001005746, EGAD00001005498 and EGAD00001007022), RD-Connect (https://rd-connect.eu/) and the Collaborative Spanish Variant Server (CSVS; http://csvs.babelomics.org/) as aggregated data. The rest of the data are available upon reasonable request.

Code availability An updated version of the bioinformatic tools of pipeline 1 and 2 is available online at https://github.com/TBLabFJD/VariantCallingFJD.

Declarations

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics approval This research has been reviewed and approved by the FJD Research Ethics Committee under approval number PIC172-20_FJD and it fulfils all the principles of the Declaration of Helsinki and its revisions.

Consent to participate All patients, or their legal guardians when necessary, signed the written informed consent forms before entering the study.

References

Abu Diab A, AlTalbishi A, Rosin B et al (2019) The combination of whole-exome sequencing and clinical analysis allows better diagnosis of rare syndromic retinal dystrophies. Acta Ophthalmol 97:e877–e886. https://doi.org/10.1111/aos.14095

Alfares A, Aloraini T, Subaie LA et al (2018) Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. Genet Med 20:1328–1333. https://doi.org/10.1038/gim.2018.41

Argyriou C, D'Agostino MD, Braverman N (2016) Peroxisome biogenesis disorders. Transl Sci Rare Dis 1:111–144. https://doi.org/10.3233/TRD-160003

Ayuso C, Millan JM (2010) Retinitis pigmentosa and allied conditions today: a paradigm of translational research. Genome Med 2:34. https://doi.org/10.1186/gm155

Boczek NJ, Hopp K, Benoit L et al (2018) Characterization of three ciliopathy pedigrees expands the phenotype associated with biallelic C2CD3 variants. Eur J Hum Genet 26:1797–1809. https://doi.org/10.1038/s41431-018-0222-3

Castro-Sánchez S, Álvarez-Satta M, Cortón M et al (2015) Exploring genotype-phenotype relationships in Bardet-Biedl syndrome families. J Med Genet 52:503–513. https://doi.org/10.1136/jmedgenet-2015-103099

Chiang JP, Trzupek K (2015) The current status of molecular diagnosis of inherited retinal dystrophies. Curr Opin Ophthalmol 26:346–351. https://doi.org/10.1097/ICU.0000000000000185

Cogné B, Latypova X, Senaratne LDS et al (2020) Mutations in the kinesin-2 motor KIF3B cause an autosomal-dominant ciliopathy. Am J Hum Genet 106:893–904. https://doi.org/10.1016/j.ajhg.2020.04.005

Ehrenberg M, Weiss S, Orenstein N et al (2019) The co-occurrence of rare non-ocular phenotypes in patients with inherited retinal degenerations. Mol vis 25:691–702

Ewans LJ, Schofield D, Shrestha R et al (2018) Whole-exome sequencing reanalysis at 12 months boosts diagnosis and is cost-effective when applied early in Mendelian disorders. Genet Med 20:1564–1574. https://doi.org/10.1038/gim.2018.39

Forsythe E, Beales PL (2013) Bardet-Biedl syndrome. Eur J Hum Genet 21:8–13. https://doi.org/10.1038/ejhg.2012.115

Frebourg T (2014) The challenge for the next generation of medical geneticists. Hum Mutat 35:909–911. https://doi.org/10.1002/humu.22592

Galbis-Martínez L, Blanco-Kelly F, García-García G et al (2021) Genotype-phenotype correlation in patients with Usher syndrome and pathogenic variants in MYO7A: implications for future clinical trials. Acta Ophthalmol. https://doi.org/10.1111/aos.14795

Gazzo AM, Daneels D, Cilia E et al (2016) DIDA: a curated and annotated digenic diseases database. Nucleic Acids Res 44:D900-907. https://doi.org/10.1093/nar/gkv1068

Guo J, Otis JM, Higginbotham H et al (2017) Primary cilia signaling shapes the development of interneuronal connectivity. Dev Cell 42:286-300.e4. https://doi.org/10.1016/j.devcel.2017.07.010

Gupta S, Chatterjee S, Mukherjee A, Mutsuddi M (2017) Whole exome sequencing: uncovering causal genetic variants for ocular diseases. Exp Eye Res 164:139–150. https://doi.org/10.1016/j.exer.2017.08.013

Hanany M, Rivolta C, Sharon D (2020) Worldwide carrier frequency and genetic prevalence of autosomal recessive inherited retinal diseases. Proc Natl Acad Sci USA 117:2710–2716. https://doi.org/10.1073/pnas.1913179117

Holtan JP, Selmer KK, Heimdal KR, Bragadóttir R (2020) Inherited retinal disease in Norway—a characterization of current clinical and genetic knowledge. Acta Ophthalmol 98:286–295. https://doi.org/10.1111/aos.14218

Jaffal L, Joumaa WH, Assi A et al (2019) Next generation sequencing identifies five novel mutations in Lebanese patients with Bardet-Biedl and Usher syndromes. Genes (Basel). https://doi.org/10.3390/genes10121047

Jalkh N, Corbani S, Haidar Z et al (2019) The added value of WES reanalysis in the field of genetic diagnosis: lessons learned from 200 exomes in the Lebanese population. BMC Med Genomics 12:11. https://doi.org/10.1186/s12920-019-0474-y

Jiman OA, Taylor RL, Lenassi E et al (2020) Diagnostic yield of panel-based genetic testing in syndromic inherited retinal disease. Eur J Hum Genet 28:576–586. https://doi.org/10.1038/s41431-019-0548-5

Johansson LF, van Dijk F, de Boer EN et al (2016) CoNVaDING: single exon variation detection in targeted NGS data. Hum Mutat 37:457–464. https://doi.org/10.1002/humu.22969

Knopp C, Rudnik-Schöneborn S, Eggermann T et al (2015) Syndromic ciliopathies: from single gene to multi gene analysis by SNP arrays and next generation sequencing. Mol Cell Probes 29:299–307. https://doi.org/10.1016/j.mcp.2015.05.008

Köhler S, Schulz MH, Krawitz P et al (2009) Clinical diagnostics in human genetics with semantic similarity searches in ontologies. Am J Hum Genet 85:457–464. https://doi.org/10.1016/j.ajhg.2009.09.003

Lee K, Garg S (2015) Navigating the current landscape of clinical genetic testing for inherited retinal dystrophies. Genet Med 17:245–252. https://doi.org/10.1038/gim.2015.15

Li J, Gao K, Yan H et al (2019) Reanalysis of whole exome sequencing data in patients with epilepsy and intellectual disability/mental retardation. Gene 700:168–175. https://doi.org/10.1016/j.gene.2019.03.037

Liu P, Meng L, Normand EA et al (2019) Reanalysis of clinical exome sequencing data. N Engl J Med 380:2478–2480. https://doi.org/10.1056/NEJMc1812033

Manara E, Paolacci S, D'Esposito F et al (2019) Mutation profile of BBS genes in patients with Bardet-Biedl syndrome: an Italian study. Ital J Pediatr 45:72. https://doi.org/10.1186/s13052-019-0659-1

Martin-Merida I, Avila-Fernandez A, Del Pozo-Valero M et al (2019) Genomic landscape of sporadic retinitis pigmentosa: findings from 877 Spanish cases. Ophthalmology 126:1181–1188. https://doi.org/10.1016/j.ophtha.2019.03.018

Matthijs G, Souche E, Alders M et al (2016) Guidelines for diagnostic next-generation sequencing. Eur J Hum Genet 24:2–5. https://doi.org/10.1038/ejhg.2015.226

Mockel A, Perdomo Y, Stutzmann F et al (2011) Retinal dystrophy in Bardet-Biedl syndrome and related syndromic ciliopathies. Prog Retin Eye Res 30:258–274. https://doi.org/10.1016/j.preteyeres.2011.03.001

Motta FL, Martin RP, Filippelli-Silva R et al (2018) Relative frequency of inherited retinal dystrophies in Brazil. Sci Rep 8:15939. https://doi.org/10.1038/s41598-018-34380-0

Perea-Romero I, Gordo G, Iancu IF et al (2021) Genetic landscape of 6089 inherited retinal dystrophies affected cases in Spain and their therapeutic and extended epidemiological implications. Sci Rep 11:1526. https://doi.org/10.1038/s41598-021-81093-y

Piñeiro-Gallego T, Cortón M, Ayuso C et al (2012) Molecular approach in the study of Alström syndrome: analysis of ten Spanish families. Mol vis 18:1794–1802

Platt FM, d'Azzo A, Davidson BL et al (2018) Lysosomal storage diseases. Nat Rev Dis Primers 4:27. https://doi.org/10.1038/s41572-018-0025-4

Posey JE, Harel T, Liu P et al (2017) Resolution of disease phenotypes resulting from multilocus genomic variation. N Engl J Med 376:21–31. https://doi.org/10.1056/NEJMoa1516767

Quinodoz M, Peter VG, Bedoni N et al (2021) AutoMap is a high performance homozygosity mapping tool using next-generation sequencing data. Nat Commun 12:518. https://doi.org/10.1038/s41467-020-20584-4

Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424. https://doi.org/10.1038/gim.2015.30

Sadagopan KA (2017) Practical approach to syndromic pediatric retinal dystrophies. Curr Opin Ophthalmol 28:416–429. https://doi.org/10.1097/ICU.0000000000000404

Sanchez-Navarro I, da SilvaBlanco-Kelly RJLF et al (2018) Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. Sci Rep 8:5285. https://doi.org/10.1038/s41598-018-23520-1

Shaheen R, Szymanska K, Basu B et al (2016) Characterizing the morbid genome of ciliopathies. Genome Biol 17:242. https://doi.org/10.1186/s13059-016-1099-5

Shamseldin HE, Shaheen R, Ewida N et al (2020) The morbid genome of ciliopathies: an update. Genet Med 22:1051–1060. https://doi.org/10.1038/s41436-020-0761-1

Sharon D, Ben-Yosef T, Goldenberg-Cohen N et al (2020) A nationwide genetic analysis of inherited retinal diseases in Israel as assessed by the Israeli inherited retinal disease consortium (IIRDC). Hum Mutat 41:140–149. https://doi.org/10.1002/humu.23903

Singh MK, Shrinkhal N, Pradhan S, Chakraborty P (2017) Bardet Biedl syndrome—a report of two cases with otolaryngologic symptoms. J Clin Diagn Res 11:ND01–ND02. https://doi.org/10.7860/JCDR/2017/24499.9466

Sundaram V, Moore AT, Ali RR, Bainbridge JW (2012) Retinal dystrophies and gene therapy. Eur J Pediatr 171:757–765. https://doi.org/10.1007/s00431-011-1615-2

Tatour Y, Ben-Yosef T (2020) Syndromic inherited retinal diseases: genetic, clinical and diagnostic aspects. Diagnost (Basel). https://doi.org/10.3390/diagnostics10100779

Tatour Y, Sanchez-Navarro I, Chervinsky E et al (2017) Mutations in SCAPER cause autosomal recessive retinitis pigmentosa with intellectual disability. J Med Genet 54:698–704. https://doi.org/10.1136/jmedgenet-2017-104632

Van Cauwenbergh C, Van Schil K, Cannoodt R et al (2017) arrEYE: a customized platform for high-resolution copy number analysis of coding and noncoding regions of known and candidate retinal dystrophy genes and retinal noncoding RNAs. Genet Med 19:457–466. https://doi.org/10.1038/gim.2016.119

Vilboux T, Doherty DA, Glass IA et al (2017) Molecular genetic findings and clinical correlations in 100 patients with Joubert syndrome and related disorders prospectively evaluated at a single center. Genet Med 19:875–882. https://doi.org/10.1038/gim.2016.204

Wenger AM, Guturu H, Bernstein JA, Bejerano G (2017) Systematic reanalysis of clinical exome data yields additional diagnoses: implications for providers. Genet Med 19:209–214. https://doi.org/10.1038/gim.2016.88

Werdich XQ, Place EM, Pierce EA (2014) Systemic diseases associated with retinal dystrophies. Semin Ophthalmol 29:319–328. https://doi.org/10.3109/08820538.2014.959202

Wright CF, Fitzgerald TW, Jones WD et al (2015) Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. Lancet 385:1305–1314. https://doi.org/10.1016/S0140-6736(14)61705-0

Wright CF, FitzPatrick DR, Firth HV (2018a) Paediatric genomics: diagnosing rare disease in children. Nat Rev Genet 19:253–268. https://doi.org/10.1038/nrg.2017.116

Wright CF, McRae JF, Clayton S et al (2018b) Making new genetic diagnoses with old data: iterative reanalysis and reporting from genome-wide data in 1,133 families with developmental disorders. Genet Med 20:1216–1223. https://doi.org/10.1038/gim.2017.246

Yang Y, Muzny DM, Xia F et al (2014) Molecular findings among patients referred for clinical whole-exome sequencing. JAMA 312:1870–1879. https://doi.org/10.1001/jama.2014.14601

Yang X-R, Benson MD, MacDonald IM, Innes AM (2020) A diagnostic approach to syndromic retinal dystrophies with intellectual disability. Am J Med Genet C Semin Med Genet 184:538–570. https://doi.org/10.1002/ajmg.c.31834

Supplementary Table S1. Clinical classification of patients with syndromic retinal diseases. Probands were classified in 7 categories. For patients with a well-recognizable SRD (i and ii), major, minor and supportive signs associated to each clinical entity and diagnostic criteria used to classify cases are indicated. The remainder ungrouped cases were classified into 5 additional categories (iii to vii), using a classification based on Human Phenotype Ontology (HPO) terms for the main extra-ocular symptoms. In a patient, the main HPO terms may be accompanied by any other clinical feature. Underlined terms corresponded to the more general parent term.

Category	Disease	Major criteria (MC)	Minor criteria (mc)	Supportive evidence (SE)	Disease diagnostic criteria	Disease-like diagnostic criteria
i) Ciliopathy (CILIOPATHY)	**ALMS** (Marshall et al. 2007)	- Vision problems: nystagmus, cone/cone-rod dystrophy, rod-cone dystrophy, blindness	- Obesity, type II diabetes mellitus and/or insulin resistance - Sensorineural hearing impairment - Dilated cardiomyopathy - Decreased liver function - Renal insufficiency - Male hypogonadism. Females: menstrual irregularities and/or abnormal circulating androgen level - Short stature	- Global developmental delay - Hypothyroidism - Growth hormone deficiency - Hyperlipidemia - Scoliosis - *Pes planus* - Normal digits[a] - Alopecia - Hypertension - Recurrent pneumonia - Recurrent urinary tract infections, abnormality of the urinary system	a) 2 MC and 2 mc or b) 1 MC and 4 mc	1 MC, 2 mc and 2 SE
	BBS (Forsythe and Beales 2013)	- Rod-cone dystrophy - Postaxial polydactyly - Obesity - Abnormality of the female and male genitalia - Abnormality of the kidney - Specific learning disability, intellectual disability	- Delayed speech and language development - Global developmental delay - Type II diabetes mellitus - Abnormality of the teeth - Abnormal heart morphology - Brachydactyly syndrome, syndactyly - Ataxia, poor coordination - Anosmia, hyposmia	- Chronic otitis media - Aganglionic megacolon (Hirschprung disease)	a) 4 MC or b) 3 MC and 2 mc	a) At least, 3 MC and 1 mc or b) less MC, but including postaxial polydactyly
	JBTS (Parisi et al. 2007)	- Molar tooth sign on MRI - Infantile muscular hypotonia	- Abnormal eye movements: oculomotor apraxia, nystagmus - Irregular respiration	- Scoliosis - Seizures - Ataxia	3 MC	a) 2 MC and 2 mc or b) 1 MC and 3 mc

		- Intellectual disability, global developmental delay	- Encephalocele - Polymicrogyria - Other CNS findings: agenesis of corpus callosum, heterotopia, Dandy-Walker malformation, cortical dysplasia - Vision problems: retinal dystrophy, congenital blindness - Polycystic kidney dysplasia - Nephronophthisis - Polydactyly			
	SLSN (Parisi et al. 2007)	- Retinal dystrophy - Nephronophthisis	- Molar tooth sign on MRI - Muscular hypotonia - Intellectual disability, global developmental delay - Abnormal eye movements: oculomotor apraxia, nystagmus - Encephalocele - Hepatic fibrosis	N/A	3 MC	Retinal dystrophy and any renal abnormality
ii) Non-ciliary specific SRD (SPECIFIC)	**ATS** (Kashtan 1993; Nozu et al. 2019)	- Renal involvement: hematuria, proteinuria, chronic kidney disease	- Bilateral sensorineural hearing impairment - Ocular abnormalities: anterior lenticonus, retinal flecks, posterior subcapsular cataract - Diffuse leiomyomatosis	- Aortic dilatation	1 MC and 2 mc	1 MC and 1 mc
	CLN (Kohlschütter et al. 2019)	- Seizures, epilepsy - Dementia - Retinopathy - Motor function impairment: involuntary movements, myoclonus, ataxia, spasticity	N/A	N/A	4 MC	At least, 2 MC

	- Morphological abnormality of the CNS: accumulation of autofluorescent lipopigment				
COH (Wang et al. 1993)	- Retinal dystrophy (childhood), severe myopia (progressive) - Microcephaly - Intellectual disability, global developmental delay - Joint hypermobility - Childhood-onset truncal obesity - Abnormal social behaviour - Neutropenia - Typical COH syndrome facial gestalt: thick hair and eyebrows, long eyelashes, downslanted palpebral fissures, broad nasal tip, smooth and short philtrum, facial hypotonia	- Muscular hypotonia - Short stature - Small and narrow hand and foot	- Growth hormone deficiency - Chronic otitis media - High-pitched and weak cry - Oral ulcers, gingival overgrowth - Oral ulcers, gingival overgrowth - Musculoskeletal features: kyphosis, scoliosis, pes planus	6 MC	4 MC and 1 mc
LCHAD deficiency (den Boer et al. 2002)	- Laboratory findings: long chain 3 hydroxyacyl CoA dehydrogenase deficiency	- Cardiomyopathy - Hepatomegaly - Hypoglycemia - Muscular hypotonia - Pigmentary retinopathy	N/A	Laboratory findings	N/A
MUL (Karlberg et al. 2004)	- Intrauterine growth retardation, postnatal growth retardation or short stature	- High pitched voice - Hepatomegaly - Nevus - Fibrous dysplasia of long bone[a]	- Ventriculomegaly - Dysarthria - Muscular hypotonia - Abnormal heart morphology	a) 3 MC and 1 mc or b) 2 MC and 3 mc	3 MC and 4 SE

		- Radiological findings: thickened cortex of long bones/slender long bones with narrow diaphyses or J-shaped *sella turcica* - Ocular findings: pigmentary retinopathy - Craniofacial features: dolichocephaly, triangular face, high and broad forehead and *telecanthus*				
	WFS (Tranebjærg et al. 1993)	- Diabetes mellitus (juvenile-onset) - Optic atrophy (juvenile-onset)	- High-frequency sensorineural hearing impairment - Truncal or gait ataxia - Dementia, intellectual disability - Psychiatric disease - Neurogenic bladder - Cardiomyopathy, abnormal heart morphology - Endocrine alterations: central diabetes insipidus, hypothyroidism, growth delay, delayed puberty, hypogonadism (in males)	- Other ophthalmologic findings: cataract, pigmentary retinopathy, nystagmus - Apnoea - Gastrointestinal dysmotility	2 MC and, at least, 1 mc (normally, hearing impairment)	a) 2 MC or b) 1 MC and 1 mc or additional SE
Category			**Main HPO terms**			
iii) RD + HL and/or ND (RD+HL±ND)			Hearing impairment (HP: 0000365) Neurodevelopmental abnormality (HP: 0012759): neurodevelopmental delay (HP: 0012758) Abnormality of nervous system physiology (HP: 0012638): abnormality of higher mental function (HP: 0011446), morphological abnormality of the central nervous system (HP: 0002011), abnormality of movement (HP: 0100022), ataxia (HP: 0001251), seizures (HP: 0001250)			

iv) Neuropathy, myopathy, or suspicion of mtDNA disorder (mtDNA)	Abnormality of metabolism/homeostasis (HP: 0001939): abnormality of carbohydrate metabolism/homeostasis (HP: 0011013), abnormality of lipid metabolism (HP: 0003119), abnormality of nitrogen compound homeostasis (HP: 0004364), abnormality of the mitochondrion (HP: 0012103) Abnormality of the musculature (HP: 0003011): abnormality of muscle physiology (HP: 0011804), abnormal muscle tone (HP: 0003808) Abnormality of coordination (HP: 0011443): poor motor coordination (HP: 0002275), incoordination (HP: 0002311)
v) RD + SD (RD+SD)	Abnormality of skeletal morphology (HP: 0011842): abnormal bone structure (HP: 0003330), abnormal axial skeleton morphology (HP: 0009121), abnormal appendicular skeleton morphology (HP: 0011844), preaxial polydactyly (HP: 0100258), abnormal joint morphology (HP: 0001367) Growth delay (HP: 0001510)
vi) RD + other (RD+OTHER)	Abnormality of the endocrine system (HP: 0000818): type I diabetes mellitus (HP: 0100651) Obesity (HP: 0001513) Abnormality of the cardiovascular system (HP: 0001626): abnormal heart morphology (HP: 0001627), abnormality of the vasculature (HP: 0002597) Abnormal genital system morphology (HP: 0012243) Abnormality of blood and blood-forming tissues (HP: 0001871) Other terms not included in the rest of categories
vii) Unclassified due to the lack of clinical information (UNCLASS/UNCLASSIFIED)	N/A

ALMS, Alström syndrome; ATS, Alport syndrome; BBS, Bardet-Bield syndrome; CLN: ceroid lipofuscinosis, neuronal; CNS, central nervous system; COH, Cohen syndrome; HL: hearing loss; JBTS, Joubert syndrome; LCHAD deficiency: long-chain 3-hydroxiacyl-CoA dehydrogenase deficiency; MRI, magnetic resonance imaging; mtDNA: mitochondrial DNA; MUL: Mulibrey nanism; N/A: non-available; ND: neurodevelopmental disorder; RD: retinal dystrophy; SD: skeletal disorder; SLSN, Senior-Løken syndrome; WFS, Wolfram syndrome. [a] Clinical entity without HPO term

Supplementary Table S2. Summary of the list of genes included on each analysis.

Name	Technique	Genes included
136-gene virtual panel	CES	*ABCC6, ABHD12, ACBD5, ADAMTS18, ADGRV1, AHI1, ALMS1, ARL13B, ARL3, ARL6, ATXN7, B9D1, B9D2, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, C2ORF71, C5ORF42, C8orf37, CC2D2A, CDH23, CENPF, CEP164, CEP290, CEP41, CIB2, CLN3, CLN5, CLN6, CLN8, CLRN1, CNNM4, COL11A1, COL2A1, COL9A1, COL9A2, COL9A3, CSPP1, CTSD, CTSF, DNAJC5, DYNC2H1, FAM161A, FLVCR1, GLIS2, GNPTG, GRN, HARS, IFT140, IFT172, IFT27, IFT57, IFT80, IFT81, INPP5E, INVS, IQCB1, JAG1, KCNJ13, KIF11, KIF7, LCA5, LZTFL1, MAK, MFSD8, MKKS, MKS1, MTTP, MYO7A, NEK8, NOTCH2, NPHP1, NPHP3, NPHP4, OFD1, OTX2, PANK2, PCDH15, PCYT1A, PDE6D, PDZD7, PEX1, PEX2, PEX7, PGK1, PHYH, PLK4, POC1B, PPT1, PRPS1, RDH11, RP1, RP1L1, RP2, RPGR, RPGRIP1, RPGRIP1L, SDCCAG8, SLC41A1, SLC9A6, TCTN1, TCTN2, TCTN3, TMEM138, TMEM216, TMEM231, TMEM237, TMEM67, TOPORS, TPP1, TREX1, TRIM32, TRPM1, TTC21B, TTC8, TTPA, TUB, TULP1, USH1C, USH1G, USH2A, VCAN, VPS13B, WDPCP, WDR19, WDR34, WDR60, WFS1, XPNPEP3, ZNF423*
377-gene virtual panel	CES	*ABCA4, ABCB6, ABCC6, ABHD12, ACBD5, ACTB, ADAM9, ADAMTS10, ADAMTS17, ADAMTS18, ADAMTSL4, ADGRV1, ADIPOR1, AFG3L2, AGK, AHI1, AHR, AIPL1, ALDH3A2, ALMS1, ALX1, ANTXR1, AP3B1, ARL13B, ARL3, ARL6, ATF6, ATOH7, ATXN7, B3GLCT, B9D1, B9D2, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BCOR, BEST1, BFSP1, BFSP2, BLOC1S6, BMP4, BMP7, C2orf71, C5orf42, C8orf37, CA4, CABP4, CACNA1F, CACNA2D4, CC2D2A, CDH23, CDH3, CDHR1, CDON, CEP164, CEP290, CEP41, CERKL, CHM, CHMP4B, CHRDL1, CIB2, CISD2, CLN3, CLN5, CLN6, CLN8, CLRN1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL11A1, COL11A2, COL18A1, COL2A1, COL4A1, COL4A2, COL9A1, COL9A2, COL9A3, CRB1, CRX, CRYAA, CRYAB, CRYBA1, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGB, CRYGD, CRYGS, CTDP1, CTSD, CYP1B1, CYP27A1, CYP4V2, DHDDS, DNAJC5, DNM1L, DTNBP1, DYNC2H1, EDNRB, EFEMP1, ELOVL4, EPHA2, ERCC1, ERCC2, ERCC5, ERCC6, EYA1, EYS, FAM161A, FBN1, FGFR1, FLVCR1, FOXC1, FOXD3, FOXE3, FOXL2, FRAS1, FREM1, FREM2, FRMD7, FSCN2, FYCO1, FZD4, GCNT2, GDF3, GDF6, GJA3, GJA8, GLA, GLI2, GLIS2, GNAT1, GNAT2, GNB3, GNPTG, GPR143, GPR179, GRIP1, GRK1, GRM6, GRN, GUCA1A, GUCA1B, GUCY2D, HADHA, HARS, HCCS, HESX1, HGSNAT, HK1, HMCN1, HMX1, HPS1, HPS3, HPS4, HPS5, HPS6, HSF4, IDH3B, IFT140, IFT172, IFT80, IFT81, IMPDH1, IMPG2, INVS, IQCB1, ITM2B, JAG1, JAM3, KCNJ13, KCNV2, KIF11, KIF7, KLHL7, LAMA1, LCA5, LIM2, LMX1B, LRAT, LRIT3, LRP5, LTBP2, LYST, LZTFL1, MAF, MAK, MC1R, MERTK, MFN2, MFRP, MFSD8, MIP, MITF, MKKS, MKS1, MLPH, MTTP, MVK, MYH9, MYO5A, MYO7A, MYOC, NAA10, NBAS, NDP, NEK8, NEUROD1, NHS, NMNAT1, NOTCH2, NPHP1, NPHP3, NPHP4, NR2E3, NR2F1, NRL, NYX, OAT, OCA2, OCRL, OFD1, OPA1, OPA3, OPN1SW, OTX2, P3H2, PANK2, PAX2, PAX3, PAX6,*

		PCDH15, PDE6A, PDE6B, PDE6C, PDE6G, PDE6H, PDZD7, PEX1, PEX2, PEX6, PEX7, PGK1, PHYH, PIGL, PIK3R1, PITPNM3, PITX2, PITX3, PLA2G5, PNPLA6, POMGNT1, PORCN, PPT1, PRCD, PROKR2, PROM1, PRPF3, PRPF31, PRPF6, PRPF8, PRPH2, PRPS1, PRSS56, PXDN, RAB18, RAB27A, RAB3GAP1, RAB3GAP2, RAX, RAX2, RB1, RBP3, RBP4, RCBTB1, RD3, RDH12, RDH5, RGR, RGS9, RGS9BP, RHO, RIMS1, RLBP1, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPGRIP1, RPGRIP1L, RS1, SAG, SALL4, SDCCAG8, SEMA3E, SEMA4A, SHH, SIL1, SIX3, SIX6, SLC16A12, SLC24A1, SLC24A5, SLC33A1, SLC41A1, SLC45A2, SLC9A6, SMOC1, SNAI2, SNRNP200, SNX3, SOX10, SOX2, SOX3, SPATA7, SPG7, STRA6, TBX22, TCTN1, TCTN2, TDRD7, TEAD1, TFAP2A, TGFB2, TIMM8A, TIMP3, TMEM114, TMEM126A, TMEM138, TMEM216, TMEM237, TMEM67, TOPORS, TPP1, TREX1, TRIM32, TRPM1, TSFM, TSPAN12, TTC21B, TTC8, TTPA, TUB, TUBGCP6, TULP1, TYR, TYRP1, UNC119, USH1C, USH1G, USH2A, VAX1, VCAN, VIM, VPS13B, VSX2, WDPCP, WDR19, WFS1,WHRN, XPNPEP3, ZNF423, ZNF513
447-gene virtual panel	WES	*ABCA4, ABCB6, ABCC6, ABHD12, ACBD5, ACO2, ACTB, ACTG1, ADAM9, ADAMTS10, ADAMTS17, ADAMTS18, ADAMTSL4, ADGRV1, ADIPOR1, AFG3L2, AGK, AHI1, AHR, AIPL1, ALDH3A2, ALMS1, ALPK1, ALX1, ANTXR1, AP3B1, ARL13B, ARL3, ARL6, ARMC9, ARSG, ATF6, ATOH7, ATXN7, B3GLCT, B9D1, B9D2, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BCOR, BEST1, BFSP1, BFSP2, BLOC1S6, BMP4, BMP7, C2CD3, C2orf71, C5orf42, C8orf37, CA4, CABP4, CACNA1F, CACNA2D4, CC2D2A, CDH23, CDH3, CDHR1, CDON, CELSR2, CENPF, CEP104, CEP120, CEP164, CEP19, CEP250, CEP290, CEP41, CERKL, CFAP410, CHM, CHMP4B, CHRDL1, CIB2, CISD2, CLN3, CLN5, CLN6, CLN8, CLRN1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL11A1, COL11A2, COL18A1, COL2A1, COL4A1, COL4A2, COL9A1, COL9A2, COL9A3, CPLANE1, CRB1, CRX, CRYAA, CRYAB, CRYBA1, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGB, CRYGD, CRYGS, CSPP1, CTDP1, CTSD, CTSF, CWC27, CYP1B1, CYP27A1, CYP4V2, DCDC2, DHDDS, DNAJC5, DNM1L, DTNBP1, DYNC2H1, EDNRB, EFEMP1, ELOVL4, EPHA2, ERCC1, ERCC2, ERCC5, ERCC6, ESPN, EXOC8, EXOSC2, EYA1, EYS, FALDH, FAM161A, FANCI, FBN1, FGFR1, FLVCR1, FOXC1, FOXD3, FOXE3, FOXL2, FRAS1, FREM1, FREM2, FRMD7, FSCN2, FYCO1, FZD4, GCNT2, GDF3, GDF6, GJA3, GJA8, GLA, GLI2, GLIS2, GNAT1, GNAT2, GNB3, GNPTG, GPR143, GPR179, GRIP1, GRK1, GRM6, GRN, GUCA1A, GUCA1B, GUCY2D, HADHA, HARS, HCCS, HESX1, HGSNAT, HK1, HMCN1, HMX1, HPS1, HPS3, HPS4, HPS5, HPS6, HSF4, HYLS1, IDH3B, IDS, IFT140, IFT172, IFT27, IFT43, IFT57, IFT74, IFT80, IFT81, IMPDH1, IMPG2, INPP5E, INVS, IQCB1, ITM2B, JAG1, JAM3, KARS1, KCNJ13, KCNV2, KIAA0556, KIAA0586, KIF11, KIF14, KIF3B, KIF7, KIZ, KLHL7, LAMA1, LCA5, LIM2, LMX1B, LRAT, LRIT3, LRP5, LTBP2, LYST, LZTFL1, MAF, MAK, MC1R, MERTK, MFN2, MFRP, MFSD8, MIP, MITF, MKKS, MKS1, MLPH, MTTP, MVK, MYH9, MYO5A, MYO7A, MYOC, NAA10, NBAS, NDP, NEK1, NEK8, NEUROD1, NHS, NMNAT1, NOTCH2, NPHP1, NPHP3, NPHP4, NR2E3, NR2F1, NRL, NYX, OAT, OCA2, OCRL, OFD1, OPA1, OPA3, OPN1SW, OTX2, P3H2, PACS1, PANK2, PAX2, PAX3, PAX6, PCARE, PCDH15, PCYT1A, PDE6A, PDE6B, PDE6C, PDE6D, PDE6G, PDE6H, PDZD7, PEX1, PEX2, PEX6, PEX7, PGK1, PHYH, PIBF1, PIGL, PIK3R1, PISD, PITPNM3, PITX2, PITX3,*

		PLA2G5, PLK4, PNPLA6, POC1B, POMGNT1, PORCN, PPT1, PRCD, PROKR2, PROM1, PRPF3, PRPF31, PRPF6, PRPF8, PRPH2, PRPS1, PRSS56, PSEN1, PTPN11, PXDN, PYGM, RAB18, RAB27A, RAB3GAP1, RAB3GAP2, RAX, RAX2, RB1, RBP3, RBP4, RCBTB1, RD3, RDH11, RDH12, RDH5, RGR, RGS9, RGS9BP, RHO, RIMS1, RIMS2, RLBP1, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPGRIP1, RPGRIP1L, RS1, RTN4IP1, SAG, SALL4, SCAPER, SCLT1, SDCCAG8, SEMA3E, SEMA4A, SGSH, SHH, SIL1, SIX3, SIX6, SLC16A12, SLC24A1, SLC24A5, SLC25A46, SLC33A1, SLC41A1, SLC45A2, SLC7A14, SLC9A6, SMARCA4, SMOC1, SNAI2, SNRNP200, SNX3, SOX10, SOX2, SOX3, SPATA7, SPG7, SSBP1, STRA6, SUFU, TBX22, TCTN1, TCTN2, TCTN3, TDRD7, TEAD1, TFAP2A, TGFB2, TIMM8A, TIMP3, TLCD3B, TMEM107, TMEM114, TMEM126A, TMEM138, TMEM216, TMEM231, TMEM237, TMEM67, TOGARAM1, TOPORS, TPP1, TREX1, TRIM32, TRNT1, TRPM1, TSFM, TSPAN12, TTC21B, TTC8, TTPA, TUB, TUBGCP4, TUBGCP6, TULP1, TYR, TYRP1, UNC119, USH1C, USH1G, USH2A, VAX1, VCAN, VIM, VPS13B, VSX2, WDPCP, WDR19, WDR34, WDR35, WDR60, WFS1, WHRN, XPNPEP3, ZNF423, ZNF513
Customized aCGH[a]	aCGH	*ABCA4, ADAM9, AIPL1, **ALMS1**, ARL2BP, ARL6, BBS1, **BBS12**, BBS2, BEST1, C1QTNF5, C21ORF2, C2ORF71, C8ORF37, CA4, CACNA1F, CACNA2D4, **CCT2**, CDHR1, **CEP290**, CERKL, CFH, CLRN1, **CLUAP1**, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, CRB1, CRX, DHDDS, DHX38, EFEMP1, ELOVL4, EMC1, EML4, EYS, FAM161A, FSCN2, GPR125, GUCA1A, GUCA1B, GUCY2D, HGSNAT, HK1, HMCN1, IDH3B, **IFT140**, IFT172, IMPDH1, IMPG1, IMPG2, **IQCB1**, **KCNJ13**, KCNV2, KIAA1549, KLHL7, **LCA5**, LRAT, MAK, MERTK, MVK, **MYO7A**, NEK2, NEUROD1, **NMNAT1**, NR2E3, NRL, OFD1, OR2W3, PDE6A, PDE6B, PDE6C, PDE6G, PDE6H, **PDZD7**, PITPNM3, PLK1S1, POC1B, PRCD, PROM1, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, PRPH2, RAB28, RAX2, RBP3, RCBTB1, **RD3**, RDH12, RDH5, RGR, RHO, RIMS1, RLBP1, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPGRIP1, SAG, **SAMD11**, SEMA4A, SF3B2, SLC7A14, SNRNP200, SPATA7, TIMP3, TOPORS, TTC8, TTLL5, TULP1, UNC119, USH2A, **VPS13B**, **WDPCP**, ZNF408, ZNF513*

aCGH: array-based comparative genomic hybridization; CES: Clinical Exome Sequencing; WES: Whole Exome Sequencing. [a] Genes in **bold** those added to the original design described in Van Cauwenberg et al. 2017 (PMID: 27608171)

Supplementary Table S3. Summary of the different in-house bioinformatic pipelines used for the study of Single Nucleotide Variations (SNV) and Copy Number Variations (CNV) in the WES analysis in our cohort, as well as the prioritization characteristics of each pipeline.

Bioinformatic pipeline		Pipeline_1	Pipeline_2	Pipeline_3
Aligning to GRCh37/hg19		BWA v0.7.12-r1039	BWA v0.7.15	Novoalign software v4.02.02
Variant calling		GATK v3.4-46	GATK v4.0.5.1	GATK v4.1.2.0
Variant annotation		ANNOVAR		
Filtering pipeline	Quality and coverage	Hard filtering: my_SNP_filter: QD < 2.0, MQ < 40.0, MQRankSum < -12.5 and ReadPosRankSum < -8.0 and my_INDEL_filter: QD < 2.0 and ReadPosRankSum < -20.0 FILTER=PASS, DP > 10, QUAL > 100		DP > 10 perc_alt > 0.25 GQ > 30
	Allelic frequency data	gnomAD, NHLBI Exome Variant Server, 1000 Genomes Project, CSVS MAX_AF < 0.05		ExAC, gnomAD, 1000 Genomes, NHLBI Exome Variant Server, GME Variome, ABraOM, in-house databases MAX_AF < 0.01; homozygous patients in ExAC < 2
	Predictors and evolutionary conservation of affected residue	Implemented splicing predictors in the Alamut software Predictors of the impact caused on the protein (SIFT, Polyphen2, Mutation Taster, M-CAP, CADD > 10, FATHMM, PROVEAN) Gene loss of function predictors (LoFtool, ExACpLI)		Predictors of the impact caused on the protein Predicted impact on messenger RNA (mRNA) splicing In-house variant frequencies and splicing predictors (MaxEntScan and SpliceAI); FS < 25
	SNPs annotation	dbSNP		
	HOMO_regions	N/A		AutoMap tool (Quinodoz et al. 2021)
CNV detection (Filters)		CoNVaDING (regionThreshold = 20, ratioCutOffLow = 0.65, ratioCutOffHigh = 1.3, zScoreCutOffLow = -3, and zScoreCutOffHigh = 3)		ExomeDepth
Year		2016 (vcf reannotated in 2018)	2018	2020

BWA: Burrows-Wheeler Aligner; CSVS: Collaborative Spanish Variant Server; DP: depth of coverage; FS: functional significance; GATK: Genome Analysis Toolkit; GQ: genotype quality; HOMO_regions: homozygosity regions; MAX_AF: highest allele frequency; MQ: mapping quality; N/A: non-available; perc_alt: frequency of the alternative allele; QD: quality of depth; QUAL: quality; WES: whole-exome sequencing. gnomAD/ExAC (https://gnomad.broadinstitute.org/), NHLBI Exome Variant Server (https://evs.gs.washington.edu/EVS/), 1000 Genomes Project (https://www.internationalgenome.org/), Collaborative Spanish Variant Server (https://csvs.babelomics.org/), GME Variome (https://igm.ucsd.edu/gme/index.php), and ABraOM (https://abraom.ib.usp.br/)

Supplementary Table S5. Diagnostic yield of the different molecular approaches for the study of the SRD cases, comparing the well-known syndromes (CILIOPATHY & SPECIFIC) vs the complete cohort.

NGS study			Well-known syndromes diagnostic rate (characterized/total probands)	Complete cohort diagnostic rate (characterized/total probands)
SNV (+CNV[a])	CES	First-tier (prospective cases)	77% (10/13)	83% (15/18)
		Retrospective cases	35% (10/29)	25% (20/79)
		Total cases with CES analysis	**48% (20/42)** SNV: 18; CNV: 2; SNV+CNV: 0	**36% (35/97)** SNV: 32; CNV: 3; SNV+CNV: 0
	WES	447-gene subpanel analysis	50% (4/8)	30% (11/37)
		Reanalysis (including hypothesis-free approach)	50% (2/4)	19% (5/26)
		Total analysis by WES	**75% (6/8)** SNV: 5; CNV: 0; SNV+CNV: 1	**43% (16/37)** SNV: 15; CNV: 0 ; SNV+CNV: 1
	mtDNA sequencing		0% (0/0)	20% (1/5)
CNV	aCGH (60K, 400K)		0% (0/4)	13% (2/16)[b]
TOTAL			**% (26/42)** SNV: 23; CNV: 2; SNV+CNV: 1	**52% (52/100)** SNV: 45; CNV: 3; SNV+CNV: 3[b]

a CNV bioinformatic detection (Commercial SOPHiA DDM platform; in-house pipeline) included. b RP-1018 and RP-1321 (dual diagnosis) were characterized using a combination of a SNV and CNV detection techniques. aCGH: array of comparative genomic hybridization; CES: clinical exome sequencing; CNV: Copy Number Variation; mtDNA: mitochondrial DNA; NGS: Next-generation sequencing; SNV: Single Nucleotide Variation; SRD: syndromic retinal diseases; VUS: variant of uncertain significance; WES: whole-exome sequencing

Supplementary Table S6. Summary of the characterized cases through WES reanalysis and the key points that led to the diagnosis.

Reanalysis	Time frame reanalysis (years)	Bioinformatic pipeline[a]	Case	Gene	Case status
New genes and associations reported in the literature since the original analysis	2	1	RP-2176	*HK1*(Okur et al. 2019)	Characterized
Hypothesis-free approach	1	2	RP-0897	*GALE*	Phenotypically partially characterized
	2	1 & 3	RP-2005	*LRRC32*	Characterized
	2	2 & 3	RP-2032	*MCOLN1*	Characterized
Compilation of new data and clinical reassessment	2	1 & 3	RP-2085	*CDH23*	Characterized

a Bioinformatic pipelines are described in the Supplementary Table S3. WES: Whole Exome Sequencing

Supplementary Table S9. Comparative table of published cohorts of syndromic retinal dystrophies.

	Cohort 1 (This work)	Cohort 2 (Jiman et al. 2020)	Cohort 3 (Manara et al. 2019)	Cohort 4 (Shaheen et al. 2016)
Clinical composition	Miscellanea of SRD	Miscellanea of SRD	BBS	Syndromic ciliopathy spectrum
Consanguinity	+/-	N/A	-	+
Study approach	Singleton	Singleton	Singleton	Singleton and familiar
NGS technique	Various	Customized panel	Customized panel	Various
Total or partially phenotypically characterized families (n)	52	58	12	225
Uncharacterized families (n)	48	48	8	40
Characterization rate	52%	55%	60%	85%

Chi-square test of independence with a significance level of 0.05:

- Cohort 1-4. X2 (3, N= 491) = 56.8418, p <0.0001. There is a significant difference between groups 1-4, so the proportion of characterized patients differ by studies.
- Cohort 1-3. X2 (2, N= 226) = 0.4728, p = 0.789478. The statistical test showed that there is no significant difference between characterization rates in the studies 1-3.

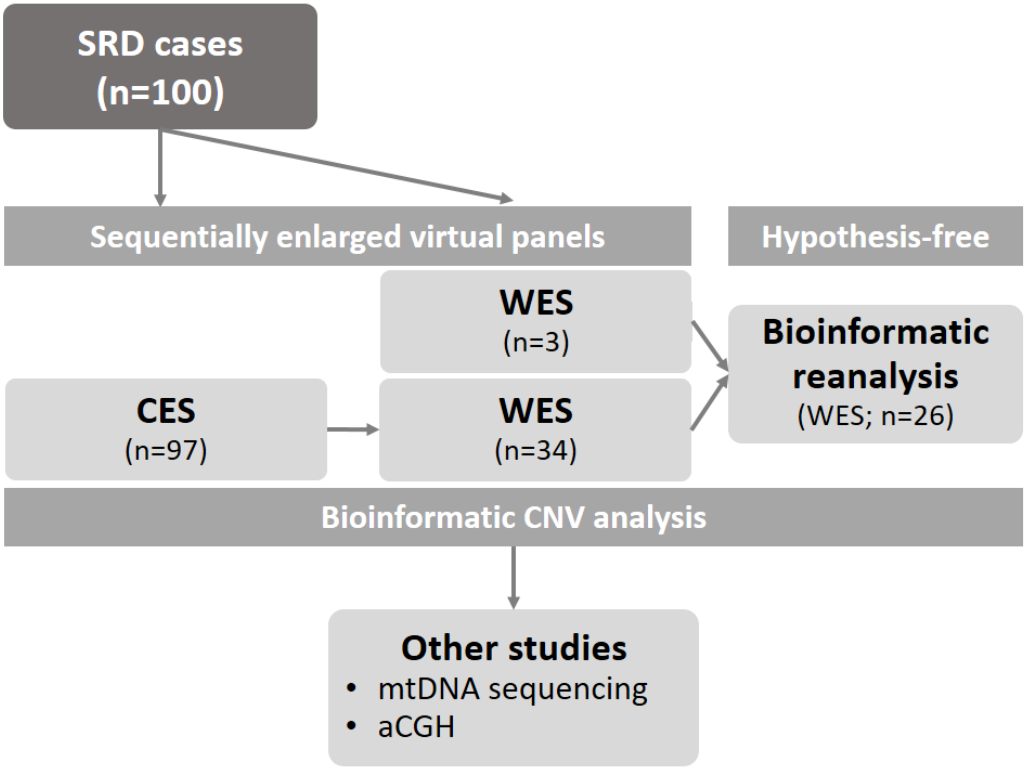
Statistical analysis using chi-square test with a significance level of $p < 0.05$. BBS: Bardet-Biedl syndrome; N/A: non-available; SRD: syndromic retinal diseases; +: yes; -: no; +/-: in some cases

Supplementary Table S10. Comparative table with different reanalysis studies.

Study	Year	Number of included cases	Diseases	Time frame since 1st analysis	Diagnostic yield of the reanalysis	Technique	Reanalysis method
(Wenger et al. 2017)	2017	40 individuals	Miscellanea	20 months	10% (4/40)	Clinical exome	Bioinformatic and literature
(Ewans et al. 2018)	2018	54 patients (of 37 families)	Miscellanea	12 months	15% (4/26)	WES (singleton and trios)	Clinical revaluation, bioinformatic and literature
(Wright et al. 2018)	2018	1133 families	Developmental disorders	3 years	Increased by 13%	WES (trios)	Bioinformatic and literature
(Jalkh et al. 2019)	2019	200 patients	Miscellanea	2 years	6.5% (13/101)	WES (singleton)	Bioinformatic and literature
(Li et al. 2019)	2019	76 families	Epilepsy and ID/MR	6 to >12 months	10.5% (8/76)	WES (trios)	Clinical revaluation, bioinformatic and literature
(Liu et al. 2019)	2019	250 patients	Miscellanea	5 years	Increased from 24.8% to 46.8%	WES (singleton)	Bioinformatic and literature ("manual")
(Liu et al. 2019)	2019	2000 cases	Miscellanea	4 years	Increased from 25.2% to 36.7%	WES (singleton)	Bioinformatic and literature (semi-automated)
This work	2021	100 cases	SRD	1-3 years	19% (5/26)	Customized panel, clinical exome, WES (singleton)	Clinical revaluation, bioinformatic and literature

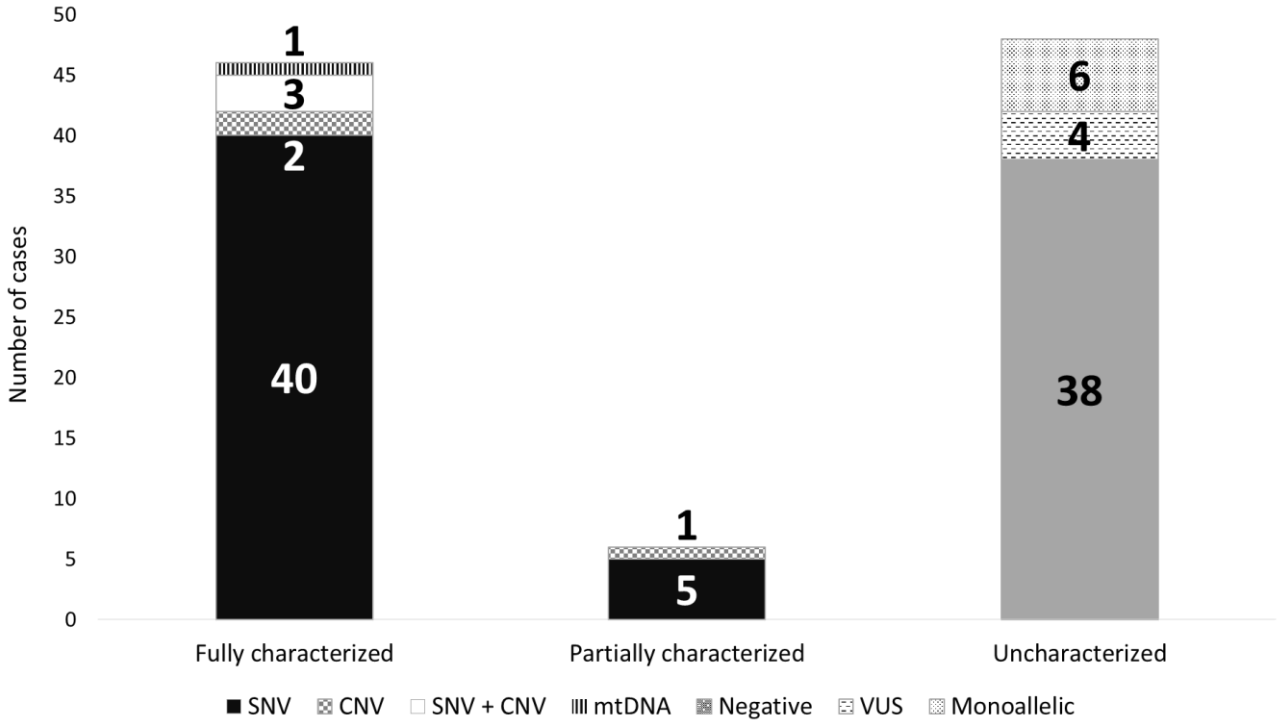
ID: intellectual disability; MR: mental retardation; SRD: syndromic retinal diseases; WES: Whole-Exome Sequencing

Supplementary Fig. S1. Working flowchart of the molecular study of the SRD cases of our cohort.



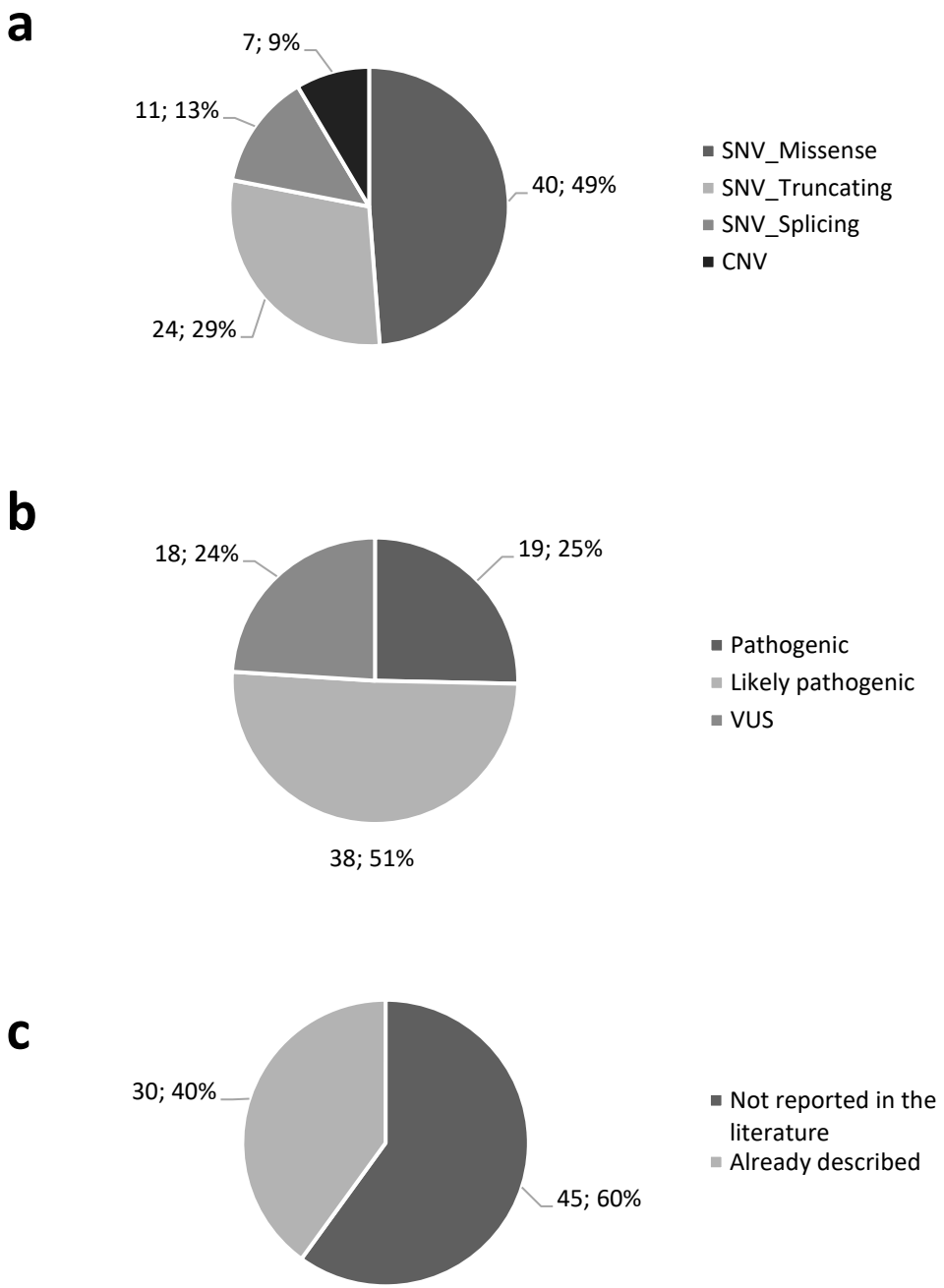
Cases have been studied by CES and/or WES, using sequential increased sizes of gene subpanels: 1) 136-gene subpanel (minimal virtual panel); 2) enlarged virtual panels of 241 and 447 genes; and 3) hypothesis-free approach. Each step of the flowchart included the bioinformatic CNV analysis of the data. In cases with suspected mtDNA disorder, mtDNA sequencing was performed. aCGH: array of comparative genomic hybridization; CES: clinical exome sequencing; CNV: Copy Number Variation; mtDNA: mitochondrial DNA; SNV: Single Nucleotide Variation; SRD: syndromic retinal diseases; WES: whole-exome sequencing

Supplementary Fig. S2 Distribution of cases after the molecular studies.



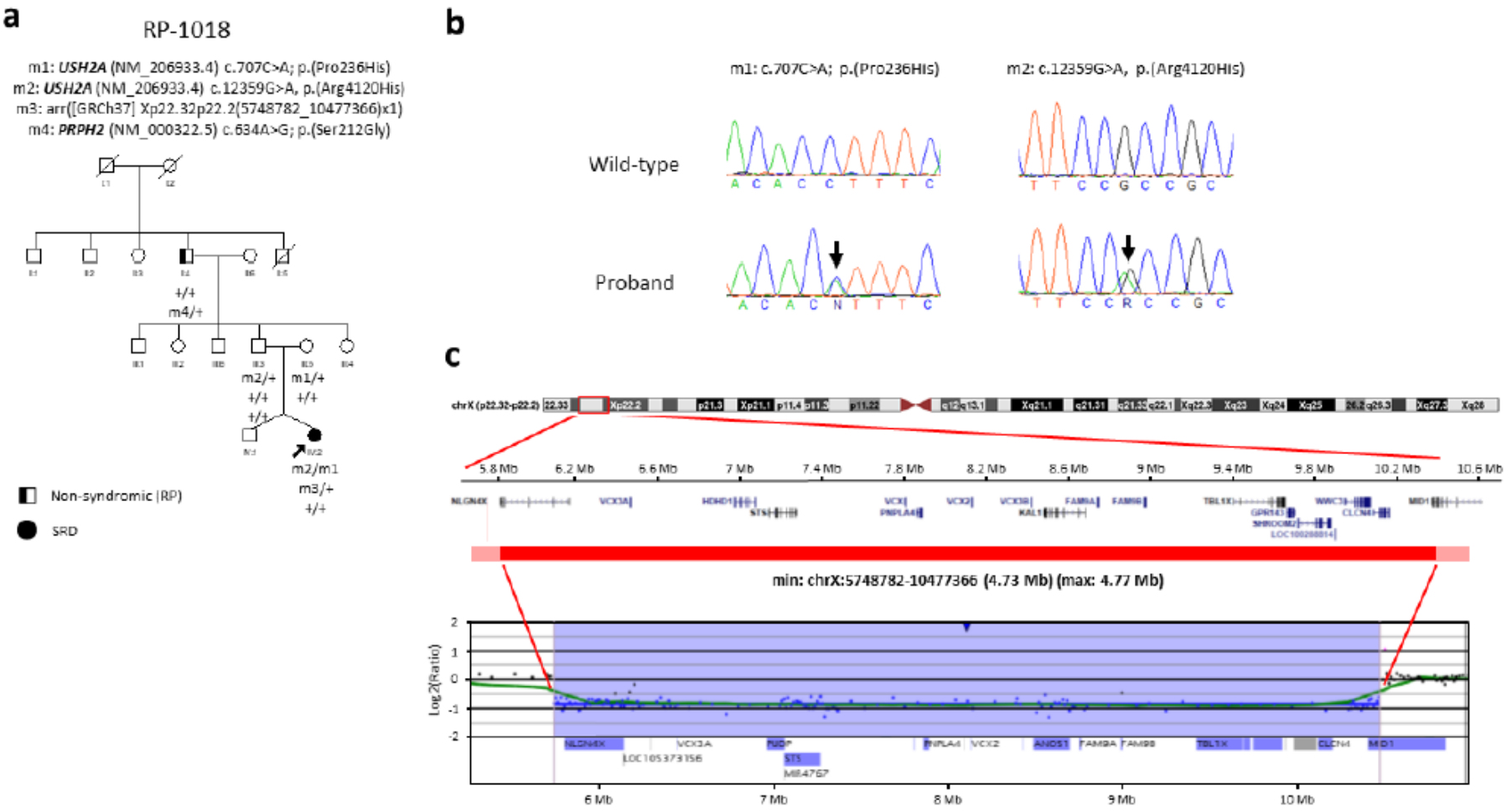
Cases were classified according to the genetic results and their type (SNV, CNV, SNV+CNV and mitochondrial DNA. CNV: Copy Number Variation; mtDNA: mitochondrial DNA; SNV: Single Nucleotide Variation

Supplementary Fig. S3 Variant analysis of the characterized and likely pathogenic/VUS monoallelic cases.



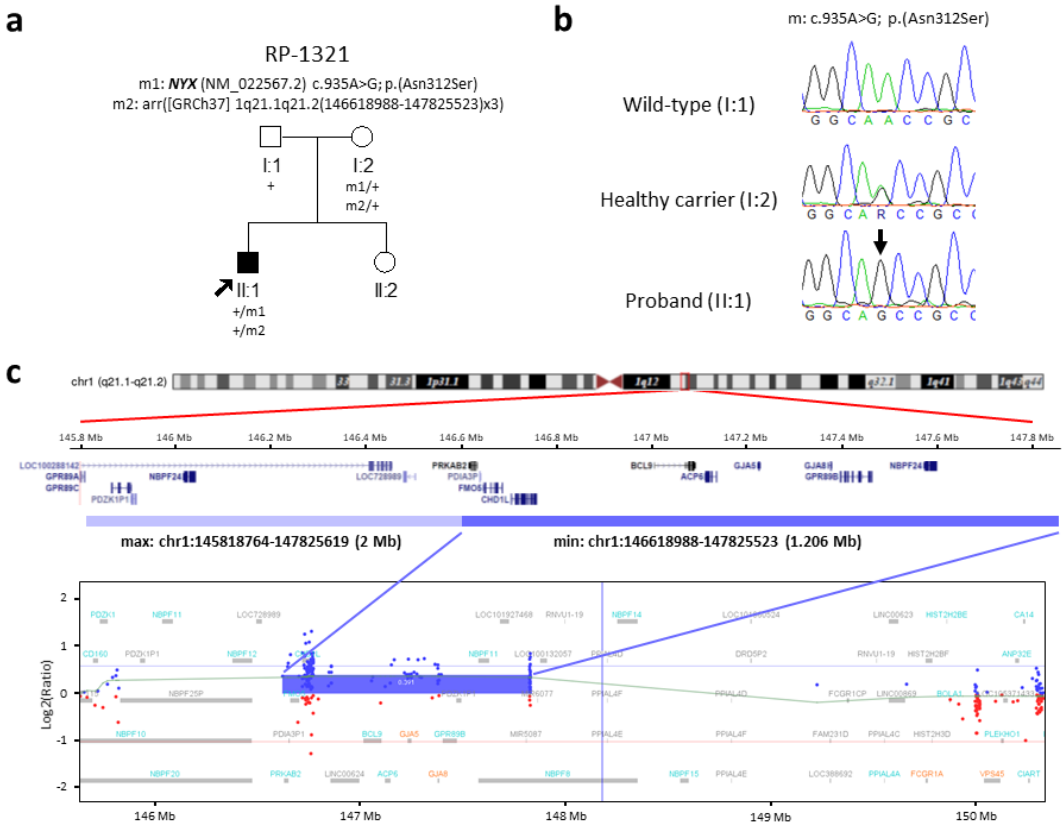
(a) Distribution of the variants. Variants could be Single Nucleotide Variations (SNVs) (missense, truncating or splicing) or Copy Number Variations (CNVs). **(b)** SNV pathogenicity distribution. Applying the guidelines of the American College of Medical Genetics and Genomics (ACMG) and the European Society of Human Genetics (ESHG), variants could be classified regarding their pathogenicity into pathogenic, likely pathogenic or variant of uncertain significance (VUS). **(c)** Distribution of new and already reported SNV variants

Supplementary Fig. S4 Dual diagnosis in RP-1018 by targeted NGS and array of comparative genomic hybridization (aCGH).



(a) Pedigree and segregation analysis within the family. Genetic findings were segregated in all the available family members and indicated with "+" for wild-type alleles and "m1, m2, m3 and m4" for each mutated allele. RP: retinitis pigmentosa; SRD: syndromic retinal disease. **(b)** Electropherograms of the two heterozygous variants in *USH2A* for wild-type and mutated allele. **(c)** Genomic rearrangement on Xq22.32-22.2 region. The presence of a 4.73 Mb de novo deletion in chromosome X was observed within Xq22.32-22.2 (minimum genomic coordinates: chrX:5748782-10477366) (dark red), involving 16 genes (*NLGN4X, VCX3A, PUDP, STS, VCX, PNPLA4, VCX2, VCX3B, ANOS1, FAM9A, FAM9B, TBL1X, GPR143, SHROOM2, CLCN4* and *MID1*), of which six were in OMIM, standing out *NLGN4* and *MID1*, associated to Mental retardation, X-linked (MIM #300495) and Opitz GBBB syndrome, type I (MIM #300000), respectively. The horizontal axis shows the genomic position along the genome (GRCh37 – hg19) and the vertical axis the log2 ratio values (-2/-1: deletion; 0: normal pattern; 1/2: duplication)

Supplementary Fig. S5 Dual diagnosis in RP-1321 by targeted NGS and array of comparative genomic hybridization (aCGH).



(a) Pedigree and segregation analysis within the family. Genetic findings were segregated in all the available family members and indicated with "+" for wild-type alleles and "m1 and m2" for each mutated allele. **(b)** Electropherograms of the *NYX* variant for wild-type, heterozygous carrier and hemizygous. **(c)** Genomic rearrangement on 1q21.1q21.2 region. A minimum 1.206 Mb duplication (genomic coordinates: chr1:146618988-147825523) (dark blue) and maximum of 2 Mb (genomic coordinates: chr1:145818764-147825619) (light blue) was observed using aCGH. This duplication comprised 13 genes (*FMO5, CHD1L, BCL9, GJA5, GJA8, PRKAB2, PDIA3P, ACP6, GPR98B, GPR98C, PDZK1P1, NBPF11* and *NBPF24*), of which 7 are included in OMIM. The horizontal axis shows the genomic position along the genome (GRCh37 – hg19) and the vertical axis the log2 ratio values (-2/-1: deletion; 0: normal pattern; 1/2: duplication).

Las **tablas suplementarias S4, S7 y S8** pueden encontrarse en:

https://link.springer.com/article/10.1007/s00439-021-02343-7

References of the Supplementary Material

1. den Boer MEJ, Wanders RJA, Morris AAM, et al (2002) Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: clinical presentation and follow-up of 50 patients. Pediatrics 109:99–104. https://doi.org/10.1542/peds.109.1.99
2. Ewans LJ, Schofield D, Shrestha R, et al (2018) Whole-exome sequencing reanalysis at 12 months boosts diagnosis and is cost-effective when applied early in Mendelian disorders. Genet Med 20:1564–1574. https://doi.org/10.1038/gim.2018.39
3. Forsythe E, Beales PL (2013) Bardet-Biedl syndrome. Eur J Hum Genet 21:8–13. https://doi.org/10.1038/ejhg.2012.115
4. Jalkh N, Corbani S, Haidar Z, et al (2019) The added value of WES reanalysis in the field of genetic diagnosis: lessons learned from 200 exomes in the Lebanese population. BMC Med Genomics 12:11. https://doi.org/10.1186/s12920-019-0474-y
5. Jiman OA, Taylor RL, Lenassi E, et al (2020) Diagnostic yield of panel-based genetic testing in syndromic inherited retinal disease. Eur J Hum Genet 28:576–586. https://doi.org/10.1038/s41431-019-0548-5
6. Karlberg N, Jalanko H, Perheentupa J, Lipsanen-Nyman M (2004) Mulibrey nanism: clinical features and diagnostic criteria. J Med Genet 41:92–98. https://doi.org/10.1136/jmg.2003.014118
7. Kashtan CE (1993) Alport Syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds) GeneReviews®. University of Washington, Seattle, Seattle (WA)
8. Kohlschütter A, Schulz A, Bartsch U, Storch S (2019) Current and Emerging Treatment Strategies for Neuronal Ceroid Lipofuscinoses. CNS Drugs 33:315–325. https://doi.org/10.1007/s40263-019-00620-8
9. Li J, Gao K, Yan H, et al (2019) Reanalysis of whole exome sequencing data in patients with epilepsy and intellectual disability/mental retardation. Gene 700:168–175. https://doi.org/10.1016/j.gene.2019.03.037
10. Liu P, Meng L, Normand EA, et al (2019) Reanalysis of Clinical Exome Sequencing Data. N Engl J Med 380:2478–2480. https://doi.org/10.1056/NEJMc1812033
11. Manara E, Paolacci S, D'Esposito F, et al (2019) Mutation profile of BBS genes in patients with Bardet-Biedl syndrome: an Italian study. Ital J Pediatr 45:72. https://doi.org/10.1186/s13052-019-0659-1
12. Marshall JD, Beck S, Maffei P, Naggert JK (2007) Alström syndrome. Eur J Hum Genet 15:1193–1202. https://doi.org/10.1038/sj.ejhg.5201933
13. Nozu K, Nakanishi K, Abe Y, et al (2019) A review of clinical characteristics and genetic backgrounds in Alport syndrome. Clin Exp Nephrol 23:158–168. https://doi.org/10.1007/s10157-018-1629-4
14. Okur V, Cho MT, van Wijk R, et al (2019) De novo variants in HK1 associated with neurodevelopmental abnormalities and visual impairment. Eur J Hum Genet 27:1081–1089. https://doi.org/10.1038/s41431-019-0366-9
15. Parisi MA, Doherty D, Chance PF, Glass IA (2007) Joubert syndrome (and related disorders) (OMIM 213300). Eur J Hum Genet 15:511–521. https://doi.org/10.1038/sj.ejhg.5201648
16. Quinodoz M, Peter VG, Bedoni N, et al (2021) AutoMap is a high performance homozygosity mapping tool using next-generation sequencing data. Nat Commun 12:518. https://doi.org/10.1038/s41467-020-20584-4
17. Shaheen R, Szymanska K, Basu B, et al (2016) Characterizing the morbid genome of ciliopathies. Genome Biol 17:242. https://doi.org/10.1186/s13059-016-1099-5

18. Tranebjærg L, Barrett T, Rendtorff ND (1993) WFS1 Wolfram Syndrome Spectrum Disorder. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds) GeneReviews®. University of Washington, Seattle, Seattle (WA)
19. Wang H, Falk MJ, Wensel C, Traboulsi EI (1993) Cohen Syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds) GeneReviews®. University of Washington, Seattle, Seattle (WA)
20. Wenger AM, Guturu H, Bernstein JA, Bejerano G (2017) Systematic reanalysis of clinical exome data yields additional diagnoses: implications for providers. Genet Med 19:209–214. https://doi.org/10.1038/gim.2016.88
21. Wright CF, McRae JF, Clayton S, et al (2018) Making new genetic diagnoses with old data: iterative reanalysis and reporting from genome-wide data in 1,133 families with developmental disorders. Genet Med 20:1216–1223. https://doi.org/10.1038/gim.2017.246

CAPÍTULO 3

Alelos modificadores y variabilidad intrafamiliar en el síndrome de Bardet-Biedl

Artículo 3: *Allelic overload and its clinical modifier effect in Bardet-Biedl syndrome*

Perea-Romero I, Solarat C, Blanco-Kelly F, *et al.*

Sometido, no publicado

Resumen

Con este trabajo se presenta el papel de la carga mutacional en la variabilidad clínica del BBS, destacando la importancia de considerar la herencia no mendeliana en el manejo clínico de este síndrome.

Se reclutó retrospectivamente una cohorte de 99 pacientes de 77 familias distintas con un diagnóstico genético final de BBS. Los datos clínicos disponibles se anotaron usando términos HPO. Después de la anotación clínica, un 77% (76/99) de los casos cumplieron *a priori* los criterios establecidos para el diagnóstico de BBS o BBS-*like*. En todas las familias con información clínica disponible excepto en una, al menos un afecto cumplía los criterios clínicos, y en todas se le hizo el estudio genético al caso con el fenotipo más severo.

Se usaron diferentes enfoques moleculares y de NGS para el estudio de la carga mutacional en los genes relacionados con BBS en los casos índice. El 48% (37/77) de los casos índice se estudiaron exclusivamente mediante secuenciación de Sanger o *microarrays* comerciales de genotipado, que no cubrían todos los genes relacionados con BBS. Mientras que en el resto de los probandos (40/77) se realizaron distintos abordajes de NGS, con los que era posible explorar la carga mutacional en genes relacionados con BBS. Finalmente, se utilizaron las herramientas *in silico* ORVAL y DiGePred para predecir el efecto oligogénico de las combinaciones alélicas candidatas encontradas, las cuales podrían estar participando en la variabilidad intrafamiliar en BBS entre los casos índice y sus hermanos afectos.

Los alelos en el gen *BBS1* fueron los más representados en nuestra cohorte, encontrándose en el 42% de las familias. Se encontraron sospechas de herencia oligogénica en el 52% de las familias estudiadas (23/45), 18 a través del análisis de datos de NGS y 5 mediante métodos tradicionales. Asimismo, se pudo excluir la presencia de alelos adicionales en el 29% de los casos índice (22/77).

No se encontraron diferencias significativas en la distribución de los síntomas entre los casos con respecto a la carga mutacional detectada.

Según las predicciones obtenidas de ORVAL y DiGePred en las familias trialélicas, el 44% de las combinaciones tendrían una herencia oligogénica en ambos métodos con un 95% de confianza en ORVAL y el umbral de confianza más alto en DiGePred. Manteniendo los umbrales de confianza, dicha posibilidad alcanzó el 91% cuando se consideraron los métodos de forma individual. La variabilidad intrafamiliar pudo confirmarse clínicamente en 6 de las familias, de las que 5 tienen combinaciones alélicas predichas como posiblemente oligogénicas en ambas herramientas empleadas.

Con este trabajo se ha profundizado en el tema de la herencia oligogénica en el BBS, encontrándose ejemplos adicionales de la posible existencia de alelos modificadores como causa de la variabilidad intrafamiliar en esta enfermedad. Además, se destaca la importancia del uso de NGS en el diagnóstico genético del BBS.

Contribución de la autora

La autora intervino en la recogida, estructuración y curación de los datos clínicos, genéticos y familiares de los 99 casos pertenecientes a 77 familias con un diagnóstico genético de BBS en al menos uno de sus miembros. Anotó todos los datos clínicos con términos HPO y realizó la clasificación fenotípica de los casos según criterios ya establecidos previamente en la literatura y en el artículo 2 de este Tesis Doctoral. Participó en el análisis y reanálisis de datos de NGS para la búsqueda de posibles alelos modificadores. Además, llevó a cabo los estudios *in silico* mediante las herramientas ORVAL y DiGePred para predecir la relación entre las combinaciones de variantes. Participó en el análisis estadístico de la distribución de las distintas características clínicas según la carga mutacional detectada en los subgrupos de casos. Finalmente, la autora escribió el manuscrito y participó en las revisiones críticas sucesivas.

Allelic overload and its clinical modifier effect in Bardet-Biedl syndrome

Perea-Romero I[1,2], Solarat C[3,4], Blanco-Kelly F[1,2], Sanchez-Navarro I[1], Bea-Mascato B[3,4], Martin-Salazar E[3,4], Lorda-Sanchez I[1,2], Tahsin-Swafiri S[1,2], Avila-Fernandez A[1,2], Martin-Merida I[1,2], Trujillo-Tiebas MJ[1,2], Carreño E[5], Jimenez-Rolando B[5], Garcia-Sandoval B[2,5], Minguez P[1,2], Corton M[1,2], Valverde D[3,4], Ayuso C[1,2]

[1] Department of Genetics, Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain.

[2] Center for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain.

[3] CINBIO, Universidade de Vigo, Vigo, Spain.

[4] Instituto de Investigación Sanitaria Galicia Sur (IIS Galicia Sur), Álvaro Cunqueiro Hospital, Vigo, Spain.

[5] Department of Ophthalmology, Fundación Jiménez Díaz University Hospital (FJD), Madrid, Spain.

Abstract

Bardet-Biedl syndrome (BBS) is an autosomal recessive ciliopathy characterized by extensive inter- and intra-familial variability, in which oligogenic interactions have been also reported. Our main goal is to elucidate the role of mutational load in the clinical variability of BBS.

A cohort of 99 patients from 77 different families with biallelic pathogenic variants in a BBS-associated gene was retrospectively recruited. Human Phenotype Ontology terms were used in the annotation of clinical symptoms. The mutational load in 39 BBS-related genes has been studied in index cases using different molecular and next-generation sequencing (NGS) approaches. Candidate allele combinations were analysed using the *in silico* tools ORVAL and DiGePred.

After clinical annotation, 76 out of the 99 cases *a priori* fulfilled established criteria for diagnosis of BBS or BBS-like. *BBS1* alleles, found in the 42% of families, were the most represented in our cohort. An increased mutational load was excluded in the 29% of the index cases (22/77). Oligogenic inheritance was suspected in 52% of the screened families (23/45), being 40 tested by means of NGS data and 5 only by traditional methods. Together, ORVAL and DiGePred platforms predicted an oligogenic effect in the 44% of the triallelic families (10/23). Intrafamilial variable severity could be clinically confirmed in 6 of the families.

Our findings show that the presence of more than two alleles in BBS-associated genes correlated in 6 families with a more severe phenotype and associated with specific findings, highlighting the role of the mutational load in the management of BBS cases.

Keywords

Bardet-Biedl syndrome; HPO; intrafamilial variability; mutational load; second-site modifiers

Introduction

Bardet-Biedl syndrome (BBS, MIM #209900) is a rare multisystemic disease that is caused by the dysfunction of primary cilia [198]. BBS is a complex ciliopathy mainly characterized by progressive retinal dystrophy, postaxial polydactyly, obesity, hypogonadism, renal anomalies, and cognitive impairment [198]. Additional findings are type 2 diabetes mellitus, speech or developmental alterations, dental anomalies, brachydactyly/syndactyly, ataxia, anosmia/hyposmia, heart malformations, or Hirschsprung disease [198,199]. Its incidence varies from 1:160,000 in northern Europe [200] to 1:13,500-18,000 in several isolated communities with higher rates of inbreeding [79,80].

Genetically, BBS is also a heterogeneous disorder with 24 loci associated to date, according to the data extracted from OMIM (Online Mendelian Inheritance in Man; last accessed February 2022) and the Human Gene Mutation Database Professional (HGMD) 2021.4 database (last accessed in February 2022). The first 21 loci (BBS1-21) account for approximately 80% of all the cases diagnosed with this syndrome [86]. BBS-related genes encode for proteins of the primary cilium and the basal body complex [36]. Besides systemic forms, some BBS genes have also been linked to non-syndromic retinopathies, as well as other systemic ciliopathies, such as Joubert (JBTS, MIM #213300), McKusick-Kaufman (MKKS, MIM #236700), Meckel (MKS, MIM #2490000), and Senior-Løken (SLSN, MIM #266900) syndromes.

BBS is usually inherited as an autosomal recessive Mendelian trait with variable intra- and inter-familial severity [199]. Twenty years ago, triallelism, i.e. three alleles in two BBS loci, was first described in a BBS family [201]. An unaffected sibling carried two variants in *BBS2* (MIM *606151), whereas the BBS-affected patient additionally presented a third allele in *MKKS* (MIM *604896) [201]. However, subsequent studies have found no evidence of its existence [202,203]. Regardless of triallelism, in other cases, the penetrance and/or phenotypic expressivity of causative biallelic BBS variants are modulated in some families by the presence of oligogenic modifiers [204–207]. In those families, a third mutation in a second gene has been correlated with an earlier onset or a more severe specific phenotype in the carriers BBS patients due to a probable modifying effect [206–208].

Variants in the secondary gene are not always straightforward predicted to be pathogenic since they can also be hypomorphic or common alleles with high population frequencies that are *a priori* considered benign. To characterize the interaction, *in vitro* [209] and *in vivo* [210] models are normally used to assess allelic combinations and their effect on modulating clinical outcome. As functional studies are not always feasible in a clinical setting, emerging *in silico* tools, such as ORVAL (Oligogenic Resource for Variants Analysis) [211] or DiGePred (DiGenic Predictor) [190], could be useful to help predict the effect of the mutational burden of rare and common variants.

This work focuses on establishing and understanding new potential oligogenic combinations that may explain the clinical variability in BBS-affected families. The identification of new possible modifier alleles may also have an important impact on genetic counselling and clinical management.

Materials and methods

Subjects and phenotypic classification

This research has been reviewed and approved by the Research Ethics Committee of the Fundación Jiménez Díaz University Hospital (FJD, Madrid, Spain) (approval number PIC172-20_FJD) and the Galician Ethical Committee for Clinical Research (Spain-no.2006/08) following the principles of the Declaration of Helsinki and its further revisions. Written informed consent was collected from all patients, or their legal guardians, when necessary, prior to inclusion in the study.

Families were retrospectively recruited from patient registries at FJD and through collaborators from different Spanish hospitals and research institutions over the last 30 years [212]. The inclusion criteria for families was a genetic diagnosis of BBS in at least one affected member. Available clinical and familial data were extracted for each patient and reviewed through medical reports, questionaries, and/or electronic health records, as previously described [213,214].

Clinical data from all affected individuals were annotated using Human Phenotype Ontology (HPO) terms. All cases, including probands (case with the more severe phenotype) and their affected relatives, were then classified into 5 different phenotypic subgroups: i) "BBS" and ii) "BBS-like", when the patient fulfilled the clinical diagnostic criteria previously specified in Perea-Romero *et al*. [215] (Supplementary Table S1); iii) "Retinal dystrophy (RD) + OTHERS", when a syndromic patient did not meet the BBS/BBS-like minimum criteria; iv) "NON-SYNDROMIC", when relatives of a BBS/BBS-like/"RD + OTHERS" index case suffered from RD without extra-ocular features; and v) "UNCLASSIFIED", which contained patients with no clinical data available.

Molecular analysis

Diagnostic genetic testing was performed in probands using different molecular approaches over the years. These included commercial genotyping microarray for known pathogenic variants in 12 BBS genes (Asper Biotech, Estonia), Sanger sequencing of the mayor BBS genes (*BBS1* (MIM *209901), *BBS10* (MIM *610148), and *BBS12* (MIM *610683)), and/or NGS approaches, such as customized targeted NGS panels, clinical exome sequencing (CES), and/or whole-exome sequencing (WES), as previously reported [214–220].

Oligogenic analysis

The available NGS data from probands were analysed to explore the mutational load in BBS-related genes. We looked for possible third alleles in other BBS genes, which could act as modulators of the recessive biallelic variants at the primary gene. Specific subpanels of 29 and 37 genes were applied for prioritizing rare variants in CES and WES analysis, respectively (Supplementary Table S2). These virtual panels were composed of already known disease-causing genes or known modifiers of BBS/BBS-like phenotypes after a literature revision, as well as retrieved from OMIM, HGMD, and RetNet databases (The Retinal Information Network, https://sph.uth.edu/retnet/; last accessed February 2022).

A monoallelic variant was considered a candidate for being a modifier allele when it was classified as class 3, 4, or 5 according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) [221], or when it had been previously hypothesised as a modifier allele in the literature. All these putative modifier alleles and the primary biallelic

disease-causing variants were confirmed and segregated by Sanger sequencing in the available affected and unaffected family members.

After genetic analysis, families were classified according to their detected mutational load in "digenic triallelic" if three mutant alleles were found in two recessive genes, or "monogenic biallelic" if only two mutant alleles were found in a recessive BBS gene.

To consider a family as possibly triallelic, it had to have at least 2 affected relatives with differences in their genotype (e.g. proband triallelic with a biallelic sibling) and in their corresponding phenotype.

The involved genes and the three mutant alleles of each "digenic triallelic" case were analysed using two different *in silico* tools, to predict candidate gene pairs and pathogenic allele combinations that could be participating in the intrafamilial variability between BBS probands and their affected siblings. All gene pairs of probands from families with suspected oligogenic inheritance were assessed using the machine learning tool DiGePred (http://www.meilerlab.org/index.php/servers; last accessed March 2022) [190], following its "random" model, which classified gene pairs as digenic when the DiGePred value was equal or greater than 0.374. Additionally, all allelic combinations of probands were analysed *in silico* using ORVAL platform (https://orval.ibsquare.be/; last accessed March 2022) [211]. Since all combinations shared a Support Score of 100% in the ORVAL tool, the Classification Score (CS) was used as the sole predictor of the probability that each specific combination was disease-causing: i) confidence of 99% of being a candidate (CS ≥ 0.83); ii) 95%-zone candidate (CS ≥ 0.64); and iii) candidate (CS ≥ 0.532).

Statistical analysis

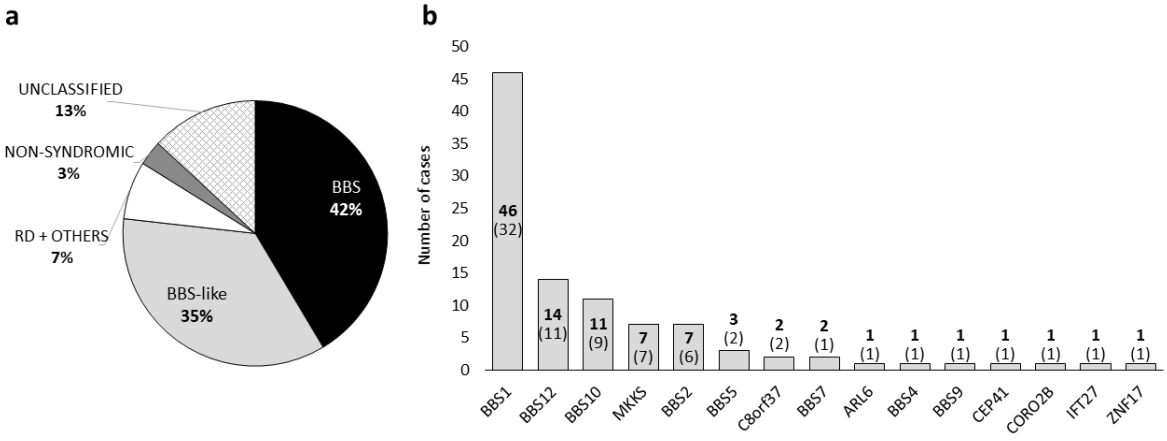
To determine the changes in the number of symptoms of the different subgroups of patients according to genetic outcome, a chi-square test was carried out and *p*-values under 0.05 were considered statistically significant.

Results

Clinical description of the cohort and genetic outcome

This study retrospectively included 99 affected individuals (77 probands and 22 affected siblings) from 77 families with a final genetic diagnosis of BBS. The cohort consisted of 54 males and 45 females (mean age: 42.2 ± 16.1 years old). Patients were phenotypically classified considering HPO terms and specific clinical criteria for BBS/BBS-like (Supplementary Table S1). After clinical annotation, the most frequent features in the whole cohort were RD, obesity and postaxial polydactyly, which appeared in more than 80% of the syndromic cases with available clinical information (n=83) (Supplementary Table S3). The 77% (76/99) of the cases in our cohort fulfilled *a priori* the diagnosis criteria for BBS or BBS-like (Fig. 1A). Among the patients who did not meet the minimum criteria for BBS/BBS-like diagnosis prior to the final molecular diagnosis, 6 cases presented visual alteration together with a combination of extra-ocular features not specific for BBS, and they were therefore classified in the group "RD+OTHERS". Besides, 3 affected siblings suffered from isolated RD and then, were classified as "NON-SYNDROMIC". Finally, no clinical data were available for 13 patients, so they were not clinically classified.

Fig. 1. Clinical classification and genetic distribution of the cohort. (A) *A priori* clinical classification. All patients have been classified into 5 categories: i) Bardet-Biedl syndrome ("BBS"); ii) suspected of BBS ("BBS-like"); iii) "RD+OTHERS"; iv) "NON-SYNDROMIC"; and v) "UNCLASSIFIED". **(B)** Distribution of the causative biallelic genes found in the complete cohort. Bold numbers indicate the total number of cases genetically diagnosed for each gene, while the number of families to which these cases belong is indicated in brackets.



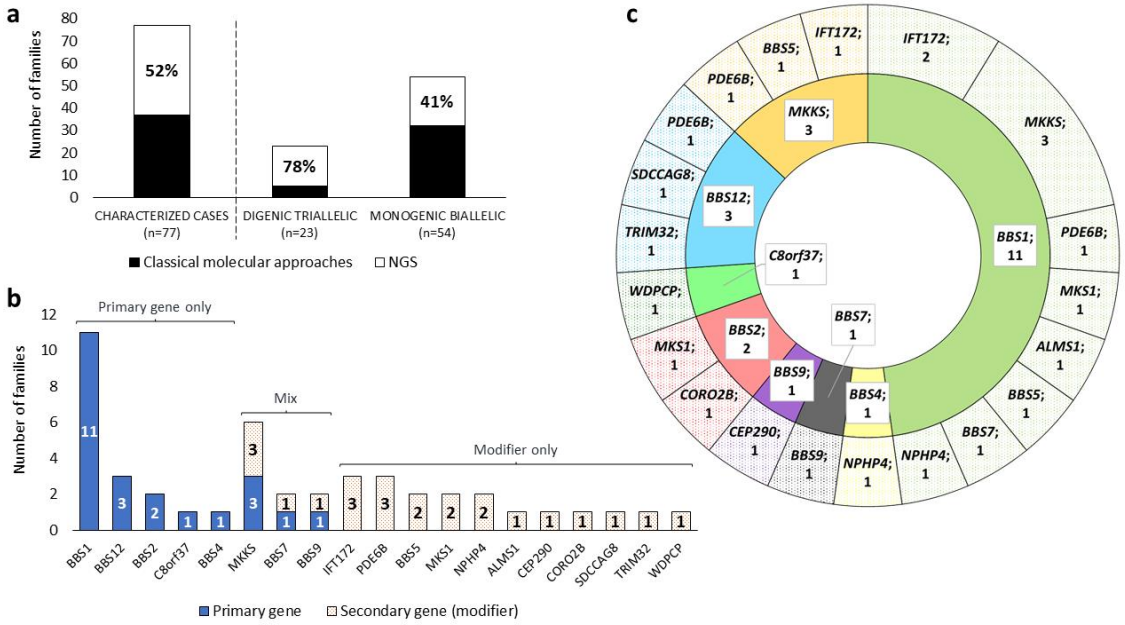
After molecular testing, a total of 15 biallelic disease-causing genes were found. The most mutated gene was *BBS1*, appearing in 42% of the families (n=32), followed by *BBS12* (14%) and *BBS10* (12%). Eight genes were found in only one family (Fig. 1B). A total of 57 different alleles were found in these 15 genes, being the missense variant p.(Met390Arg) in *BBS1* the most frequently identified among the causative alleles (32%; 64/198). The 6 more frequently identified alleles accounted for half of all alleles found (100/198) (Supplementary Table S4).

Oligogenic outcome

In our cohort of families carrying biallelic variants in a primary BBS gene, we assessed the existence of third alleles in other BBS genes to be candidate as genetic modulators. In 40 probands with available NGS data from almost all BBS genes, a NGS reanalysis was performed to explore their mutational load in BBS-related genes. For the remaining 37 index cases, the search of third-alleles has been restricted to those BBS genes screened by genotyping microarrays and Sanger sequencing (Fig. 2A). Families were grouped according to their mutational load after all the genetic studies in "digenic triallelic" (n=23) or "monogenic biallelic" (n=54) families. A total of 21 different potential monoallelic modifiers were found in 14 genes. The missense variant p.(Ala242Ser) in *MKKS* was the most prevalent variant among modifier alleles (16%; 4/25) (Supplementary Table S4).

Fig. 2. Mutational load in BBS-related genes. (A) Distribution of BBS families according to the molecular screening and mutational load. Families are grouped as "digenic triallelic" (three mutant alleles in two recessive genes) and "monogenic biallelic" (two mutant alleles in a recessive gene). NGS includes customized targeted NGS panel, clinical exome sequencing (CES), and/or whole-exome sequencing (WES). **(B)** Mutational load and gene role in the allelic combination. The genes found in the "digenic triallelic" families are grouped considering if they are primary (recessive biallelic gene) or secondary (monoallelic modifier) genes. **(C)** Distribution of the oligogenic cases according to the primary and secondary genes found in the 23 families with suspected triallelism. Only the genetic outcome of each proband is shown. The inner and

outer circles represent the biallelic primary gene and the monoallelic gene (or possible modifier) accompanying the biallelic variants, respectively.



NGS data allowed to screen a larger number of genes than traditional methods. As a result, in the cases studied, there was a higher proportion of families falling into the "digenic triallelic" subgroup (78%) than into the "monogenic biallelic" subgroup (41%) (Fig. 2A).

The overall oligogenic rate was 51% (23/45). After NGS reanalysis, 18 families were positive for a third allele in a secondary BBS-related gene, plus 5 additional oligogenic positive families that were identified by traditional methods. The presence of a potential modifier allele could only be excluded in the 29% (22/77) of the cases, which had been analysed by NGS as first-tier approach or reanalysed to assess the presence of additional alleles in already known BBS-related genes.

Regarding mutational load, *BBS1*, *BBS12*, *BBS2*, *C8orf37* (MIM *614477), and *BBS4* (MIM *600374) always appeared as the main cause of disease, e. g. primary gene, in the "digenic triallelic" cases (Fig. 2B), whereas *MKKS*, *BBS7* (MIM *607590), and *BBS9* (MIM *607968) could take all allelic roles. Besides, eleven genes have only been found as possible modifiers.

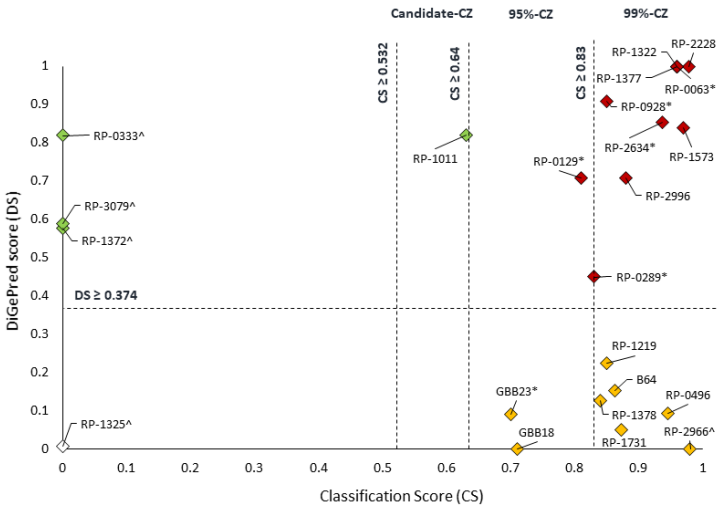
Twenty different combinations of two BBS genes were identified within the triallelic families, being *BBS1* the most frequently involved in 11 of them as the primary gene. Three genes were overrepresented as modifiers compared to the rest, which were *MKKS*, *IFT172* (MIM *607386), and *PDE6B* (MIM *180072). In addition, only the combination of *BBS1_MKKS* (n=3) and *BBS1_IFT172* (n=2) appeared more than once (Fig. 2C). However, each allelic combination was private, so no common distribution of alleles between families was found (Supplementary Table S5 and S6). All these gene pairs and allelic combinations were rated using DiGePred classifier and ORVAL platform, respectively. However, predictions could not be made for one of the 23 gene pairs and for 4 of the 23 allelic combinations (Fig.3A). According to the potential oligogenic pathogenic effects, 44% (10/23) of the identified triallelic combinations were predicted to have an oligogenic inheritance with both methods with a 95% of confidence in ORVAL and the highest confidence threshold in DiGePred. Besides, the possibility of oligogenic inheritance reached 91% at the same confidence when only one method was considered (Fig. 3A-B).

Fig. 3. Predicted oligogenic effects of allelic combinations in BBS-related genes. (A) List of the allelic combination of the 23 families and their DiGePred and ORVAL scores. The families were grouped according to the scores obtained in those two *in silico* tools in: i) high confidence in both methods (red colour), gene pair with digenic disease potential (DiGePred Score (DS) ≥ 0.374) and predicted oligogenic combination of variants with a confidence of at least 95% (Classification Score (CS) ≥ 0.64); ii) high confidence for genes with digenic potential, DS ≥ 0.374 (green); iii) high confidence for the oligogenic combination, but in genes with lower potential for digenism, 95%-zone candidate in ORVAL (CS ≥ 0.64) (orange); and iv) combinations in which

a

	ID_Primary gene_Modifier	CS_ORVAL	DS_DiGePred
*	RP-2228_*BBS1_MKKS*	0.979	1
*	RP-0063_*BBS1_MKKS*	0.96	1
	RP-1377_*BBS1_MKKS*	0.96	1
	RP-1322_*BBS1_BBS7*	0.96	1
*	RP-0928_*BBS7_BBS9*	0.8507	0.908
*	RP-2634_*BBS1_BBS5*	0.9372	0.854
	RP-1573_*MKKS_BBS5*	0.9698	0.84
	RP-2996_*BBS1_IFT172*	0.88	0.707
*	RP-0129_*BBS1_IFT172*	0.81	0.707
*	RP-0289_*BBS1_MKS1*	0.83	0.45
	RP-1219_*BBS2_MKS1*	0,85	0.224
	B64_*BBS12_PDE6B*	0.8635	0.152
	RP-1378_*BBS1_PDE6B*	0.84	0.127
	RP-0496_*MKKS_PDE6B*	0.946	0.0927
*	GBB23_*BBS1_ALMS1*	0.7	0.0895
	RP-1731_*BBS1_NPHP4*	0.8735	0.05
	GBB18_*BBS2_CORO2B*	0.71	0.000913
^	RP-2966_*C8orf37_WDPCP*	0.98	NA
	RP-1011_*BBS12_SDCCAG8*	0.63	0.819
^	RP-0333_*BBS12_TRIM32*	NA	0.819
^	RP-3079_*BBS9_CEP290*	NA	0.59
^	RP-1372_*MKKS_IFT172*	NA	0.577
^	RP-1325_*BBS4_NPHP4*	NA	0.00669

b



digenic potential is discarded (white). ^Predictions in one of the methods could not be made for 5 allelic combinations. *Six families in which phenotypic and allelic differences between affected siblings were reported. **(B)** Graphic representation of the scores obtained in DiGePred and ORVAL.

There were no significant differences in the distribution of symptoms between the cases with respect to their detected mutational load. However, there was slight enrichment in the frequency of aganglionic megacolon in “digenic triallelic” cases, and brachydactyly in “monogenic biallelic” (Supplementary Table S3).

Evidence of modifier alleles in our cohort

Among the 23 families in which a third allele apart from biallelic disease-causing variants was identified, we assessed the possibility of being true oligogenic modifiers in view of several facts, such as the oligogenic effect predicted by ORVAL and DiGePred (Fig. 3), the informativity of the family, and intrafamilial differences in the penetrance of major BBS features and/or the severity within the affected individuals.

We found 3 families with differences in the severity of their syndromic-associated symptoms (family IDs GBB23, RP-0063, and RP-0928), having the triallelic index case a more severe presentation than its biallelic sibling (Supplementary Table S5). First, in the family GBB23 with two siblings with biallelic *BBS1* variants causing a clinical diagnosis of BBS, the proband who also carry a third missense allele in *ALMS1* (p.(His3880Tyr)), developed type 2 diabetes mellitus (T2DM). Only ORVAL predicted the *BBS1_ALMS1* combination as oligogenic with a 95% of

confidence (CS = 0.7). Secondly, biallelic *BBS1* variants were found in the two siblings affected of BBS of family RP-0063. The proband, which also presented polydactyly, intellectual disability, renal anomalies, asthma, and seizures, carried a third allele in *MKKS* (p.(Ala242Ser)). This combination was predicted to be oligogenic by ORVAL with 99% of confidence (CS = 0.96) and DiGePred (DS = 1). Finally, family RP-0928 was formed by two BBS-affected siblings with biallelic *BBS7* variants. In the proband, who also exhibited hepatic steatosis, an additional allele in *BBS9* (p.(Met126Leu)) was found. This combination had a CS and a DS of 0.8507 and 0.908, respectively. It was therefore predicted as oligogenic with 99% of confidence by ORVAL platform.

Interestingly, an oligogenic effect was also suspected in 3 other families (family IDs RP-0129, RP-0289, and RP-2634) due to the absence of syndromic features in one biallelic affected sibling, who only suffered from isolated RD, while the triallelic proband had syndromic clinical features (Supplementary Table S5). ORVAL with 95% confidence and DiGePred predicted a possible oligogenic effect in all combinations with possible triallelic inheritance in the probands of these families (Supplementary Table S5 and Fig. 3).

The modifier effect in 17 out of 23 suspected of triallelic inheritance remained unclear due to the lack of familial informativeness and/or clinical data. While individuals from the families RP-1322, RP-1377, and RP-1378, both presented the same genotype and/or syndromic phenotype (Supplementary Table S5), triallelic inheritance was found in 13 sporadic cases with no other affected relative. Furthermore, there was a lack of clinical information in an additional family (B64). However, ORVAL (95%-candidate zone) together with DiGePred predicted a likely oligogenic effect in 5 allelic combinations found in these families, and only one of those platforms in 11 more (Fig. 3).

DISCUSSION

BBS is an extremely clinically and genetically heterogeneous ciliopathy characterized by intra- and inter-familial variability [199,222]. Generally, BBS presents with an autosomal recessive inheritance, but in some cases, an oligogenic inheritance has been proposed, in the form of triallelism or second-site modifiers [100,201,204,205,223]. In these families, some unaffected or less severely affected individuals have 2 pathogenic variants in a BBS-related gene, whereas the BBS-diagnosed or more severely affected relatives carry 3 alleles in 2 different BBS-related genes. Although the involvement of triallelism in BBS families is controversial [201–203,206], there is further evidence for the possible existence of third modifier alleles [204–207]. Hence, our study presents a comprehensive study of the mutational burden in BBS and highlights the importance of considering non-Mendelian inheritance to improve the clinical management of BBS.

First, we recruited a large cohort of 99 cases from 77 families with genetic suspicion of BBS after molecular testing. Although other studies only consider those cases that met the diagnostic criteria for BBS [203,205,206], we have included also patients who did not fulfil *a priori* the clinical criteria of BBS described by Forsythe *et al*., 2013 [198]. In fact, half of the syndromic cases with available clinical information (41/83) were not first classified as BBS but after genetic testing. Thus, for some of the clinical features, a slight bias was observed in our cohort compared to the distribution in other reports [198,199,224], which can be explained by the inclusion of cases with a diagnosis of “BBS-like” or even more unspecific systemic findings (“RD-OTHERS”), or a poor clinical acquisition of some of the features.

Nowadays, NGS is the technology of choice for the study of BBS [225–227]. It allows the identification of new causative variants and further reanalysis to assess new BBS genes that may have been identified after a primary analysis or had not been covered by any of the classical methods initially used [228,229]. This statement is consistent with the fact that 78% of the suspected oligogenic families in our cohort were discovered through NGS approaches.

It has been estimated that oligogenic inheritance is present in less than 10% of the BBS families [230]. This value is confirmed by our triallelic distribution, with a rate of 13% in the informative triallelic families. However, it increases to 51% when all families with suspected modifiers alleles were included. We cannot elucidate if the third allele triggers a modifier effect in most families, because both siblings presented the same genotype-phenotype, the index case was the only affected in the family, or clinical information was unavailable.

BBS1 can cause both BBS and non-syndromic inherited RD. This gene is the most frequent source of BBS, accounting for 23%-51% of characterized families [205,231,232], which agrees with 42% of molecularly characterized families with *BBS1* as the major primary gene in our cohort. The variant p.(Met390Arg) has been reported in up to 80% of *BBS1*-related alleles across different worldwide populations [231], being mostly associated with BBS as only 21% of *BBS1* carriers showed non-syndromic presentations [212]. In our cohort, this variant is accordingly the most represented with an allelic frequency of 70%. The reason for this slight decrease may be that we only included BBS-associated families but excluded those with only non-syndromic affected individuals.

The implication of *BBS1* in oligogenic inheritance remains unclear. Some reports claim that *BBS1* is rarely involved in complex inheritance [231], but *BBS1* has been reported in a triallelic inheritance in 15% of families, being the primary gene instead of acting as modifier in only 4% of cases [205]. However, our results go further, showing that only 65% of our *BBS1*-characterized families fit in an autosomal recessive inheritance, while in the remaining families, *BBS1* might participate in oligogenic inheritance as the primary gene in 11 families, together with *MKKS*, *IFT172*, or other 6 BBS-related genes. This same behaviour was observed for *BBS12* in 3 families from our cohort, but in this case, there are no reports related to the likelihood of its participation in complex inheritance. In those families, biallelic *BBS12* variants were found together with the missense variants p.(Gly352Val) in *PDE6B*, p.(Arg82Leu) in *TRIM32* (MIM *602290), and p.(Arg400Cys) in *SDCCAG8* (MIM *613524), respectively. While the combinations *BBS12_SDCCAG8* and *BBS12_TRIM32* were predicted only with DiGePred, only the combination *BBS12_PDE6B* was predicted as oligogenic with ORVAL platform with a 99% of confidence.

Generally, BBS-associated proteins are located at the base of the cilium and participate in ciliary biogenesis and in cilia function [198,233], but a variety of specific locations and functions have been described [81,234]. In our work, the involvement of BBS proteins in a specific complex, structure, or process does not seem to be related to the level of involvement of each gene in the triallelic inheritance. However, we have seen that the genes that encode for the chaperonin-like complex (*BBS10*, *BBS12,* and *MKKS*) are mostly implied in a recessive inheritance. Moreover, *BBS12* and *MKKS* are normally the primary gene when are involved in oligogenic inheritance. Therefore, these genes are usually the principal gene causing the disease regardless of the type of inheritance. It has been reported that the activity of genes encoding BBSome components (*ARL6, BBIP1, BBS1, BBS2, BBS5, BBS7, BBS9* and *TTC8*) may be dependent on the chaperonin-like genes [235] and families with variants in the chaperonin-like complex present a more severe phenotype [219], so these chaperonin-like genes may not normally require a second-site modifier. Nevertheless, we found 3 families from our cohort carrying the variant p.(Ala242Ser)

in *MKKS* as a possible modifier allele in combination with biallelic *BBS1* variants. This non-synonymous change, which has been previously described as a dominant-negative allele [210,236], disrupt the protein conformation of the BBSome, thus preventing them from doing their proper function [210,236]. Intrafamilial variable severity has been seen in one of these 3 families carrying the p.(Ala242Ser) variant in combination with homozygous p.(Met390Arg) in *BBS1*. Our result suggests that the increase in the detected mutational load may correlate with a more severe phenotype, which could be explained by its chaperone function [214]. Variable expressivity between siblings involving other genetic combinations can be also found in other 5 families in our cohort.

The presence of modifier alleles can determine the phenotype, since they may influence the presentation of the BBS phenotype [210]. This can be the scenario for family RP-2634, in which the syndromic index case carries two compound heterozygous variants in *BBS1* and the heterozygous missense p.(Arg207His) in *BBS5*, whereas her non-syndromic sister is just biallelic for the *BBS1* variants. This *BBS5* variant, presenting a minor-allele frequency of 0.9% in Europeans, has been predicted as a null mutation [210].

Specific heterozygous variants acting as modifiers has previously been associated with the existence of particular findings (e.g. ocular, neurological, or renal features) [237–239]. A sibling from family GBB23 carries the homozygous pathogenic variant p.(Met390Arg) in *BBS1*, and additionally, the index case suffering from T2DM also carries the heterozygous variant p.(His3880Tyr) in *ALMS1*. Mutations in *ALMS1* are the cause of Alström syndrome (ALMS; MIM #203800), an ultra-rare metabolic ciliopathy associated with severe visual impairment, sensorineural deafness, obesity, insulin resistance, T2DM, and hypogonadism, among other features [240]. One of the explanations of glucose metabolism alterations in this syndrome are defects in the ALMS1 protein, which participates in the insulin-regulated glucose transport [241]. In our family, the variant identified in *ALMS1* may be acting as a second-site modifier altering the possibility of suffering T2DM. However, the high frequency of diabetes mellitus in the general population [242] might also be a plausible explanation for its presence in this case.

Some of the possible modifier alleles in the BBS-causing genes found in our cohort could be good candidates for functional studies to analyse a possible modifying effect on the BBS phenotype, e.g. *IFT172*, *TRIM32*, or *WDPCP*. Furthermore, we identified third alleles in other genes previously reported as possible candidates or modifiers of BBS, e.g. *ALMS1*, *CORO2B*, *NPHP4*, or *PDE6B* [219,243–245]. Therefore, our findings could also support a possible involvement of these genes in the pathogenesis of BBS. For example, *PDE6B* is a gene associated with non-syndromic RD, but also reported to BBS phenotype in a consanguineous family with homozygous pathogenic mutations in *BBS10* and *PDE6B* [245]. Here, three different heterozygous variants in *PDE6B* were found in 3 families (family IDs RP-1378, RP-0496, and B64), accompanying biallelic variants in *BBS1*, *MKKS*, and *BBS12*, respectively. It is unknown how the effect of the mutational load detected in these families may be affecting their phenotype, but *PDE6B* and other genes, which are involved in the phototransduction and visual transduction pathways, are downregulated in BBS and ALMS in zebrafish models and may be drivers of the retinal degeneration [246]. Nonetheless, the hypothetical role of *PDE6B* and other unclear modifiers in BBS should be further studied functionally.

The effect of modifier alleles on clinical manifestation needs to be assessed usually by means of *in vitro* and *in vivo* strategies [209,210]. However, due to technical limitations it is not always possible to perform these analyses in a clinical setting. Alternatively, *in silico* tools can help to discover and predict combinations of variants that can be affecting the patients' phenotype

[190,211]. According to our data, ORVAL and DiGePred together support an oligogenic inheritance in 44% of our families with more than two alleles, reaching 91% with only one positive method.

To understand the phenotypic variability in BBS families, both genetic and environmental factors should be considered. Recently, multi-omics analyses are being considered to understand and elucidate the role of the mutational load in BBS-associated mechanisms through the integration of multiple analyses (mutational load, differential gene and/or protein expression, epigenetic, and/or metabolome-based signatures). Therefore, obtaining larger data sets would help to clarify the role of possible modifiers in BBS and related ciliopathies [225,247].

In summary, this work deepens into the controversial topic of oligogenic inheritance in BBS, finding new evidence for existence of second-site genetic modifiers as cause of intrafamilial variability in this disease. Besides, it highlights the importance of the use of NGS in the genetic diagnosis of BBS.

References of the Supplementary Material

1. Forsythe, E. & Beales, P. L. Bardet-Biedl syndrome. Eur. J. Hum. Genet. EJHG 21, 8–13 (2013).
2. M'hamdi, O., Ouertani, I. & Chaabouni-Bouhamed, H. Update on the genetics of bardet-biedl syndrome. Mol. Syndromol. 5, 51–56 (2014).
3. Klein, D. & Ammann, F. The syndrome of Laurence-Moon-Bardet-Biedl and allied diseases in Switzerland. Clinical, genetic and epidemiological studies. J. Neurol. Sci. 9, 479–513 (1969).
4. Farag, T. I. & Teebi, A. S. High incidence of Bardet Biedl syndrome among the Bedouin. Clin. Genet. 36, 463–464 (1989).
5. Moore, S. J. et al. Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. Am. J. Med. Genet. A. 132A, 352–360 (2005).
6. Forsyth, R. & Gunay-Aygun, M. Bardet-Biedl Syndrome Overview. in GeneReviews® (eds. Adam, M. P. et al.) (University of Washington, Seattle, 1993).
7. Reiter, J. F. & Leroux, M. R. Genes and molecular pathways underpinning ciliopathies. Nat. Rev. Mol. Cell Biol. 18, 533–547 (2017).
8. Katsanis, N. et al. Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. Science 293, 2256–2259 (2001).
9. Smaoui, N. et al. Screening of the eight BBS genes in Tunisian families: no evidence of triallelism. Invest. Ophthalmol. Vis. Sci. 47, 3487–3495 (2006).
10. Abu-Safieh, L. et al. In search of triallelism in Bardet-Biedl syndrome. Eur. J. Hum. Genet. EJHG 20, 420–427 (2012).
11. Katsanis, N. et al. BBS4 is a minor contributor to Bardet-Biedl syndrome and may also participate in triallelic inheritance. Am. J. Hum. Genet. 71, 22–29 (2002).
12. Beales, P. L. et al. Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. Am. J. Hum. Genet. 72, 1187–1199 (2003).
13. Badano, J. L. et al. Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. Hum. Mol. Genet. 12, 1651–1659 (2003).
14. Bin, J. et al. BBS7 and TTC8 (BBS8) mutations play a minor role in the mutational load of Bardet-Biedl syndrome in a multiethnic population. Hum. Mutat. 30, E737-746 (2009).

15. Katsanis, N. The oligogenic properties of Bardet-Biedl syndrome. Hum. Mol. Genet. 13 Spec No 1, R65-71 (2004).
16. Woodsmith, J. et al. Protein interaction perturbation profiling at amino-acid resolution. Nat. Methods 14, 1213–1221 (2017).
17. Zaghloul, N. A. et al. Functional analyses of variants reveal a significant role for dominant negative and common alleles in oligogenic Bardet-Biedl syndrome. Proc. Natl. Acad. Sci. U. S. A. 107, 10602–10607 (2010).
18. Renaux, A. et al. ORVAL: a novel platform for the prediction and exploration of disease-causing oligogenic variant combinations. Nucleic Acids Res. 47, W93–W98 (2019).
19. Mukherjee, S. et al. Identifying digenic disease genes via machine learning in the Undiagnosed Diseases Network. Am. J. Hum. Genet. 108, 1946–1963 (2021).
20. Perea-Romero, I. et al. Genetic landscape of 6089 inherited retinal dystrophies affected cases in Spain and their therapeutic and extended epidemiological implications. Sci. Rep. 11, 1526 (2021).
21. Beales, P. L., Elcioglu, N., Woolf, A. S., Parker, D. & Flinter, F. A. New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. J. Med. Genet. 36, 437–446 (1999).
22. Castro-Sánchez, S. et al. Exploring genotype-phenotype relationships in Bardet-Biedl syndrome families. J. Med. Genet. 52, 503–513 (2015).
23. Perea-Romero, I. et al. NGS and phenotypic ontology-based approaches increase the diagnostic yield in syndromic retinal diseases. Hum. Genet. 140, 1665–1678 (2021).
24. Pereiro, I. et al. New mutations in BBS genes in small consanguineous families with Bardet-Biedl syndrome: detection of candidate regions by homozygosity mapping. Mol. Vis. 16, 137–143 (2010).
25. Pereiro, I. et al. Arrayed primer extension technology simplifies mutation detection in Bardet-Biedl and Alström syndrome. Eur. J. Hum. Genet. EJHG 19, 485–488 (2011).
26. Álvarez-Satta, M. et al. Overview of Bardet-Biedl syndrome in Spain: identification of novel mutations in BBS1, BBS10 and BBS12 genes. Clin. Genet. 86, 601–602 (2014).
27. Castro-Sánchez, S. et al. Whole exome sequencing as a diagnostic tool for patients with ciliopathy-like phenotypes. PloS One 12, e0183081 (2017).
28. Sanchez-Navarro, I. et al. Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. Sci. Rep. 8, 5285 (2018).
29. Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. Off. J. Am. Coll. Med. Genet. 17, 405–424 (2015).
30. Riise, R. et al. Intrafamilial variation of the phenotype in Bardet-Biedl syndrome. Br. J. Ophthalmol. 81, 378–385 (1997).
31. Eichers, E. R., Lewis, R. A., Katsanis, N. & Lupski, J. R. Triallelic inheritance: a bridge between Mendelian and multifactorial traits. Ann. Med. 36, 262–272 (2004).
32. Badano, J. L. et al. Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. Hum. Mol. Genet. 12, 1651–1659 (2003).
33. Rooryck, C. & Lacombe, D. [Bardet-Biedl syndrome]. Ann. Endocrinol. 69, 463–471 (2008).
34. Forsythe, E., Kenny, J., Bacchelli, C. & Beales, P. L. Managing Bardet-Biedl Syndrome-Now and in the Future. Front. Pediatr. 6, 23 (2018).

35. Jeziorny, K. et al. Next-Generation Sequencing in the Diagnosis of Patients with Bardet-Biedl Syndrome-New Variants and Relationship with Hyperglycemia and Insulin Resistance. Genes 11, E1283 (2020).
36. Castro-Sánchez, S., Álvarez-Satta, M., Pereiro, I., Piñeiro-Gallego, M. T. & Valverde, D. [Algorithm for the molecular analysis of Bardet-Biedl syndrome in Spain]. Med. Clin. (Barc.) 145, 147–152 (2015).
37. Marion, V. et al. Exome sequencing identifies mutations in LZTFL1, a BBSome and smoothened trafficking regulator, in a family with Bardet--Biedl syndrome with situs inversus and insertional polydactyly. J. Med. Genet. 49, 317–321 (2012).
38. Ajmal, M. et al. Exome sequencing identifies a novel and a recurrent BBS1 mutation in Pakistani families with Bardet-Biedl syndrome. Mol. Vis. 19, 644–653 (2013).
39. Khan, S. A. et al. Genetics of human Bardet-Biedl syndrome, an updates. Clin. Genet. 90, 3–15 (2016).
40. Mykytyn, K. et al. Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (BBS1). Am. J. Hum. Genet. 72, 429–437 (2003).
41. Forsythe, E. et al. Risk Factors for Severe Renal Disease in Bardet-Biedl Syndrome. J. Am. Soc. Nephrol. JASN 28, 963–970 (2017).
42. Focșa, I. O. et al. A case of Bardet-Biedl syndrome caused by a recurrent variant in BBS12: A case report. Biomed. Rep. 15, 103 (2021).
43. Suspitsin, E. N. & Imyanitov, E. N. Bardet-Biedl Syndrome. Mol. Syndromol. 7, 62–71 (2016).
44. Florea, L., Caba, L. & Gorduza, E. V. Bardet-Biedl Syndrome-Multiple Kaleidoscope Images: Insight into Mechanisms of Genotype-Phenotype Correlations. Genes 12, 1353 (2021).
45. Niederlova, V., Modrak, M., Tsyklauri, O., Huranova, M. & Stepanek, O. Meta-analysis of genotype-phenotype associations in Bardet-Biedl syndrome uncovers differences among causative genes. Hum. Mutat. 40, 2068–2087 (2019).
46. Nachury, M. V. et al. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. Cell 129, 1201–1213 (2007).
47. Tory, K. et al. High NPHP1 and NPHP6 mutation rate in patients with Joubert syndrome and nephronophthisis: potential epistatic effect of NPHP6 and AHI1 mutations in patients with NPHP1 mutations. J. Am. Soc. Nephrol. JASN 18, 1566–1575 (2007).
48. Khanna, H. et al. A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. Nat. Genet. 41, 739–745 (2009).
49. Louie, C. M. et al. AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. Nat. Genet. 42, 175–180 (2010).
50. Marshall, J. D., Beck, S., Maffei, P. & Naggert, J. K. Alström syndrome. Eur. J. Hum. Genet. EJHG 15, 1193–1202 (2007).
51. Dassie, F. et al. Alström syndrome: an ultra-rare monogenic disorder as a model for insulin resistance, type 2 diabetes mellitus and obesity. Endocrine 71, 618–625 (2021).
52. Cho, N. H. et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. 138, 271–281 (2018).
53. González-Del Pozo, M. et al. Deciphering intrafamilial phenotypic variability by exome sequencing in a Bardet-Biedl family. Mol. Genet. Genomic Med. 2, 124–133 (2014).
54. Lindstrand, A. et al. Copy-Number Variation Contributes to the Mutational Load of Bardet-Biedl Syndrome. Am. J. Hum. Genet. 99, 318–336 (2016).
55. Chakrabarty, S., Savantre, S. B., Ramachandra Bhat, C. & Satyamoorthy, K. Multiple genetic mutations implicate spectrum of phenotypes in Bardet-Biedl syndrome. Gene 725, 144164 (2020).

56. Hostelley, T. L., Lodh, S. & Zaghloul, N. A. Whole organism transcriptome analysis of zebrafish models of Bardet-Biedl Syndrome and Alström Syndrome provides mechanistic insight into shared and divergent phenotypes. BMC Genomics 17, 318 (2016).
57. Kenny, J., Forsythe, E., Beales, P. & Bacchelli, C. Toward personalized medicine in Bardet-Biedl syndrome. Pers. Med. 14, 447–456 (2017).

Supplementary Table S1. Clinical criteria for the diagnosis of Bardet-Biedl syndrome (BBS) or BBS-like [1].

	Major finding (MC)	Minor finding (mc)	Supportive evidence (SE)	Disease diagnostic criteria	Disease-like diagnostic criteria
BBS [2]	- Rod-cone dystrophy - Postaxial polydactyly - Obesity - Abnormality of the female and male genitalia - Abnormality of the kidney - Specific learning disability, intellectual disability	- Delayed speech and language development - Global developmental delay - Type II diabetes mellitus - Abnormality of the teeth - Abnormal heart morphology - Brachydactyly syndrome, syndactyly - Ataxia, poor coordination - Anosmia, hyposmia	- Chronic otitis media - Aganglionic megacolon (Hirschprung disease)	a) 4 MC or b) 3 MC and 2 mc	a) At least, 3 MC and 1 mc or b) less MC, but including postaxial polydactyly

1. Perea-Romero, I. *et al.* NGS and phenotypic ontology-based approaches increase the diagnostic yield in syndromic retinal diseases. *Hum Genet* **140**, 1665–1678 (2021).

2. Forsythe, E. & Beales, P. L. Bardet-Biedl syndrome. *Eur J Hum Genet* **21**, 8–13 (2013).

Supplementary Table S2. **List of the genes included in the 29-gene subpanel for clinical exome sequencing (CES) and 37-gene subpanel for whole exome sequencing (WES).** Already reported disease-causing genes and modifiers of BBS have been included. From the 40 out of 77 families that were studied using NGS, 33 had only CES and the remaining 7 had WES.

	Disease-causing gene	Modifier	CES	WES
ADIPOR1 **(NM_015999.6)**	X		X	X
ALMS1 **(NM_015120.4)**	X	X	X	X
ARL6 **(NM_177976.3)**	X	X	X	X
BBIP1 **(NM_001195306.1)**	X			X
BBS1 **(NM_024649.5)**	X	X	X	X
BBS10 **(NM_024685.4)**	X		X	X
BBS12 **(NM_152618.3)**	X		X	X
BBS2 **(NM_031885.5)**	X		X	X
BBS4 **(NM_033028.5)**	X	X	X	X
BBS5 **(NM_152384.3)**	X	X	X	X
BBS7 **(NM_176824.3)**	X	X	X	X
BBS9 **(NM_198428.3)**	X	X	X	X
C8orf37 **(NM_177965.3)**	X	X	X	X
CCDC28B **(NM_024296.5)**		X	X	X
CEP164 **(NM_014956.5)**	X			X
CEP19 **(NM_032898.5)**	X			X
CEP290 **(NM_025114.4)**	X	X	X	X
CEP41 **(NM_018718.3)**	X		X	X
CORO2B **(NM_006091.5)**	X			X
IFT172 **(NM_015662.3)**	X		X	X
IFT27 **(NM_006860.5)**	X			X
IFT74 **(NM_025103.4)**	X			X
KIF7 **(NM_198525.3)**	X		X	X
LZTFL1 **(NM_020347.4)**	X		X	X
MKKS **(NM_170784.3)**	X	X	X	X
MKS1 **(NM_017777.4)**	X		X	X
NPHP1 **(NM_001128178.3)**	X		X	X
NPHP4 **(NM_015102.5)**	X	X	X	X
PDE6B **(NM_000283.4)**	X		X	X
SCAPER **(NM_020843.4)**	X			X
SCLT1 **(NM_144643.4)**	X			X
SDCCAG8 **(NM_006642.5)**	X	X	X	X
TMEM67 **(NM_153704.6)**	X	X	X	X
TRIM32 **(NM_001099679.2)**	X		X	X
TTC21B **(NM_024753.5)**	X		X	X
TTC8 **(NM_144596.4)**	X		X	X
WDPCP **(NM_015910.7)**	X		X	X

Supplementary Table S3. **Summary of the clinical data of the 83 patients presenting systemic features.** Three non-syndromic cases of retinal dystrophies and 13 unclassified cases due to the lack of clinical information were not included. After oligogenic analyses of BBS-related gene, cases were classified according to their detected mutational load in: i) "digenic triallelic (n = 24) or ii) "monogenic biallelic" (n = 21). In the remaining 38 cases, no oligogenic studies were performed. Clinical presentation of all patients was annotated using Human Phenotype Ontology (HPO) terms. The *p-value* of the chi-square test has to be under 0.05 to be considered statistically significant. Freq, frequency; NA, non-applicable; SE, supportive evidence.

	Clinical feature	HPO number	Freq - cohort	Freq - digenic triallelic cases (1)	Freq - monogenic biallelic cases (2)	Freq - unknown cases (3)	*p-value* [1-3]	*p-value* [1-2]
Major criteria	Retinal dystrophy	HP:0000556	98% (78/80)	100% (24/24)	95% (18/19)	97% (36/37)	0.544	0.256
	Obesity	HP:0001513	90% (70/78)	91% (21/23)	84% (16/19)	92% (33/36)	0.658	0.480
	Postaxial polydactyly	HP:0100259	83% (66/80)	78% (18/23)	70% (14/20)	92% (34/37)	0.095	0.536
	Intellectual disability / Specific learning disability	HP:0001249 / HP:0001328	54% (38/71)	48% (11/23)	53% (8/15)	58% (19/33)	0.772	0.74
	Abnormality of genital system	HP:0000078	48% (24/50)	44% (7/16)	56% (5/9)	48% (12/25)	0.852	0.571
	Abnormality of the kidney	HP:0000077	31% (15/49)	28% (5/18)	46% (5/11)	25% (5/20)	0.471	0.331
Minor feature	Global developmental delay	HP:0001263	50% (27/54)	47% (7/15)	43% (6/14)	54% (14/25)	0.700	0.837
	Brachydactyly	HP:0001156	47% (15/32)	33% (5/15)	75% (3/4)	54% (7/13)	0.269	0.134
	Abnormal heart morphology	HP:0001627	25% (10/40)	21% (3/14)	22% (2/9)	29% (5/17)	0.857	0.964
	Delayed speech and language development	HP:0000750	15% (4/27)	10% (1/10)	25% (3/12)	0% (0/5)	0.361	0.364
	Type II diabetes mellitus	HP:0005978	4% (3/78)	4% (1/24)	5% (1/20)	3% (1/34)	0.926	0.895
	Abnormality of the dentition	HP:0000164	3% (2/78)	0% (0/24)	0% (0/20)	6% (2/34)	0.265	NA
	Ataxia	HP:0001251	NA	NA	NA	NA	NA	NA
	Anosmia	HP:0000458	NA	NA	NA	NA	NA	NA
SE	Hearing abnormality	HP:0000364	8% (6/77)	4% (1/24)	5% (1/20)	12% (4/33)	0.468	0.895
	Aganglionic megacolon	HP:0002251	3% (2/78)	8% (2/24)	0% (0/20)	0% (0/34)	0.099	0.186

Supplementary Table S4. Allelic load in BBS genes in the complete cohort. A total of 223 alleles, 198 in the primary gene and 25 potential modifiers, were identified in the 99 affected cases, which corresponded to 79 different alleles. Allelic frequencies of the general population and Southern European (SE) were searched in gnomAD version v2.1.1. NA, non-applicable.

Nº of alleles	Gene	Variant	Allele found in BBS and non-syndromic	Role in disease	Cohort allele frequency	GnomAD allelic frequency_ALL	GnomAD allelic frequency_SE
Alleles in primary genes							
64	*BBS1*	NM_024649.5: c.1169T>G; p.(Met390Arg)	X	Primary	2.87E-1	1.57E-3	2.5E-3
11	*BBS1*	NM_024649.5: c.1645G>T; p.(Glu549*)		Primary	4.93E-2	1.59E-5	0.000
8	*BBS12*	NM_152618.3: c.1115_1116del; p.(Phe372*)		Primary	3.59E-2	8.64E-5	0.000
6	*BBS1*	NM_024649.5: c.1097T>A; p.(Val366Asp)		Primary	2.69E-2	NA	NA
6	*BBS2*	NM_031885.5: c.823C>T; p.(Arg275*)		Primary	2.69E-2	1.91E-4	8.69E-5
5	*BBS12*	NM_152618.3: c.263T>G; p.(Leu88Arg)		Primary	2.24E-2	NA	NA
4	*BBS10*	NM_024685.4: c.1677C>A; p.(Tyr559*)		Primary	1.79E-2	3.99E-5	0.000
4	*BBS10*	NM_024685.4: c.271dupT; p.(Cys91Leufs*5)		Primary	1.79E-2	5.79E-4	1.75E-4
4	*BBS10*	NM_024685.4: c.850C>T; p.(Gln284*)		Primary	1.79E-2	8.02E-6	0.000
4	*BBS2*	NM_031885.5: c.565C>T; p.(Arg189*)		Primary	1.79E-2	7.96E-6	0.000
4	*BBS12*	NM_152618.3: c.323C>T; p.(Pro108Leu)		Primary	1.79E-2	NA	NA
3	*BBS10*	NM_024685.4: c.992T>C; p.(Val331Ala)		Primary	1.35E-2	NA	NA
3	*BBS12*	NM_152618.3: c.1616G>A; p.(Gly539Asp)		Primary	1.35E-2	NA	NA
3	*MKKS*	NM_170784.3: c.1530G>A; p.(Trp510*)		Primary	1.35E-2	3.98E-6	0.000
3	*MKKS*	NM_170784.3: c.748G>A; p.(Gly250Arg)		Primary	1.35E-2	1.59E-5	0.000
2	*BBS4*	arr([GRCh37] 15q24.1(72,685,818-72,686,251)x0)		Primary	8.97E-3	NA	NA
2	*CORO2B*	NM_006091.5: c.581T>A; p.(Leu194Gln)		Primary	8.97E-3	2.51E-3	6.69E-3
2	*ZNF17*	NM_006959.2: c.1903G>T; p.(Glu635*)		Primary	8.97E-3	2.44E-4	2.61E-4
2	*CEP41*	NM_018718.3: c.5C>T; p.(Ser2Phe)		Primary	8.97E-3	2.72E-4	8.7E-5
2	*BBS1*	NM_024649.5: c.69G>A; p.(Trp23*)		Primary	8.97E-3	NA	NA
2	*BBS1*	NM_024649.5: c.837del; p.(Lys280Serfs*9)		Primary	8.97E-3	NA	NA
2	*BBS1*	NM_024649.5: c.951+58C>T; p.(?)	X	Primary	8.97E-3	NA	NA

Nº of alleles	Gene	Variant	Allele found in BBS and non-syndromic	Role in disease	Cohort allele frequency	GnomAD allelic frequency_ALL	GnomAD allelic frequency_SE
2	*BBS2*	NM_031885.5: c.1932T>A; p.(Tyr644*)		Primary	8.97E-3	NA	NA
2	*BBS5*	NM_152384.3: c.143-1G>C; p.(?)		Primary	8.97E-3	3.98E-5	0.000
2	*BBS5*	NM_152384.3: c.412C>T; p.(Arg138Cys)		Primary	8.97E-3	3.98E-6	0.000
2	*BBS12*	NM_152618.3: c.1619G>A; p.(Gly540Asp)		Primary	8.97E-3	0.000	0.000
2	*BBS12*	NM_152618.3: c.1627G>A; p.(Glu543Lys)		Primary	8.97E-3	NA	NA
2	*MKKS*	NM_170784.3: c.1232G>C; p.(Gly411Ala)		Primary	8.97E-3	NA	NA
2	*MKKS*	NM_170784.3: c.432_435delTAGT; p.(Phe144Leufs*14)		Primary	8.97E-3	NA	NA
2	*MKKS*	NM_170784.3: c.748G>A; p.(Gly250Arg)		Primary	8.97E-3	1.59E-5	0.000
2	*MKKS*	NM_170784.3: c.1361T>C; p.(Leu454Pro)		Primary	8.97E-3	NA	NA
2	*BBS7*	NM_176824.3: c.1372-1G>C; p.(?)		Primary	8.97E-3	NA	NA
2	*BBS7*	NM_176824.3: c.896_897delAA; p.(Lys299Argfs*4)		Primary	8.97E-3	NA	NA
2	*C8orf37*	NM_177965.4: c.156-1G>T; p.(?)		Primary	8.97E-3	NA	NA
2	*C8orf37*	NM_177965.4: c.28_31del; p.(Asp10Lysfs*12)		Primary	8.97E-3	NA	NA
2	*ARL6*	NM_177976.3: c.4G>T; p.(Gly2*)		Primary	8.97E-3	3.98E-6	8.7E-5
2	*BBS5*	NM_152384.3: c.543del; p.(Phe181Leufs*5)		Primary	8.97E-3	NA	NA
2	*BBS1*	NM_024649.5: c.1515_1525del; p.(Gln506Asnfs*48)		Primary	8.97E-3	NA	NA
2	*BBS10*	NM_024685.4: c.1510_1511del; p.(Ile504Serfs*17)		Primary	8.97E-3	NA	NA
1	*IFT27*	NM_006860.5: c.104A>G; p.(Tyr35Cys)		Primary	4.48E-3	NA	NA
1	*IFT27*	NM_006860.5: c.350-2A>G; p.(?)		Primary	4.48E-3	NA	NA
1	*BBS1*	NM_024649.5: c.118delT; p.(Cys40Alafs*2)		Primary	4.48E-3	8E-6	0.000
1	*BBS1*	NM_024649.5: c.436C>T; p.(Arg146*)		Primary	4.48E-3	7.95E-6	0.000
1	*BBS1*	NM_024649.5: c.863T>G; p.(Leu288Arg)		Primary	4.48E-3	NA	NA
1	*BBS10*	NM_024685.4: c.1013_1015delAAG; p.(Glu388del)		Primary	4.48E-3	1.61E-5	0.000

Nº of alleles	Gene	Variant	Allele found in BBS and non-syndromic	Role in disease	Cohort allele frequency	GnomAD allelic frequency_ALL	GnomAD allelic frequency_SE
1	*BBS10*	NM_024685.4: c.1241T>C; p.(Leu414Ser)		Primary	4.48E-3	3.98E-6	0.000
1	*BBS10*	NM_024685.4: c.273C>G; p.(Cys91Trp)		Primary	4.48E-3	2.82E-5	1.75E-4
1	*BBS10*	NM_024685.4: c.360G>A; p.(Trp120*)		Primary	4.48E-3	NA	NA
1	*BBS2*	NM_031885.5: c.1237C>T; p.(Arg413*)		Primary	4.48E-3	1.99E-5	0.000
1	*BBS2*	NM_031885.5: c.471G>A; p.(Thr157=)		Primary	4.48E-3	3.98E-6	0.000
1	*BBS12*	NM_152618.3: c.1082del; p.(Gly361Valfs*382)		Primary	4.48E-3	3.98E-6	0.000
1	*BBS9*	NM_198428.3: c.1789C>T; p.(Gln597*)		Primary	4.48E-3	NA	NA
1	*BBS9*	NM_198428.3: c.2349C>A; p.(Cys783*)		Primary	4.48E-3	NA	NA
1	*BBS12*	NM_152618.3: c.1383_1386del; p.(Asn461Lysfs*10)		Primary	4.48E-3	NA	NA
1	*BBS10*	NM_024685.4: c.1599_1602del; p.(Thr534Ilefs*21)		Primary	4.48E-3	7.96E-6	8.7E-5
1	*BBS12*	NM_152618.3: c.1893_1894del; p.(Pro632Phefs*7)		Primary	4.48E-3	3.98E-6	0.000
1	*BBS12*	NM_152618.3: c.1140del; p.(Val381Tyrfs*2)		Primary	4.48E-3	NA	NA
Potential modifier alleles in secondary genes							
4	*MKKS*	NM_170784.3: c.724G>T; p.(Ala242Ser)		Secondary	1.79E-2	5.21E-3	3.92E-3
2	*PDE6B*	NM_000283.4: c.1798G>A; p.(Asp600Asn)		Secondary	8.97E-3	5.58E-5	3.48E-4
1	*PDE6B*	NM_000283.4: c.1055G>T; p.(Gly352Val)		Secondary	4.48E-3	3.99E-6	0.000
1	*PDE6B*	NM_000283.4: c.292C>T; p.(Arg98Cys)		Secondary	4.48E-3	1.99E-4	2.13E-4
1	*TRIM32*	NM_001099679.2: c.1198C>T; p.(Arg400Cys)		Secondary	4.48E-3	3.58E-5	0.000
1	*CORO2B*	NM_006091.5: c.386C>T; p.(Ala129Val)		Secondary	4.48E-3	4.07E-6	0.000
1	*SDCCAG8*	NM_006642.5: c.245G>T; p.(Arg82Leu)		Secondary	4.48E-3	3.98E-6	0.000
1	*NPHP4*	NM_015102.5: c.1571C>A; p.(Ser524*)		Secondary	4.48E-3	NA	NA
1	*NPHP4*	NM_015102.5: c.2962G>A; p.(Ala988Thr)		Secondary	4.48E-3	6.04E-5	0.000
1	*ALMS1*	NM_015120.4: c.11638C>T; p.(His3880Tyr)		Secondary	4.48E-3	1.02E-3	0.000
1	*IFT172*	NM_015662.3: c.2015G>A; p.(Arg672Gln)		Secondary	4.48E-3	5.45E-4	1.3E-3

Nº of alleles	Gene	Variant	Allele found in BBS and non-syndromic	Role in disease	Cohort allele frequency	GnomAD allelic frequency_ALL	GnomAD allelic frequency_SE
1	*IFT172*	NM_015662.3: c.3073C>G; p.(Pro1025Ala)		Secondary	4.48E-3	1.3E-3	7.83E-4
1	*IFT172*	NM_015662.3: c.4363C>T; p.(Arg1455Trp)		Secondary	4.48E-3	3.02E-4	4.35E-4
1	*WDPCP*	NM_015910.7: c.1032C>A; p.(Cys344*)		Secondary	4.48E-3	NA	NA
1	*MKS1*	NM_017777.4: c.1136C>G; p.(Ala379Gly)		Secondary	4.48E-3	NA	NA
1	*MKS1*	NM_017777.4: c.1318G>A; p.(Gly440Ser)		Secondary	4.48E-3	NA	NA
1	*CEP290*	NM_025114.4: c.7311_7313delGAA; p.(Lys2437del)		Secondary	4.48E-3	1.22E-5	0.000
1	*BBS5*	NM_152384.3: c.551A>G; p.(Asn184Ser)		Secondary	4.48E-3	4.19E-3	5.66E-3
1	*BBS5*	NM_152384.3: c.620G>A; p.(Arg207His)		Secondary	4.48E-3	6.56E-3	5.31E-3
1	*BBS7*	NM_176824.3: c.1235A>G; p.(Asp412Gly)		Secondary	4.48E-3	1.49E-3	1.82E-3
1	*BBS9*	NM_198428.3: c.376A>T; p.(Met126Leu)		Secondary	4.48E-3	NA	NA

Las **tablas suplementarias S5 y S6** pueden encontrarse en:

https://drive.google.com/drive/folders/10_eu3Vg3bUXWpl0D3rLNyNNI6fFkeFsZ?usp=sharing

CAPÍTULO 4

Descripción genética y fenotípica de la cohorte de pacientes de USH diagnosticados con *MYO7A*

Artículo 4: *Genotype-phenotype correlation in patients with Usher syndrome and pathogenic variants in MYO7A: implications for future clinical trials*

Galbis-Martínez L, Blanco-Kelly F, García-García G, Ávila-Fernández A, Jaijo T, Fuster-García C, Perea-Romero I, *et al.*

Publicado en *Acta Ophthalmologica*, 2021

Resumen

El propósito de este trabajo fue establecer correlaciones entre las características clínicas de una cohorte de pacientes con USH diagnosticados genéticamente con variantes patogénicas en *MYO7A*, el tipo de variante patogénica y su ubicación en el dominio de la proteína.

Para ello, se seleccionaron 62 pacientes con USH pertenecientes a 46 familias que estuviesen caracterizados con variantes bialélicas en *MYO7A*. Los casos se evaluaron considerando su historia oftalmológica autoinformada y las pruebas oftalmológicas realizadas (campo visual computarizado, mejor agudeza visual corregida y examen oftalmoscópico y electrofisiológico). Cuando fue posible, se realizaron también tomografías de coherencia óptica y autofluorescencia del fondo de ojo. Además, se evaluaron las funciones auditivas y vestibulares de los pacientes.

La mayoría de los pacientes presentaban un fenotipo típico de USH1, es decir, hipoacusia neurosensorial de tipo prelingual y severa-profunda, disfunción vestibular y RP prepuberal. No se observaron diferencias estadísticamente significativas para las variables analizadas, excepto para edad de aparición de la hipoacusia. Esto puede deberse a la existencia de dos casos con USH2 en la cohorte, caracterizados por hipoacusia neurosensorial de tipo poslingual, ausencia de disfunción vestibular y RP de inicio postpuberal; así como de otro caso adicional de USH atípico.

Con este trabajo no se ha podido encontrar una correlación entre el genotipo y el fenotipo para *MYO7A*. Sin embargo, los hallazgos podrían resultar útiles para la evaluación de la eficacia en los ensayos clínicos, ya que el tipo de variante de *MYO7A* no parece cambiar el inicio, gravedad o curso de la enfermedad visual.

Contribución de la autora

La autora participó en la recogida, estructuración y revisión de los datos clínicos, genéticos demográficos y familiares de los 62 casos incluidos con USH y caracterizados con variantes bialélicas en *MYO7A*. Elaboró los árboles genealógicos de las familias con las segregaciones. Intervino en el análisis *in silico* de las variantes y en el análisis de los haplotipos de las variantes más frecuentes.

Además, participó en la escritura del manuscrito y en su revisión crítica y aprobación final.

El artículo y el material suplementario se encuentran publicados en:

https://onlinelibrary.wiley.com/doi/10.1111/aos.14795

Acta Ophthalmologica

ACTA OPHTHALMOLOGICA 2021

Genotype–phenotype correlation in patients with Usher syndrome and pathogenic variants in *MYO7A*: implications for future clinical trials

Lilián Galbis-Martínez,[1,2] Fiona Blanco-Kelly,[1,2] Gema García-García,[2,3,4] Almudena Ávila-Fernández,[1,2] Teresa Jaijo,[2,3,4] Carla Fuster-García,[2,3,4] Irene Perea-Romero,[1,2] Olga Zurita-Muñoz,[1,2] Belén Jimenez-Rolando,[2,5] Ester Carreño,[2,5] Blanca García-Sandoval,[2,5] José M. Millán[2,3,4] and Carmen Ayuso[1,2]

[1]Department of Genetics, University Hospital Fundacion Jimenez Diaz, IIS-FJD, UAM, Madrid, Spain
[2]CIBERER, ISCIII, Madrid, Spain
[3]Unit of Genetics, University Hospital La Fe – IIS La Fe, Valencia, Spain
[4]Joint Unit for Rare Diseases IIS La Fe-CIPF, Valencia, Spain
[5]Department of Ophthalmology, University Hospital Fundacion Jimenez Diaz, IIS-FJD, UAM, Madrid, Spain

ABSTRACT.

Purpose: **We aimed to establish correlations between the clinical features of a cohort of Usher syndrome (USH) patients with pathogenic variants in *MYO7A*, type of pathogenic variant, and location on the protein domain.**

Methods: **Sixty-two USH patients from 46 families with biallelic variants in *MYO7A* were examined for visual and audiological features. Participants were evaluated based on self-reported ophthalmological history and ophthalmological investigations (computerized visual field testing, best-corrected visual acuity, and ophthalmoscopic and electrophysiological examination). Optical coherence tomography and fundus autofluorescence imaging were performed when possible. Auditory and vestibular functions were evaluated. Patients were classified according to the type of variant and the protein domain where the variants were located.**

Results: **Most patients displayed a typical USH1 phenotype, that is, prelingual severe-profound sensorineural hearing loss, prepubertal retinitis pigmentosa (RP) and vestibular dysfunction. No statistically significant differences were observed for the variables analysed except for the onset of hearing loss due to the existence of two USH2 cases, defined as postlingual sensorineural hearing loss, postpubertal onset of RP, and absence of vestibular dysfunction, and one atypical case of USH.**

Conclusion: **We were unable to find a correlation between genotype and phenotype for *MYO7A*. However, our findings could prove useful for the assessment of efficacy in clinical trials, since the type of *MYO7A* variant does not seem to change the onset, severity or course of visual disease.**

Key words: clinical trials – genetic screening – *MYO7A* – genotype–phenotype correlation – Usher syndrome

†These authors contributed equally.

Acta Ophthalmol.

doi: 10.1111/aos.14795

Introduction

Usher syndrome (USH) is an autosomal recessive and genetically heterogeneous disorder characterized by bilateral sensorineural hearing loss, retinitis pigmentosa (RP), and, sometimes, vestibular dysfunction (Millán et al. 2011). It is the main cause of blindness in combination with deafness (Vernon 1969), and its prevalence ranges from 3.2 to 6.2 per 100,000 people, depending on the population (Boughman et al. 1983; Hope 1997; Espinós et al. 1998).

The three clinical types of USH are USH type 1 (USH1, MIM#276900), USH type 2 (USH2, MIM#276901) and USH type 3 (USH3, MIM#276903), which are distinguishable by their hearing and vestibular phenotypes (Saihan et al. 2009; Millán et al. 2011). USH2 is characterized by stable, moderate-to-severe hearing loss and normal vestibular function. The USH3 phenotype is characterized by progressive loss of hearing and variable degrees of vestibular dysfunction (Kimberling & Moller 1995). Of the three clinical groups, USH1 is the most disabling, since it is characterized by profound congenital hearing loss, absence of vestibular function, and earlier onset of RP than in and USH2

and USH3 (Blanco-Kelly et al. 2015). Children with USH1 have delayed motor development secondary to abnormal vestibular and hearing function. Speech acquisition may be further affected if deafness is not corrected early (Petit 2001). While cochlear implantation has proven to be an effective approach to correct hearing loss (Jatana et al. 2013), there is currently no cure for RP in USH patients.

The six genes involved in USH1 are *MYO7A* (MIM #276903), *USH1C* (MIM #605242), *CDH23* (MIM #605516), *PCDH15* (MIM #605514), *USH1G* (MIM #607696) and *CIB2* (MIM #605564). *MYO7A*, is the most frequently mutated in USH1, accounting for 29–50% of all cases (Weil et al. 1995; Bharadwaj et al. 2000; Ouyang et al. 2005; Jaijo et al. 2006; Roux et al. 2006; Oshima et al. 2008; Le Quesne Stabej et al. 2012; Bonnet et al. 2016). Mutations in *MYO7A* cause not only USH1, but also dominant or recessive isolated sensorineural hearing loss (Tamagawa et al. 2002; Riazuddin et al. 2008).

MYO7A encodes myosin VIIa, an actin-based motor protein comprising several functional domains, as follows: (1) the N-terminal motor (or head) domain, which contains the actin-binding site and the ATP-binding site; (2) the neck region, with five IQ that bind calmodulin; and (3) the tail region, including two large tandem repeats separated by an SH3 (*src homology-3*) motif and containing the MyTH4 (*myosin tail homology 4*) and FERM (*4.1 ezrin, readixin, moesin*) domains. FERM mediates attachment to the plasma membrane through its interaction with harmonin (Reiners et al. 2006; Yu et al. 2017) (Fig. 1).

Myosin VIIa is expressed in the inner ear and in the retina. In the ear, it is essential for the development of the hair bundle of the cochlea and vestibular system, and for mechanoelectrical transduction in the hair cells (Self et al. 1998; Boëda et al. 2002; Kros et al. 2002). In the retina, it is expressed in rod and cone photoreceptor cells and in the retinal pigment epithelium, where it plays an important role in photoreceptor opsin transport through the connecting cilium and in the movement of melanosomes and phagosomes (Liu et al. 1998; Liu et al. 1999; Gibbs et al. 2004).

Here, we retrospectively describe the phenotype of a cohort of 62 USH patients genetically diagnosed with mutations in *MYO7A* in order to outline a retrospective cross-sectional study of the disease and to detect correlations with genotype.

Methods

Patients

The study population comprised 62 patients from 46 families with a clinical diagnosis of USH1 or USH2 and carrying biallelic causative variants in *MYO7A*. The patients were referred from the Federación de Asociaciones de Distrofias de Retina de España (FARPE) and from several Spanish hospitals and were recruited at the Genetics Department of the Fundación Jiménez Díaz Hospital (Madrid, Spain). Molecular analyses were performed by the Genetics Department of University Hospital La Fe (Valencia, Spain) or by the Clinical Genetics Department of Fundación Jiménez Díaz Hospital (Madrid, Spain).

Whenever possible (29 families), samples from family members were

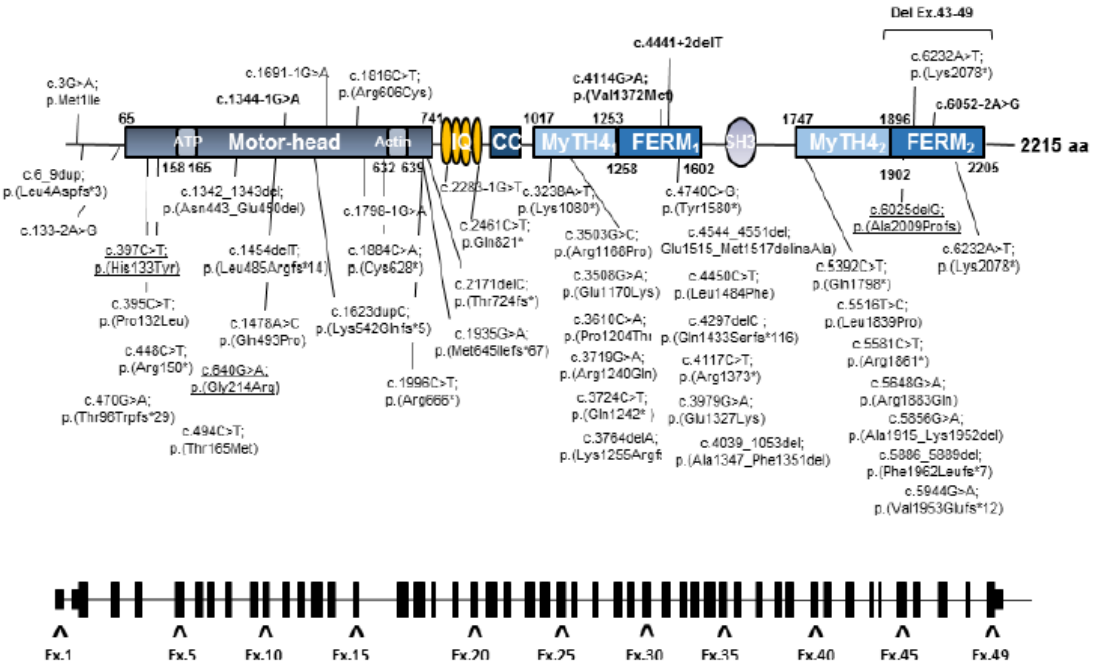


Fig. 1. MYO7A protein structure showing its functional domains.

used for segregation analysis of the variants identified in the index patient. Family pedigrees of segregated families are shown in Fig. S1.

Written informed consent was obtained from all patients and their relatives. All investigations were reviewed and approved by the Ethics Committee of the hospital and conducted in accordance with the principles of the Declaration of Helsinki and its revisions.

Clinical evaluation

Usher syndrome was diagnosed on the basis of simultaneous occurrence of sensorineural deafness and retinal dystrophy. Phenotypic classification into USH clinical types was performed as described previously (Smith et al. 1994).

Fifty-four patients (39 families) and 55 cases (40 families) were evaluated to determine their ophthalmological and audiological phenotype, respectively.

The ophthalmological evaluation consisted of a self-reported ophthalmological history recorded from questionnaires (including onset of symptoms, age at diagnosis, and family history), and objective ophthalmological studies, which included computerized central and peripheral visual field testing, best-corrected visual acuity, ophthalmoscopic examination after pupillary dilation, and electrophysiological examination (full-field electroretinogram). In some cases, optical coherence tomography (OCT) and fundus autofluorescence (FAF) imaging were recorded.

Auditory and vestibular functions were assessed by complete examination whenever possible. Patients with no islands of hearing and no response better than 90 dB along the standard test frequencies were classified as USH1.

Genetic analysis

Peripheral blood samples of index cases and family members were collected in EDTA tubes. DNA was extracted as described elsewhere (Blanco-Kelly et al. 2015).

Several approaches were used for molecular characterization, since USH patients were genetically diagnosed during a period when various molecular techniques were developed and implemented in the laboratory, as follows: (1) sequencing of all exons and flanking intronic regions of *MYO7A*; (2) a specific USH genotyping microarray (AsperBiotech, Tartu, Estonia); (3) comparative genomic hybridization microarray (CGH-microarray) chip including the USH genes for detecting large genomic rearrangements; (4) targeted next-generation sequencing (NGS); (5) Clinical exome: TruSight-One (Illumina) or Clinical Exome Solution (Sophia Genetics, Saint Sulpice, Switzerland), using the NextSeq platform (Illumina, Inc, San Diego, CA, USA).

Suspected pathogenic variants and familial segregation studies were confirmed using Sanger sequencing.

In silico analysis of variants

Variants found in *MYO7A* were checked against the following databases: LOVD (https://grenada.lumc.nl/LSDB_list/lsdbs/MYO7A), Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ Professional 2018.2) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). The pathogenicity of novel missense variants was analysed using four predictive software programs: (1) SIFT (http://sift.bii.a-star.edu.sg/), (2) Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), (3) M-CAP (http://bejerano.stanford.edu/mcap/) and (4) Mutation Taster. Putative variants affecting the splicing process were studied using NNSPLICE v0.9 (http://www.fruitfly.org/seq_tools/splice.html), Human Splicing Finder (HSF; Human Splicing Finder), Splice Site Finder, MaxEntScan and Gene Splicer. Each software application uses an algorithm that gives a range of values. For instance, consensus values go from 0 to 100 for Human Splicing Finder, –20 to +20 for MaxEntScan, and 0 to 1 for NNSplice. The threshold is defined at 65 for HSF, 3 for MaxEnt, and according to the operator for NNSplice. Therefore, every signal with a score above the threshold is considered to be a splice site (donor or acceptor). A value below the threshold is predicted to abolish the splice site.

Novel rare variants were checked against the Genome Aggregation Database (gnomAD Exomes and gnomAD Genomes), 1000 Genomes Project, Exome Variant Server (EVS), Exome Aggregation Consortium (ExAC), and Collaborative Spanish Variant Server (created by CIBERER-BIER: http://csvs.babelomics.org).

The nomenclature of *MYO7A* variants was drafted according to the reference sequence NM_000260.3.

Statistical analysis

Patients were grouped based on mutation type and mutation protein location.

Group 1: According to genotype mutation, as follows: category A, missense/missense; B, missense/truncating; and C, truncating/truncating.
Group 2: According to the protein domain where the mutation was located, as follows: category 1, motor /motor; 2, motor/tail$_1$; 3, tail$_1$/ tail$_1$; 4, motor/neck; 5, neck/neck; 6, neck-tail$_1$; 7, motor/-tail$_2$; 8, tail$_2$/ tail$_2$; and 9, tail$_1$/tail$_2$ ('motor', motor-head domain; 'neck', region linking the motor-head and tail regions; 'tail$_1$', region comprising the MyTH4$_1$-FERM$_1$ domains; and 'tail$_2$', fragment containing the MyTH4$_2$-FERM$_2$ domains).

The survival curves for years free of visual acuity (VA) under 0.1 (decimal), years free of visual field lower than 10°, and years free of cataracts were estimated using the Kaplan–Meier method. These analyses were based on available clinical data obtained from ophthalmological examinations (not self-reported). Curves were compared by means of the log-rank test.

The variables age at diagnosis, onset of night blindness, onset of visual field constriction, onset of visual acuity reduction, onset of hearing loss and age of onset (months) at unaided walking (based on self-reported data) were compared for all categories according to group 1 and group 2 using the Kruskal–Wallis test.

Haplotype analysis

To determine whether the most prevalent mutations in our cohort shared haplotypes among affected families, we selected the following for haplotype analysis: three single intragenic nucleotide polymorphisms (SNPs), namely, rs6592706, rs948972 and rs11237122 (Roberts et al. 2015), and three polymorphic markers spanning the USHB1 locus, namely, D11S787,

D11S527 and D11S4186 (Adato et al. 1997). The primers used are listed in Table S1.

The location of each marker referring to the *MYO7A* gene and to the most prevalent *MYO7A* variants found in this study is depicted in Fig. S2.

Results

Clinical features

Clinical information was limited for some patients, since they were referred to our hospital from other regions of Spain. Electroretinography data were available for 46 patients, of whom 34 (74%) had a nonrecordable ERG and 12 (26%) had recordings with abnormal values. Optical coherence tomography (OCT) was carried out in 51 patients (82%). Only five (8%) patients had cystoid macular oedema.

Most patients displayed a typical USH1 phenotype; three were classified as atypical USH or USH2 (Table S2). One patient from the RP-1218 family was diagnosed with atypical USH based on the auditory phenotype, since he referred progressive hearing loss beginning at 15 years and his audiometry at 23 years showed a down-sloping pattern, with mild-moderate hypoacusis at low-middle frequencies and moderate-severe hypoacusis at high frequencies (Fig. S3). Patients FRP-159 and RP-2947 were classified as USH2. The FRP-159 patient presented with moderate hearing loss and intelligible speech, and patient RP-2947 had more severe hypoacusis, although this was diagnosed later (at 3 years) and corrected with hearing aids at the age of 9 years. This patient had a more attenuated ophthalmological phenotype, with no symptoms of loss of visual acuity at the age of 65 years.

When available, OCT disclosed the expected loss of outer retina concentrically in the periphery of the macular area, with total disorganization of the ellipsoid layer and outer retina in the most severe cases. Some patients had macular oedema with intraretinal fluid in areas overlying a preserved ellipsoid layer. Blue-peak FAF—when available—revealed peripheral loss of FAF with a hyperautofluorescent concentric halo in all patients.

The overall mean age at diagnosis for patients with mutations in *MYO7A* was 14.86 ± 10.37 years. Age of onset by condition was as follows: night blindness, 13.23 ± 9.47 years; onset of reduced visual field, 13.87 ± 8.91 years; loss of visual acuity, 19.88 ± 11.83 years; diagnosis of hearing loss, 11.83 ± 9.1 months; and unaided walking, 22.89 ± 10.8.88 months.

Molecular characterization

Forty-nine distinct pathogenic/likely pathogenic variants were identified in the 46 families. Patients from 22 of these families were homozygous, and 24 were compound heterozygous. Consanguinity was observed in 10 of the 22 homozygous families. Changes included missense, frameshift, nonsense, small and gross deletions and variants likely affecting splicing, the most frequent being those leading to a truncated protein (57 out of 92 alleles). The mutations observed in the present report are detailed in Table S3.

Patients were distributed as follows:

Grouping 1: type of mutation

1 Categories (cases):

a A: missense/missense (15)
b B: missense/truncating (15)
c C: truncating/truncating (32)

Grouping 2: protein domain affected by the mutation

1 Categories (cases):

a 1: motor /motor (22)
b 2: motor/tail$_1$ (4)
c 3: tail$_1$/ tail$_1$ (8)
d 4: motor/neck (2)
e 5: neck-neck (4)
f 6: neck-tail$_1$ (3)
g 7: motor-tail$_2$ (5)
h 8: tail$_2$/ tail$_2$ (8)
i 9: tail$_1$/ tail$_2$ (6)

The positions of the variants observed along the myosin VIIA structural domains are depicted in Fig. 1.

Only three of the detected mutations were located upstream of the motor domain, and two were located at the IQ motif. These last five changes were severe mutations that may prevent the correct start or produce a premature termination of the protein translation or create an aberrant transcript.

Table 1. Pathogenic predictions of novel (exonic and intronic) variants by several computational programs. 'X → Y' indicates the reduction in the score for the main donor or acceptor site from 'X' to 'Y'.

MYO7A variant	Protein	SIFT (score)	PolyPhen-2 (score)	MutationTaster	M-CAP (score)
c.4114G>A	p.(Val1372Met)	Deleterious (0.02)	Prob. damaging (0.999)	Disease-causing	Possibly pathogenic (0.086)
delE43-E49	c.5857-?_*544+?del				

MYO7A variant	Intron	Splice Site Finder	MaxEntScan	NNSPLICE	Gene Splicer	Human Splicing Finder	Predicted consequence
c.1344-1G>A	12	82 → 0	10 → 0	1 → 0	11 → 0	88 → 0	Possible effect at nearest splice site
c.4441+2delT	33	71 → 0	9 → 0	1 → 0	3 → 0	99 → 0	Possible effect at nearest splice site
c.6052-2A>G	44	90 → 0	10 → 0	1 → 0	12 → 0	89 → 0	Possible effect at nearest splice site

Each software uses an algorithm that provides a range of values. For instance, consensus values run from 0 to 100 for Human Splicing Finder, from −20 to +20 for MaxEntScan, and from 0 to 1 for NNSplice. The threshold is defined at 65 for HSF, 3 for MaxEnt, and according to the operator for NNSplice. Consequently, every signal with a score above the threshold is considered to be a splice site (donor or acceptor). A value below the threshold is predicted to abolish the splice site.

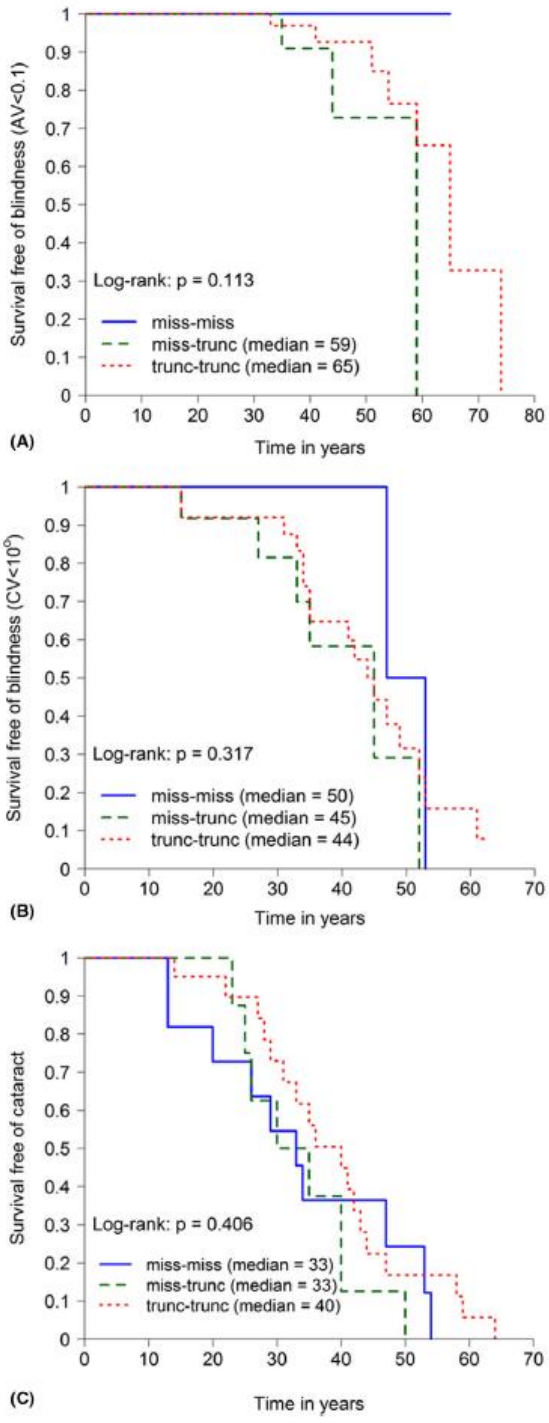


Fig. 2. Survival curves. (A) Blindness-free survival on the basis of visual acuity (blindness VA < 0.1). (B) Blindness-free survival on the basis of visual field (blindness VF < 10°). (C) Blindness-free survival on the basis of age of onset of cataracts.

The most prevalent variants detected were as follows: c.397C>T and p.(His133Tyr), found in three homozygous patients (six alleles); c.640G>A and p.(Gly214Arg), found in four unrelated patients (five alleles); and c.6025delG and p.(Ala2009Profs*32), found in two homozygous and in one heterozygous patient (five alleles). Only 13 of the variants were found in more than one of the families (36 of the variants were found in only one family in our cohort).

Five of the alleles identified in *MYO7A* (10%) had not been previously reported. Screening for potentially causative missense and splice novel variants was performed using prediction software programs. In the case of missense variants, those predicted as damaging by at least two different prediction programs in an evolutionary conserved residue (Table 1), and not found in the general population, and cosegregating with the disease (when DNA of relatives were available) were initially considered potentially pathogenic (Table S4). For splice variants, the software programs applied used algorithms with a threshold for prediction of the effect on the splice site; Table 1 shows how the high scores obtained for the wild-type variants decrease to zero for the mutations c.1344-1G>A, c.4441+2delT and c.6052-2A>G, thus implying that they alter the nearest splice site; therefore, we considered them to be pathogenic.

Statistical analysis

Blindness-free survival in years is shown in Fig. 2. A visual acuity value under 0.1 was reached at a median of 65 years by patients who carried two truncating mutations and at almost 60 years by those who had a combination of one missense mutation and one truncating mutation (Fig. 2A). A visual field lower than 10° was reached at 44 and 45 years for patients with two truncating mutations and a combination of a missense and a truncating mutation, respectively, and at 50 years of age for those bearing two missense mutations (Fig. 2B). Cataracts appeared at a median age of 40 years for patients with two truncating mutations and 33 years for those carrying two missense mutations and those with one missense mutation and one truncating mutation (Fig. 2C).

A tendency towards later onset of symptoms in patients carrying two truncating mutations was observed; however, these differences were not statistically significant. The p values were 0.113, 0.317 and 0.406 for visual acuity, visual field and cataract, respectively.

No statistically significant differences were observed for any visual variable (age at diagnosis, onset of night blindness, reduction of visual field or visual acuity) except for onset of hearing loss (mean 0.7–17.9) due to atypical USH and USH2 (Table 2).

Similarly, no statistically significant differences were found when variables were categorized according to the altered protein domain (Table 3).

Haplotype analysis

In order to explore the possible founder effect of the most frequent *MYO7A* variants in the Spanish population, we performed haplotype studies on the most recurrent *MYO7A* variants found in this study (c.640G>A, c.397C<T and c.6025delG) (Fig. S4). Families carrying the c.640G>A mutation were from the same Spanish region (Castilla-La Mancha), whereas families with c.397C<T or c.6025delG variants came from different, distant Spanish locations. A haplotype was associated with each of the three mutations. In the families in the present study, haplotypes were shared by all the patients who carried the same mutation, thus suggesting a common origin for each of them.

Discussion

More than 500 variants within the gene encoding myosin VIIA have been described to date, and most are private mutations (HGMD, Human Gene Mutation Database, https://portal.biobase-international.com/hgmd/pro/gene.php?gene=MYO7A). We screened for mutations in 46 families with USH, mainly type I, and detected 49 different

Table 2. Mean and standard deviation, median (in years, except for onset of hearing loss and unaided walking [in months]) and p value of the age of onset for the analysed features among patients categorized on the basis of the mutation type.

Variable	Group	*n*	Mean ± SD	Median (IQR)	p value
Age at diagnosis	miss-miss	13	11.9 ± 5.9	11.0 (6.0)	0.268
	miss-trunc	11	18.8 ± 10.1	13.0 (14.0)	
	trunc-trunc	23	15.1 ± 11.2	12.0 (7.5)	
Onset of night blindness	miss-miss	7	8.6 ± 3.9	8.0 (4.5)	0.298
	miss-trunc	8	12.6 ± 4.8	10.5 (5.0)	
	trunc-trunc	22	14.0 ± 11.0	11.5 (6.8)	
Onset of visual field constriction	miss-miss	7	9.9 ± 5.7	10.0 (9.0)	0.333
	miss-trunc	9	17.3 ± 10.6	14.0 (9.0)	
	trunc-trunc	24	13.5 ± 8.7	12.0 (4.8)	
Onset of visual acuity reduction	miss-miss	7	17.4 ± 10.8	14.0 (13.0)	0.738
	miss-trunc	9	21.1 ± 11.6	20.0 (20.0)	
	trunc-trunc	17	22.0 ± 14.5	18.0 (22.0)	
Onset of hearing los (months)	miss-miss	13	3.0 ± 5.7	0.0 (3.0)	0.008
	miss-trunc	12	17.9 ± 51.2	1.5 (8.0)	
	trunc-trunc	25	0.7 ± 3.6	0.0 (0.0)	
Unaided walking (months)	miss-miss	3	27.7 ± 9.7	30.0 (9.5)	0.220
	miss-trunc	6	17.8 ± 1.5	18.5 (2.5)	
	trunc-trunc	8	25.4 ± 11.0	21.5 (9.0)	

miss-miss = missense-missense genotype, miss-trunc = missense-truncating genotype, IQR = interquartile range, SD = standard deviation, trunc-trunc = truncating-truncating genotype.

Table 3. Mean and standard deviation, median (in years, except for hearing loss and unaided walking [in months]) and p value of the onset for the features analysed categorized on the basis of the protein domain that is affected by the mutations (only missense mutations).

Variable	Group	*n*	Mean ± SD (years)	Median (IQR) (years)	p value
Age at diagnosis	motor-motor	14	12.8 ± 7.7	11.0 (5.8)	0.277
	motor-tail$_1$	4	18.0 ± 9.6	19 (11.5)	
	tail$_1$-tail$_1$	7	10.0 ± 6.1	10.0 (2.5)	
	motor-neck	2	11.0 ± 1.4	11.0 (1.0)	
	neck-neck	2	12.0 ± 0.0	12.0 (0.0)	
	neck-tail$_1$	2	14.0 ± 1.4	14.0 (1.0)	
	motor-tail$_2$	4	22.0 ± 7.4	25.0 (4.0)	
	tail$_2$-tail$_2$	6	17.0 ± 12.3	13.0 (7.3)	
	tail$_1$-tail$_2$	5	9.8 ± 3.4	11.0 (1.0)	
Onset of night blindness	motor-motor	12	9.5 ± 9.0	7.5 (5.5)	0.068
	motor-tail$_1$	3	12.0 ± 2.0	12.0 (2.0)	
	tail$_1$-tail$_1$	7	16.0 ± 11.6	12.0 (3.5)	
	motor-neck	2	9.0 ± 1.4	9.0 (1.0)	
	neck-neck	2	28.5 ± 14.8	28.5 (10.5)	
	neck-tail$_1$	2	14.0 ± 1.4	14.0 (1.0)	
	motor-tail$_2$	4	14.5 ± 6.4	15 (10.5)	
	tail$_2$-tail$_2$	3	8.3 ± 3.1	9.0 (3.0)	
	tail$_1$-tail$_2$	3	10.3 ± 3.8	12.0 (3.5)	
Onset of visual field constriction	motor-motor	12	12.0 ± 9.1	11.5 (9.5)	0.242
	motor-tail$_1$	3	8.3 ± 4.9	6.0 (4.5)	
	tail$_1$-tail$_1$	7	11.6 ± 2.6	11.0 (3.5)	
	motor-neck	2	9.3 ± 1.8	9.3 (1.3)	
	neck-neck	2	15.0 ± 4.2	15.0 (3.0)	
	neck-tail$_1$	2	14.0 ± 1.4	14.0 (1.0)	
	motor-tail$_2$	4	20.3 ± 7.8	20 (4.8)	
	tail$_2$-tail$_2$	5	18.0 ± 13.6	14.0 (7.0)	
	tail$_1$-tail$_2$	3	8.7 ± 4.2	10.0 (4.0)	
Onset of visual acuity reduction	motor-motor	12	23.7 ± 16.6	18.5 (23.0)	0.238
	motor-tail$_1$	2	31.5 ± 2.1	31.5 (1.5)	
	tail$_1$-tail$_1$	4	9.5 ± 4.2	10.0 (4.0)	
	neck-neck	2	26.5 ± 12	26.5 (8.5)	
	neck-tail$_1$	2	14.0 ± 1.4	14.0 (1.0)	
	motor-tail$_2$	4	17.8 ± 10.7	18.5 (8.8)	
	tail$_2$-tail$_2$	6	14.5 ± 8.3	14.0 (13.3)	
	tail$_1$-tail$_2$	1	35.0 ± NC	35.0 (0.0)	
Onset of hearing los (months)	motor-motor	16	2.3 ± 5.2	0.0 (0.0)	0.296
	motor-tail$_1$	4	5.3 ± 8.6	1.5 (6.8)	
	tail$_1$-tail$_1$	8	3.3 ± 3.8	1.5 (7.3)	
	motor-neck	2	0.0 ± 0.0	0.0 (0.0)	
	neck-neck	2	0.0 ± 0.0	0.0 (0.0)	
	neck-tail$_1$	3	0.0 ± 0.0	0.0 (0.0)	
	motor-tail$_2$	4	47 ± 88.7	4.0 (51.0)	
	tail$_2$-tail$_2$	6	0.0 ± 0.0	0.0 (0.0)	
	tail$_1$-tail$_2$	5	0.6 ± 1.3	0.0 (0.0)	
Start walking (months)	motor-motor	5	24.2 ± 8.6	22.0 (13.0)	0.324
	tail$_1$-tail$_1$	4	20.0 ± 2.7	19.0 (1.5)	
	motor-neck	1	36.0 ± NC	36.0 (0.0)	
	neck-neck	1	18.0 ± NC	18.0 (0.0)	
	neck-tail$_1$	1	48.0 ± NC	48.0 (0.0)	
	motor-tail$_2$	1	18.0 ± NC	18.0 (0.0)	
	tail$_1$-tail$_2$	4	17.8 ± 2.4	17.0 (2.8)	

IQR = interquartile range, SD = standard deviation.

mutations. Of these, five were novel, with absence of hot spot mutations. The results illustrate the high diversity of mutations responsible for USH1B and the absence of a predominant mutation, at least in the Spanish population.

The three recurrent mutations (c.640G>A, c.397C>T and c.6025delG) in our study have also been identified worldwide in different populations (https://databases.lovd.nl/shared/genes/MYO7A). We found that each was associated with a common haplotype, suggesting a common ancestral origin, at least in Spain.

Our phenotyping results for *MYO7A* patients are very similar to those reported by Blanco-Kelly (Blanco-Kelly et al. 2015) in a study carried out in the Spanish population including USH1 patients with mutations in *MYO7A, CDH23, PCDH15, USH1C* and *SANS*, in which the mean age at onset of hearing loss was 1.1 ± 0.5 years and the onset of visual symptoms was 9.5 ± 8 years (Blanco-

Kelly et al. 2015). This is not striking, since more than 50% of cases of USH1 in our study were due to *MYO7A* mutations.

In genes such as the *USH2A* gene, missense mutations lead to a less severe phenotype than truncating mutations (Pérez-Carro et al. 2018). While this might have been expected for *MYO7A*, our results indicate that there is no straightforward correlation between the severity of the USH1 phenotype and the type of mutation in *MYO7A* (Rong et al. 2014).

No statistically significant differences were recorded for the variables analysed, although this could be because of insufficient statistical power. As can be observed, there is a slight difference for carriers of two missense mutations, in whom onset of visual symptoms seems to be earlier than in those with two truncating mutations. The lack of genotype–phenotype correlation, however, could also be due to the absence of genotype–phenotype for *MYO7A*.

The only significant variable (p value: 0.007) was age at onset of hearing loss. It is well known that USH1 is defined by congenital or very early profound sensorineural hearing loss. In our series, hearing loss was detected within the first year of life in most cases. But the differences observed in the present series are due to three cases diagnosed of either atypical USH or USH2, mainly patient RP-1218.

Another bias to be borne in mind is that this study was based to a certain degree on patient recall for some variables, and this might reduce the likelihood of detecting any real difference, especially if the difference is small.

Patients with an atypical phenotype associated with *MYO7A* are not infrequent, and mutations in *MYO7A* have been associated with diverse clinical phenotypes. A number of studies have reported patients with mutations in *MYO7A* who are clinically USH2 (Bonnet et al. 2011, 2016; Rong et al. 2014).

Despite the efforts of many researchers to establish a genotype–phenotype correlation for inherited retinal dystrophies, this correlation has proven elusive for most of the genes involved (Sorrentino et al. 2016).

For the last few years, next-generation sequencing has made it possible to screen large series of patients and a large number of genes, thus enabling a correlation with phenotype to be established for mutations in some genes (eg, *ABCA4, RHO*, *EYS* and *CRBI*) (Boulanger-Scemama et al. 2015; Fernandez-San Jose et al. 2015; Nassisi et al. 2018).

A similar phenotype–genotype correlation has been defined for the p.(Cys579Phe) variant, for which, depending on its zygosity in the *USH2A* gene, distinguishable phenotypes can be identified (Pérez-Carro et al. 2018).

In contrast, a genotype–phenotype correlation seems to be difficult to establish for other genes, such as *MYO7A*. Thus, the current study could be useful for further assessment of efficacy in clinical trials, as it provides a detailed phenotype description for *MYO7A* and suggests that the onset, severity and progression of visual disease is not determined by the type of mutation in *MYO7A* or its location.

In most cases, mutations in *MYO7A* lead to USH1. However, the phenotypic range of *MYO7A* is wide, leading to USH2 and nonsyndromic hearing loss. The study of *MYO7A* must be borne in mind in USH2 patients who do not harbour mutations in the USH2 genes, since they may benefit from current and forthcoming retinal therapeutic strategies based on gene therapy.

In the era of gene-based therapeutic strategies, our study could be useful for the assessment of efficacy in clinical trials. Furthermore, our data will improve counselling of patients and relatives on the clinical onset and prognosis of the disease.

References

Adato A, Weil D, Kalinski H, Pel-Or Y, Ayadi H, Petit C, Korostishevsky M & Bonne-Tamir B (1997): Mutation profile of all 49 exons of the human myosin VIIA gene, and haplotype analysis, in Usher 1B families from diverse origins. Am J Hum Genet **61**: 813–821.

Bharadwaj AK, Kasztejna JP, Huq S, Berson EL & Dryja TP (2000): Evaluation of the myosin VIIA gene and visual function in patients with Usher syndrome type I. Exp Eye Res **71**: 173–181.

Blanco-Kelly F, Jaijo T, Aller E et al. (2015): Clinical aspects of usher syndrome and the USH2A gene in a cohort of 433 patients. JAMA Ophthalmol **133**: 157–164.

Boëda B, El-Amraoui A, Bahloul A et al. (2002): Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. EMBO J **21**: 6689–6699.

Bonnet C, Grati M, Marlin S et al. (2011): Complete exon sequencing of all known Usher syndrome genes greatly improves molecular diagnosis. Orphanet J Rare Dis **6**: 21.

Bonnet C, Riahi Z, Chantot-Bastaraud S et al. (2016): An innovative strategy for the molecular diagnosis of Usher syndrome identifies causal biallelic mutations in 93% of European patients. Eur J Hum Genet **24**: 1730–1738.

Boughman JA, Vernon M & Shaver KA (1983): Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis **36**: 595–603.

Boulanger-Scemama E, El Shamieh S, Démontant V et al. (2015): Next-generation sequencing applied to a large French cone and cone-rod dystrophy cohort: Mutation spectrum and new genotype-phenotype correlation. Orphanet J Rare Dis **10**: 85.

Espinós C, Millán JM, Beneyto M & Nájera C (1998): Epidemiology of Usher syndrome in Valencia and Spain. Community Genet **1**: 223–228.

Fernandez-San Jose P, Blanco-Kelly F, Corton M et al. (2015): Prevalence of Rhodopsin mutations in autosomal dominant Retinitis Pigmentosa in Spain: clinical and analytical review in 200 families. Acta Ophthalmol **93**: e38–e44.

Gibbs D, Azarian SM, Lillo C, Kitamoto J, Klomp AE, Steel KP, Libby RT & Williams DS (2004): Role of myosin VIIa and Rab27a in the motility and localization of RPE melanosomes. J Cell Sci **117**: 6473–6483.

Hope CI (1997): Usher syndrome in the city of Birmingham – prevalence and clinical classification. Br J Ophthalmol **81**: 46–53.

Jaijo T, Aller E, Oltra S et al. (2006): Mutation profile of the MYO7A gene in Spanish patients with Usher syndrome type I. Hum Mutat **27**: 290–291.

Jatana KR, Thomas D, Weber L, Mets MB, Silverman JB & Young NM (2013): Usher syndrome: characteristics and outcomes of pediatric cochlear implant recipients. Otol Neurotol **34**: 484–489.

Kimberling WJ & Moller C (1995): Clinical and molecular genetics of Usher syndrome. J Am Acad Audiol **6**: 63–72.

Kros CJ, Marcotti W, Van Netten SM, Self TJ, Libby RT, Brown SDM, Richardson GP & Steel KP (2002): Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations. Nat Neurosci **5**: 41–47.

Le Quesne Stabej P, Saihan Z, Rangesh N et al. (2012): Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study. J Med Genet **49**: 27–36.

Liu X, Ondek B & Williams DS (1998): Mutant myosin VIIa causes defective

melanosome distribution in the RPE of shaker-1 mice. Nat Genet **19**: 117–118.

Liu X, Udovichenko IP, Brown SD, Self TJ, Libby RT, Steel KP & Williams DS (1999): Myosin VIIa participates in opsin transport through the photoreceptor cilium. J Neurosci **19**: 6267–6274.

Millán JM, Aller E, Jaijo T, Blanco-Kelly F, Gimenez-Pardo A & Ayuso A (2011): An update on the genetics of Usher syndrome. J Ophthalmol **2011**: 1–8.

Nassisi M, Mohand-Saïd S, Dhaenens CM et al. (2018): Expanding the mutation spectrum in ABCA4: sixty novel disease causing variants and their associated phenotype in a large French stargardt cohort. Int J Mol Sci **19**: E2196.

Oshima A, Jaijo T, Aller E, Millan JM, Carney C, Usami S, Moller C & Kimberling WJ (2008): Mutation profile of the CDH23 gene in 56 probands with Usher syndrome type I. Hum Mutat **29**: 1–11.

Ouyang XM, Yan D, Du LL et al. (2005): Characterization of Usher syndrome type I gene mutations in an Usher syndrome patient population. Hum Genet **116**: 292–299.

Pérez-Carro R, Blanco-Kelly F, Galbis-Martínez L et al. (2018): Unravelling the pathogenic role and genotype-phenotype correlation of the USH2A p. (Cys759Phe) variant among Spanish families. PLoS One **13**: 1–18.

Petit C (2001): Usher syndrome: from Genetics to Pathogenesis. Annu Rev Genomics Hum Genet **2**: 271–297.

Reiners J, Nagel-Wolfrum K, Jürgens K, Märker T & Wolfrum U (2006): Molecular basis of human Usher syndrome: deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease. Exp Eye Res **83**: 97–119.

Riazuddin S, Nazli S, Ahmed ZM et al. (2008): Mutation spectrum of MYO7A and evaluation of a novel nonsyndromic deafness DFNB2 allele with residual function. Hum Mutat **29**: 502–511.

Roberts L, George S, Greenberg J & Ramesar RS (2015): A founder mutation in MYO7A underlies a significant proportion of usher syndrome in indigenous south africans: Implications for the African diaspora. Invest Ophthalmol Vis Sci **56**: 6671–6678.

Rong W, Chen X, Zhao K et al. (2014): Novel and recurrent MYO7A mutations in Usher syndrome type 1 and type 2. PLoS One **9**: e97808.

Roux AF, Fougère V, Le Guédard S et al. (2006): Survey of the frequency of USH1 gene mutations in a cohort of Usher patients shows the importance of cadherin 23 and protocadherin 15 genes and establishes a detection rate of above 90%. J Med Genet **43**: 763–768.

Saihan Z, Webster AR, Luxon L & Bitner-Glindzicz M (2009): Update on Usher syndrome. Curr Opin Neurol **22**: 19–27.

Self T, Mahony M, Fleming J, Walsh J, Brown SD & Steel KP (1998): Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. Development **125**: 557–566.

Smith RJH, Berlin CI, Hejtmancik JF et al. (1994): Clinical diagnosis of the Usher syndromes. Usher syndrome Consortium. Am J Med Genet **50**: 32–38.

Sorrentino FS, Gallenga CE, Bonifazzi C & Perri P (2016): A challenge to the striking genotypic heterogeneity of retinitis pigmentosa: a better understanding of the pathophysiology using the newest genetic strategies. Eye **30**: 1542–1548.

Tamagawa Y, Ishikawa K, Ishikawa K, Ishida T, Kitamura K, Makino S, Tsuru T & Ichimura K (2002): Phenotype of DFNA11: a nonsyndromic hearing loss caused by a myosin VIIA mutation. Laryngoscope **112**: 292–297.

Vernon M (1969): Usher's syndrome: deafness and progressive blindness. J Chronic Dis **22**: 133–151.

Weil D, Blanchard S, Kaplan J et al. (1995): Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature **374**: 60–61.

Yu IM, Planelles-Herrero VJ, Sourigues Y et al. (2017): Myosin 7 and its adaptors link cadherins to actin. Nat Commun **8**: 1–14.

Received on July 13th, 2020.
Accepted on January 22nd, 2021.

Correspondence:
Carmen Ayuso
Department of Medical Genetics
University Hospital Fundación Jiménez Díaz
Avda. Reyes Católicos, 2
28040 Madrid
Spain
Tel: +34 915 504 872
Fax: +34 961 246 620
Email: Cayuso@fjd.es
José M. Millán
Molecular, Cellular and Genomics Biomedicine
University Hospital La Fe. Tower A, Floor 4
Avda. Fernando Abril Martorell, 106
46026 Valencia
Spain
Tel: +34 961 246 677
Fax: +34 915 504 849
Email: millan_jos@gva.es

This project was financially supported by the Center for Biomedical Network Research on Rare Diseases (CIBERER), FIS (PI16/00425, PI16/00539 and IIS-FJD Biobank PT13/0010/0012). LG-M and IPR were supported by the Río Hortega and predoctoral Programs (CM16/00126 and FI17/00192, respectively) from Institute of Health Carlos III (ISCIII, Spanish Ministry of the Economy, Industry and Competitiveness), Regional Government of Madrid (CAM, B2017/BMD37), and Regional Government of the Valencian Community (PROMETEU/2018/135), with partial support from the European Regional Development Fund (ERDF). Additional support was received from the Ramon Areces Foundation, the University Chair UAM-IIS-FJD of Genomic Medicine, ONCE Foundation and the Spanish National Organization of the Blind (ONCE). Drafting of this manuscript was possible thanks to the UshTher project (Clinical trial of gene therapy with dual AAV vectors for retinitis pigmentosa in patients with Usher syndrome type IB), which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 754848. The authors are grateful to the families that participated in this study and to the colleagues who referred patients to us. We also thank the Genetics and Ophthalmology Departments of Fundación Jimenez Diaz University Hospital (FJD, Madrid) and Asunción Giménez, Cristina Villaverde, and Ignacio Mahillo for their technical assistance.

†These authors contributed equally.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Name and details of the primers for the amplification of the markers used in the haplotype.
Table S2. Genotype and phenotype details of the cohort studied.
Table S3. Mutations found in the cohort studied in this work.
Table S4. Novel mutations found in this study and their possible effect on the protein.
Figure S1. Pedigrees of the 29 segregated families.
Figure S2. Diagram representing the *MYO7A* gene and surrounding regions and the relative position of the markers selected for haplotyping. Location of the recurrent mutations is shown by an arrow. umbers in brackets indicate chromosomal position in chromosome 11.
Figure S3. Audiograms from patient belonging to family RP-1218 family diagnosed with atypical USH.
Figure S4. Haplotype analysis of the three most recurrent *MYO7A* mutations found in this study.

Table S1. Name and details of the primers for the amplification of the markers used in the haplotype

Marker	Forward primer (5´- 3´)	Reverse primer (5´®3´)	Amplification T (° C)	Amplification size (bp)	chromosome position
D11S527	gcccctctacttgtctggag	atgcggctccaagacaagttc	60	142-166	Chr11: 76398461-76398644
D11S787	gtgggcttattgtggtagtagtc	caagaggaggcaggagagtc	55	164-182	Chr11: 76380332-76380503
D11S4186	attctcccaatctatcgctc	gggcagtaatgatgatgtg	55	154-175	Chr11: 76968447-76968718
rs948972	agtccaagctcacagaggag	acactcctgtctgcctgatc	60	210	Chr11: 76894235
rs6592706	cttgaaggtggtctagttctca	atgtggattcaacagggcca	60	250	Chr11: 76840156
rs11237122	tgctgtactttggccctgaa	gcagaatctcgaagtcagagg	58	247	Chr11: 76920003

Table S2. Genotype and phenotype details of the cohort studied.

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
RP-2552	16/0469	c.1344-1G>A	p.?	MD/sp/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	M					Temporal S, peripheral hypopigmentation , normal ON + M (8yr)	No (10yr)	ERG: NR Sco BE. Pho NR OD/ normal OS, ab flicker BE (8yr)
FRP-477		c.1623dupC	p.(Lys542Glnfs*5)	MD/fs/t	c.6232A>T	p.(Lys2078*)	FERM 2/ns/t	Usher I	P	M						Y (surgery at 22yr)	
FRP-463		c.1996C>T	p.(Arg666*)	MD/ns/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	F	36	36	36	36		Y (36yr)	
RP-2982	18/1956	c.4441+2delT	p.?	FERM 1/sp/t	c.5886_5889 delCTTT	p.(Phe1962Leuf s*7)	MyTH4 2/fs/t	Usher I	P	F					T RP + RPE A (47yr)	Y (47yr)	ERG: NR Sco, reduced cones (41yr)
RP-1072	06/0494	c.3719G>A	p.(Arg1240Gln)	MyTH4 1/ms/ms	c.3724C>T	p.(Gln1242*)	MyTH4 1/ns/t	Usher I	P	M	47	Y	Y	Y	T RP + normal M (41yr and 59yr)	Y (40yr)	ERG: NR OD/ minimal OS
FRP-575	38175	c.5392C>T	p.(Gln1798*)	MyTH4 2/ns/ t	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	Usher I	P	F					At V+ slight ON pallor		
RP-0207	1869	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	P	M	19	15	15	35	T RP + foveal atrophy (63yr)	pseudofa quia (59yr)	
RP-0207	1899	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M	45	12	12		T RP (64yr)	Y (58yr)	
RP-0207	1897	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M		4	4	49	T RP + normal M (51yr)	Y (64yr)	ERG: NR BE (45yr)
RP-0405	97/0574	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	Usher I	P	F	10	9	10	34	T RP (12yr)->T RP + M degeneration (33yr)->T RP + M drusen (48yr)	Y (26yr)	ERG: NR BE (45yr)
RP-1915	12/0446	c.4297delC	p.(Gln1433Serfs* 116)	FERM 1/fs/t	c.4297delC	p.(Gln1433Serfs *116)	FERM 1/fs/t	Usher I	P	F	0 (4 months)	13	13		T RP + chorioretinal atrophy+ normal M (44yr)	Y (44yr)	ERG: NR BE (44yr)
RP-0473	98/0492	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.3979G>A	p.(Glu1327Lys)	FERM 1_U/ms/ms	Usher I	P	M	15	12	5	30	At V + S + normal ON and M (37yr). At V, S + RPE A (53yr)	Y (33yr)	ERG: NR BE (34yr)
RP-1229	08/0057	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.640G>A	p.(Gly214Arg)	MH/ms/ms	Usher I	P	F	8	4	3	7	T RP + normal M (9yr)	Incipient OS (13yr)	ERG: NR rods, reduced amplitudes cones (10yr). /NR BE (13yr)
RP-2475	15/1774	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	Usher I	P	M	8	5	6	5	At V + S + normal ON (8yr)	No (8yr)	ERG: NR BE (8yr)
RP-0367	97/0006	c.1478A>C	p.(Gln493Pro)	MH/ms/ms	c.4450C>T	p.(Leu1484Phe)	FERM 1/ms/ms	Usher I	P	M	28		Y		T RP + normal M (28yr)	No (29yr)	ERG: NR / VEP NR (BE (28yr)/)
RP-0237	2322	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	P	F	8	8	18	14	Ad RP (53yr)	No (28yr)->Y (53yr)	

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
RP-0237	2323	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	S	F	11	7	13	14	Ad RP (53yr)	No (22yr)->Y (47yr)	
RP-2934	18/1272	c.5944G>A	p.(Gly1982Arg)	FERM 2/fs-sp/t	c.5944G>A	p.(Val1953Glufs*12)	FERM 2/fs-sp/t	Usher I	P	M			18	15			
RP-2947	18/1411	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	Usher II / RP+hearing loss	P	M	9	15	15	No (65)	Pale ON + S (65yr)	Y (34yr)	
RP-1808	11/1123	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F	4	35	No		T RP + normal M (26yr)	No (35yr)	
RP-0284	17/3679	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	c.6052-2A>G	p.?	FERM 2/sp/t	Usher I	P	M	10	13	4	35	T RP (18yr). M oedema, (35yr	Y (50yr)	ERG: NR Flicker OD/reduced amplitudes + normal latencies OI 35yr), NR (49yr)
RP-1218	07/0994	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3G>A	p.(Met1Ile)	MD/ms/t	Atypical Usher	P	M	27	8	20	17	T RP + normal M (35yr)	Y (26yr)	ERG: NR BE (25yr)
RP-1710	10/1915	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	P	F	7	Y	Y	Y	T RP (20yr)	Y (incipient 20yr)	ERG: NR BE (25yr). / VEP normal (20yr)
RP-1710	10/1968	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	S	F	14	Y	Y	Y			
RP-1716	10/2009	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I	P	M	14	5	5	17	At V + slight ON pallor, ab M and RPE (16yr). T RP + M oedema (22yr)	Y (incipient 13yr, full 23yr)	ERG: NR (22yr)
RP-0026	1357	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	P	M	13	4	14	6	Peripheral pigment deposits	Y (29yr)	ERG: NR (31yr)
RP-0026	1358	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	B	M	9	8	12	20	RP + normal M (28yr)->T RP + M RPE atrophy (44yr)	Y (28yr)	ERG: NR (30yr) BE
RP-0279	2737	c.2171delC	p.(Thr724fs*)	MH/fs/t	c.2171delC	p.(Thr724fs*)	MD/fs/t	Usher I	P	M	9	11	11	25	T RP (28yr)-> T RP + M atrophy + ab RPE	Y (40yr)	
RP-0393	97/0465	c.4039_4053del	p.(Ala1347_Phe1351del)	FERM 1/in-fld/t	c.4117C>T	p.(Arg1373*)	FERM 1/fs/t	Usher I	P	M	8	12	8		At V + S + normal ON (11yr)->T RP + M RPE A (27yr)	Y (27yr)	ERG: and VEP: NR BE (9yr)
RP-0848	04/0188	c.2283-1G>T	p.?	IQ1/sp/t	c.2283-1G>T	p.?	IQ1/sp/t	Usher I	P	F	12				T RP (12yr)		ERG: severe alteration (12yr)/ VEP normal (12yr)
RP-0868	04/0972	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	Usher I	P	F	21	42	14	14	T RP + RPE diffuse alteration (33yr)->RPE M degeneration (57yr)	Y (43yr)	ERG: NR BE (57yr) / reduced amplitudes (57yr)

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
RP-1966	12/1049	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	Usher I	P	F	12		Y	6	TRP (39yr)	Y (54yr)	
RP-2781	17/2194	c.133-2A>G	p.?	MD 5'/sp/t	c.3979G>A	p.(Glu1327Lys)	FERM 1/ms/ms	Usher I	P	M	23	10	14	33	TRP + ab RPE M (44yr)	Y (25yr)	ERG: NR (45yr)
RP-0594	00/0369	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.5856G>A	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I	P	F	11	10	11	4	TRP + M normal OD / cellophane reflex OS (30yr)	Y (30yr)	
RP-2401	15/0075	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F	15						
RP-2632	16/2173	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	Usher I	P	F	41		41	13		Y (41yr)	
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M	10	8	8	No (16 y)	TRP + normal M (15yr)	Y (13yr)	
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M					At V + S (22yr)	N (21yr)	NR (21yr)
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		F	12	39	12	35	TRP (32yr and 41yr)	Y (32yr)	ERG: NR (29yr)/ VEP: Delayed latencies + reduced amplitudes (29yr)
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M		18	18	18	TRP (34yr)	Y (incipient 34yr)	ERG: NR (9yr) / VEP: NR (31yr)
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M	12	10	10-11	No (22 y)	TRP	N (22yr)	ERG:NR (31yr)
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F	15	15	15	15	TRP (42yr)	Y (42yr)	ERG: NR (39yr)/VEP: Delayed latencies + reduced amplitudes (39yr)
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		M							
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F	13	13	13	13	TRP (31yr)	Y (31yr)	ERG: NR (31yr) / VEP: delayed latencies + reduced amplitudes (31yr)
FRP-159		c.3610C>A	p.(Pro1204Thr)	MyTH4 1/ms/ms	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	Usher II		M	41		37	31	TRP (41yr)	Y (40yr)	ERG. reduced amplitudes (42yr)
FRP-175		c.448C>T	p.(Arg150*)	MD/ns/t	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs-sp/t	Usher I		M	6	14	6		TRP (35yr)	Y (35yr)	
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I		F	25	20	20	20	At V + S + normal ON + M (23yr).	Y (23yr) OD	ERG: ab (23yr)
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2sp/t	Usher I		M	25	20	30	30	ON pallor, pigment OD/papillae atrophy glaucoma OI (45yr)	Y (25yr OS , 30yr OD)	ERG: NR (45yr)/ VEP: reduced amplitudes + normal latencies OD/NR OI (45yr)
FRP-246		c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F	11	6	10		TRP (22yr)	No	ERG and VEP NR (23yr)

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
FRP-286		c.6025delG	p.(Ala2009Profs)	FERM 2/fs/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F	18				Pigment mobilization (26yr)		ERG and VEP: NR (42yr)
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		M	11	9	9	9	At V + few S (21yr)	No	ERG: NR (25yr) / VEP: Delayed response + reduced amplitudes (17yr)
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		F	10	11	11	11	At V + few S (18yr). TRP (31yr)	Y (incipient 35yr),	ERG: Delayed latencies + reduced amplitudes (10yr)-> minimal response (23yr) / VEP: Highly delayed response (23yr)
FRP-342		c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F	14	9	14	23		Y (33yr)	ERG: NR (23yr)/VEP: Delayed latencies + normal amplitudes (23yr)
FRP-451		c.4544_4551delAGATCATGinsCA	p.(Glu1515_Met1517delinsAla)	FERM 1/in-fd/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F	4	12	12	No (14y)	TRP + normal M (11, 13 and 14yr)	Y (incipient 14yr)	
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		F							
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		M							
FRP-550		c.1935G>A	p.(Met645Ilefs*67)	MD/fs-sp/t	c.1691-1G>A	p.?	MD/sp/t	Usher I		M	11	11	11				NR (12yr)
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M					TRP (33yr)		ERG and VEP: NR (34yr)
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M					TRP (27yr)		ERG: NR (8yr)/VEP: OD delayed latencies/OS:NR (28yr)
FRP-78		c.470G>A	p.(Ser157Asn)	MH/fs-sp/t	c.1454delT	p.(Leu485Argfs*14)	MD/fs/t	Usher I		M		3	3	2		N (18yr)	
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		M	11						
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		F	13						
Family	Gene	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of	Usher type*	K	Gen	BCVA (dec)	OMS AV*	BCVA, # examination	VF	VF, # examination	VF class	Other

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
				of variant-A /B (A1)			variant-A /B (A2)										
RP-2552	16/0469	c.1344-1G>A	p.?	MD/sp/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	M	OD 0.6/OS 0.7	0	8	cent island (VF >20° BE)	8	1	
FRP-477		c.1623dupC	p.(Lys542Glnfs*5)	MD/fs/t	c.6232A>T	p.(Lys2078*)	FERM 2/ns/t	Usher I	P	M	CF (OD)/0.2 (OS)	1/3	34	5° BE	34	3	OCT:OS macular hole
FRP-463		c.1996C>T	p.(Arg666*)	MD/ns/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	F	OD 0.117/OS 0.067	1/2	46	10°	46	2	
RP-2982	18/1956	c.4441+2delT	p.?	FERM 1/sp/t	c.5886_5889 delCTTT	p.(Phe1962Leuf s*7)	MyTH4 2/fs/t	Usher I	P	F	OD 0.5/ OS0.3	0/1	47	>10° BE	50	2	OCT: loss of photoreceptor layer, respect of central area (47yr)
RP-1072	06/0494	c.3719G>A	p.(Arg1240Gln)	MyTH4 1/ms/ms	c.3724C>T	p.(Gln1242*)	MyTH4 1/ns/t	Usher I	P	M	0.4 OD/0.4 OS-> 0.2 OD/ 0.2 OI -> LP	0->1->3	41->42->59				
FRP-575	38175	c.5392C>T	p.(Gln1798*)	MyTH4 2/ns/t	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	Usher I	P	F	0.8 OD/0.4 OS cor	0	13	20 OD°/15°OS	14	1/2	IOP:normal BE
RP-0207	1869	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	P	M	BE 0.4 -> 0.4 OD/0.2 OS-> 0.4 OD/0.16 OS	0->0/1->0/1	59->63->63	10 ° cent	63	2	Glaucoma. OCT: loss of foveal depression (63yr). Thickening of internal limiting membrane preserved central external segments (71yr).
RP-0207	1899	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M	OD 0.1/0.3 OS -> OD HM/0.3 OS	2/1->3/1	60->63	OD / OS 5° cent	61	3	IOP: Normal (60 y).OCT (66 y) : OD slight foveal atrophy
RP-0207	1897	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M	OD FC/OS 0.3 dif ->OD HM /OS FC ->OD HM/ OS LP	3/1->3	49->51->74		49	3	IOP: 30)mmHg (45yr) OCT: macular atrophy (64yr)
RP-0405	97/0574	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	Usher I	P	F	BE 0.5 ->0.4 BE -> OD 0.4/OS 0.3 ->OD	0->0/1->1/1	25->31->34->48	OD 15°/OS 14°	45	2	Photophobia (34yr). Photopsia + dyschormatopsia (48yr).OCT: epiretinal

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
											0.2/OS 0.16						membrane, foveal intrarretinal cysts, ab external layers
RP-1915	12/0446	c.4297delC	p.(Gln1433Serfs*116)	FERM 1/fs/t	c.4297delC	p.(Gln1433Serfs*116)	FERM 1/fs/t	Usher I	P	F	BE 0.8 dif ->OD 0.7/ OS 0.3 dif	0->0/1	38->44	OD 26°/ OS 22°->OD 22°/ OS 16°	40->44	1	Photophobia (39yr)
RP-0473	98/0492	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.3979G>A	p.(Glu1327Lys)	FERM 1_U/ms/ms	Usher I	P	M	OD 0.5/OS 0.6-> OD 0.5 dif/OS 0.5-> OD0.5/OS 0.16	0->0->0/1	37->44->53	OD absolute scotoma/OS 10°	44	2->3	OCT: macular cysts Photophobia and photopsia (5yr)
RP-1229	08/0057	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.640G>A	p.(Gly214Arg)	MH/ms/ms	Usher I	P	F	BE 1 dif->OD 0.9/OS 0.7-> BE 0.7	0->0->0	7->12->19	BE 60°->OD 40°/OS 50°-> BE 10-15°-> BE 10-15°	9->12->13->19	1->2	Subfoveal Cysts Normal Ishihara (13yr)
RP-2475	15/1774	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	Usher I	P	M	OD 0.7/OS 0.8	0	8	OD 64°/OS 43°	8		
RP-0367	97/0006	c.1478A>C	p.(Gln493Pro)	MH/ms/ms	c.4450C>T	p.(Leu1484Phe)	FERM 1/ms/ms	Usher I	P	M	BE 0.8	0	28	20°	28	1	Normal colour vision
RP-0237	2322	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	P	F	0.5 BE ->0.1 BE	0->2	28->53	20° cent-> 2° (53yr)	28->53	2->3	
RP-0237	2323	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	S	F	0.5 BE->0.3 BE	0->1	22->47	50% vision-> 2° (47yr)	22->47	2->3	
RP-2934	18/1272	c.5944G>A	p.(Gly1982Arg)	FERM 2/fs-sp/t	c.5944G>A	p.(Val1953Glufs*12)	FERM 2/fs-sp/t	Usher I	P	M							
RP-2947	18/1411	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	Usher II / RP+HL	P	M	OD 0.6/ OS 0.5	0	65				OCT: external layers Atrophy
RP-1808	11/1123	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F	BE 0.6 ->OD 0.6/OS 0.3 -> 0.4 BE	0-->0/1	26->31->35				
RP-0284	17/3679	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	c.6052-2A>G	p.?	FERM 2/sp/t	Usher I	P	M	OD 0.5/OS 0.6-> BE 0.35->OD 0.15/OS 0.4dif->0.05/0.2 -> OD CF/OS 0.2	0/0->1/1->1/0->2/1->3/1	18->30->35-> 49->52	OD10°/OS5°-> absolute scotomae/ cent island	35->52	2/3->3	Photophobia (46yr). OCT (50yr): normal M, OCT (58yr): foveal atrophy.
RP-1218	07/0994	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3G>A	p.(Met1Ile)	MD/ms/t	Atypical Usher	P	M	OD 0.7/OS 0.7-> OD	0→0	25→35	20° BE→15° BE	31→35	2	OCY: ON atrophy OS

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotid e (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
											0.5/OS 0.7						(25yr)Photophobi a (25 y)
RP-1710	10/1915	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	P	F	0.3 BE	1	20	OD 15°/OS 15°-20°	20	½	
RP-1710	10/1968	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	S	F	OD 0.2/OSFC	1/3	29				
RP-1716	10/2009	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I	P	M	OD 0.8/OS 0.5-> OD 0.7/OS 0.5-> OD 0.6/OS 0.4	0	14->16-> 22	10°	22	2	Photopsia (22yr). Normal colour vision. OCT: subfoveal cysts, ab external layers, epiretinal membrane (22yr)
RP-0026	1357	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	P	M	OD 0.5/OS 0.4-> OD 0.5/OS 0.8	0	26->31	cent + temporal island BE	31		Astigmatism and myopia (9yr)
RP-0026	1358	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	B	M	OD 0.7/OS 0.8-> OD 0.7/OS 0.6-> OD 0.2/OS 0.3-> OD 0.16/OS 0.05	0->0->1-> ½	28->30-> 44->47	OD temporal relative scotomae->5-10°	30→44	1->3	
RP-0279	2737	c.2171delC	p.(Thr724fs*)	MH/fs/t	c.2171delC	p.(Thr724fs*)	MD/fs/t	Usher I	P	M	BE 0.3->0.1 BE->LP BE	1->2->3	28->53-> 54	10° cent BE->< 10° BE-> absolute scotoma	28->52->53	2->3	Photophobia + dyschromatopsia (25yr). OCT: macular atrophy (54yr). FAF: retinal atrophy, hipofluorescent 'posterior pole + translucent choroidal vessels (55yr)
RP-0393	97/0465	c.4039_4053d el	p.(Ala1347_Phe1 351del)	FERM 1/in-f id/t	c.4117C>T	p.(Arg1373*)	FERM 1/fs/t	Usher I	P	M	BE 0.5-> OD FC/ OD 0.1-> BE 0.05	1->2->2	11->24-> 33	10° BE	11	2	Dyschromatopsia (11yr). FAF hyperautofluores cent fovea : OD / perifoveal OS (33 y)
RP-0848	04/0188	c.2283-1G>T	p.?	IQ1/sp/t	c.2283-1G>T	p.?	IQ1/sp/t	Usher I	P	F							
RP-0868	04/0972	c.3764delA	p.(Lys1255Argfs* 8)	MyTH4 1_FERM1/fs /t	c.3764delA	p.(Lys1255Argfs *8)	MyTH4 1_FERM1/fs/t	Usher I	P	F	OD 0.5/ OS 0.6dif-> BE 0.4-> OD 0.16/ OS 0.3dif-	0->1->1/2	33-> 57-> 58-> 59	10°BE->5°	33 and 45->47	2->3	OCT:M atrophy no oedema (57yr). Photophobia,

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
											> OD 0.16 OS /0.05->BE CF						normal colour vision (57 r)
RP-1966	12/1049	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	Usher I	P	F	0.1 BE	2	39				
RP-2781	17/2194	c.133-2A>G	p.?	MD 5'/sp/t	c.3979G>A	p.(Glu1327Lys)	FERM 1/ms/ms	Usher I	P	M	LP BE	3	44	Tubular cent	45		Photophobia y dyschromatopsia (44yr).OCT: no cysts (45yr)
RP-0594	00/0369	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.5856G>A	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I	P	F	OS 0.5/ OD 0.6	0	30	20°BE	19		OCT: OD-intratreretinal cysts BE, OS: epiretinal membrane (30yr)
RP-2401	15/0075	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F							
RP-2632	16/2173	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	Usher I	P	F	BE 0.05	2	41	<10° BE	41	3	
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M	BE 0.7	0	15	>8 ° BE	15	2-3	OCT: preserved central M
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M	BE 0.6	0	22	Tubular cent	21	2	
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		F	OD 0.4/ OS CF	0/3	32				
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M	OD 0.4/ OS 0.3	0/1	40	Tubular cent	31	3	
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M	BE 0.6	0					OCT M preserved central thickness
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F	OD 0.2/ OS 0.15->LP BE	1/1->3	42->65	<10° BE	42	3	
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		M							
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F	OD 0.2/ OS 0.15	1/1	31	<10° BE	34	3	
FRP-159		c.3610C>A	p.(Pro1204Thr)	MyTH4 1/ms/ms	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	Usher II		M	OD 0.7 OS /0.3	0/1	40	20°BE	41	1	
FRP-175		c.448C>T	p.(Arg150*)	MD/ns/t	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs-sp/t	Usher I		M	BE 0.8->BE 0.6->BE 0.32	0/0->0/0->1/1	15->22->35	5°BE->3°BE	15->35	3→3	
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I		F	OD 0.7 / OS 0.5 → OD 0.7 / OS 0.4	0/0->0/0	23-> 28	<20°->10°	23	2	
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2sp/t	Usher I		M	OD 0.2/ OS 0	1/3	45	7°/0°	45	3/3	
FRP-246		c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F	0.8 BE -> 0,7 BE	0/0	22->23	10° BE	23	2	OCT: slight thickening of internal limiting membrane
FRP-286		c.6025delG	p.(Ala2009Profs)	FERM 2/fs/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F	OD 0.15/ OS 0.3	1/1	36				

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		M	0.8 BE	0/0	21	Tubular cent	21		
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		F	0.8 BE-> OD 0.4/ OS 0.6- >0.2 BE-> LP	0/0	15->31- >32-> 35	<10° BE->0°	15->35	3	OCT: retinal thinning + RPE atrophy (38yr).
FRP-342		c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F	0.2/0.05	½	33→35	<10° BE→ <10°	33->35	3->3	OCT: atrophy + retinal thinning, of central retina.
FRP-451		c.4544_4551delAGATCATGinsCA	p.(Glu1515_Met1517delinsAla)	FERM 1/in-fd/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F	BE 1	0	11-> 12-> 13-> 14	Normal	11->12	0->0	
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		F							
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		M							
FRP-550		c.1935G>A	p.(Met645Ilefs*67)	MD/fs-sp/t	c.1691-1G>A	p.?	MD/sp/t	Usher I		M				15°OD/>20° OS	12	2/1	
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M	OD 0.2/ OS 0.4	1/0	33	Absolute scotomae	33	3	
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M	OD 0.4/ OS 0.1	0/2	27	5°	27	3	
FRP-78		c.470G>A	p.(Ser157Asn)	MH/fs-sp/t	c.1454delT	p.(Leu485Argfs*14)	MD/fs/t	Usher I		M							Glaucoma
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		M							
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		F							retinal detachment (3yr)
RP family	**Gene**	**Nucleotide (Allele1)**	**Amino acid (Allele1)**	**Prot Domain (A1)/ Type of variant-A /B (A1)**	**Nucleotide (Allele2)**	**Amino acid (Allele2)**	**Prot Domain (A2))/ Type of variant-A /B (A2)**	**Usher type***	**K**	**G**	**HL**	**HL# diag**	**HL degree**	**HL: Stab/Prog**			
RP-2552	16/0469	c.1344-1G>A	p.?	MD/sp/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	M	Y	1- 2yr	sev/prof				
FRP-477		c.1623dupC	p.(Lys542Glnfs*5)	MD/fs/t	c.6232A>T	p.(Lys2078*)	FERM 2/ns/t	Usher I	P	M	Y						
FRP-463		c.1996C>T	p.(Arg666*)	MD/ns/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	F	Y	0yr	sev/prof				
RP-2982	18/1956	c.4441+2delT	p.?	FERM 1/sp/t	c.5886_5889 delCTTT	p.(Phe1962Leufs*7)	MyTH4 2/fs/t	Usher I	P	F	Y						
RP-1072	06/0494	c.3719G>A	p.(Arg1240Gln)	MyTH4 1/ms/ms	c.3724C>T	p.(Gln1242*)	MyTH4 1/ns/t	Usher I	P	M	Y	0yr	sev/prof	Stable			
FRP-575	38175	c.5392C>T	p.(Gln1798*)	MyTH4 2/ns/t	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	Usher I	P	F							
RP-0207	1869	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	P	M	Y	0yr					
RP-0207	1899	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M	Y		severe				
RP-0207	1897	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M	Y		severe				
RP-0405	97/0574	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	Usher I	P	F	Y	8-9	severe	Stable			

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
RP-1915	12/0446	c.4297delC	p.(Gln1433Serfs*116)	FERM 1/fs/t	c.4297delC	p.(Gln1433Serfs*116)	FERM 1/fs/t	Usher I	P	F	Y	0yr	sev/prof				
RP-0473	98/0492	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.3979G>A	p.(Glu1327Lys)	FERM 1_U/ms/ms	Usher I	P	M	Y	18	sev/prof	Stable			
RP-1229	08/0057	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.640G>A	p.(Gly214Arg)	MH/ms/ms	Usher I	P	F		0yr	sev/prof	Stable			
RP-2475	15/1774	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	Usher I	P	M	Y	0yr	sev/prof	Stable			
RP-0367	97/0006	c.1478A>C	p.(Gln493Pro)	MH/ms/ms	c.4450C>T	p.(Leu1484Phe)	FERM 1/ms/ms	Usher I	P	M	Y	3 months					
RP-0237	2322	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	P	F	Y	0yr	sev/prof				
RP-0237	2323	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	S	F	Y	0yr	sev/prof				
RP-2934	18/1272	c.5944G>A	p.(Gly1982Arg)	FERM 2/fs-sp/t	c.5944G>A	p.(Val1953Glufs*12)	FERM 2/fs-sp/t	Usher I	P	M	Y	0yr					
RP-2947	18/1411	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	Usher II / RP+HL	P	M	Y	3yr	severe				
RP-1808	11/1123	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F	Y	10 months	sev/prof	Stable			
RP-0284	17/3679	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	c.6052-2A>G	p.?	FERM 2/sp/t	Usher I	P	M	Y	3 months	sev/prof	Stable			
RP-1218	07/0994	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3G>A	p.(Met1Ile)	MD/ms/t	Atypical Usher	P	M	Y	15yr	Mod (25yr)-> sev(41yr)	Progressive			
RP-1710	10/1915	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	P	F	Y	0yr		Stable			
RP-1710	10/1968	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	S	F	Y	0yr					
RP-1716	10/2009	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I	P	M	Y	0yr	sev/prof	Stable			
RP-0026	1357	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	P	M	Y	0yr	sev/prof	Stable			
RP-0026	1358	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	B	M	Y	0yr	sev/prof	Stable			
RP-0279	2737	c.2171delC	p.(Thr724fs*)	MH/fs/t	c.2171delC	p.(Thr724fs*)	MD/fs/t	Usher I	P	M	Y	0yr	sev/prof				
RP-0393	97/0465	c.4039_4053del	p.(Ala1347_Phe1351del)	FERM 1/lin-fid/t	c.4117C>T	p.(Arg1373*)	FERM 1/fs/t	Usher I	P	M	Y	0yr	sev/prof				
RP-0848	04/0188	c.2283-1G>T	p.?	IQ1/sp/t	c.2283-1G>T	p.?	IQ1/sp/t	Usher I	P	F	Y						
RP-0868	04/0972	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	Usher I	P	F	Y	0yr	sev/prof				
RP-1966	12/1049	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	Usher I	P	F	Y	0yr					
RP-2781	17/2194	c.133-2A>G	p.?	MD 5'/sp/t	c.3979G>A	p.(Glu1327Lys)	FERM 1/ms/ms	Usher I	P	M	Y	0yr	sev/prof	Stable			
RP-0594	00/0369	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.5856G>A	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I	P	F	Y	8 months					
RP-2401	15/0075	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F	Y	0yr					
RP-2632	16/2173	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	Usher I	P	F	Y	0yr					

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M	Y	0yr	profound				
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M	Y						
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		F	Y	0yr	profound				
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M	Y	0yr					
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M	Y	0yr	profound				
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F	Y	0yr	profound	Stable			
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		M	Y	0yr	profound	Stable			
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F	Y	0yr	profound	Stable			
FRP-159		c.3610C>A	p.(Pro1204Thr)	MyTH4 1/ms/ms	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	Usher II		M	Y	8 months	moderate	Stable			
FRP-175		c.448C>T	p.(Arg150*)	MD/ns/t	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs-sp/t	Usher I		M	Y	0yr	profound	Stable			
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I		F	Y	0yr		Stable			
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2sp/t	Usher I		M	Y	0yr		Stable			
FRP-246		c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F	Y	0yr	profound	Stable			
FRP-286		c.6025delG	p.(Ala2009Profs)	FERM 2/fs/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F							
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		M	Y	8 months	profound	Stable			
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		F	Y	7 months	profound	Stable			
FRP-342		c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F	Y	0yr	profound	Stable			
FRP-451		c.4544_4551delAGATCATGinsCA	p.(Glu1515_Met1517delinsAla)	FERM 1/in-fd/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F	Y	0yr	profound	Stable			
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		F	Y						
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		M	Y						
FRP-550		c.1935G>A	p.(Met645Ilefs*67)	MD/fs-sp/t	c.1691-1G>A	p.?	MD/sp/t	Usher I		M	Y	0yr	profound	Stable			
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M	Y		profound				
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M	Y		profound				
FRP-78		c.470G>A	p.(Ser157Asn)	MH/fs-sp/t	c.1454delT	p.(Leu485Argfs*14)	MD/fs/t	Usher I		M	Y	0yr		Stable			

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		M	Y	0yr	profound				
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		F	Y	0yr	profound				
Family	Gene	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	# standing (months)	Delayed unaided walk	Unaided walking # (months)	Cochlear implantation	# cochlear implant	verbal	language acquisition (yr)
RP-2552	16/0469	c.1344-1G>A	p.?	MD/sp/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	M	12	Y	16	Y	3 (RE)/6 (LE)	Y	>3
FRP-477		c.1623dupC	p.(Lys542Glnfs*5)	MD/fs/t	c.6232A>T	p.(Lys2078*)	FERM 2/ns/t	Usher I	P	M							
FRP-463		c.1996C>T	p.(Arg666*)	MD/ns/t	c.1996C>T	p.(Arg666*)	MD/ns/t	Usher I	P	F							
RP-2982	18/1956	c.4441+2delT	p.?	FERM 1/sp/t	c.5886_5889 delCTTT	p.(Phe1962Leufs*7)	MyTH4 2/fs/t	Usher I	P	F						N	
RP-1072	06/0494	c.3719G>A	p.(Arg1240Gln)	MyTH4 1/ms/ms	c.3724C>T	p.(Gln1242*)	MyTH4 1/ns/t	Usher I	P	M							
FRP-575	38175	c.5392C>T	p.(Gln1798*)	MyTH4 2/ns/t	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	Usher I	P	F							
RP-0207	1869	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	P	M							
RP-0207	1899	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M							
RP-0207	1897	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	c.1884C>A	p.(Cys628*)	MD Ac/ns/t	Usher I	B	M							
RP-0405	97/0574	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.1190C>A	p.(Ala397Asp)	MD/ms/ms	Usher I	P	F		Y	30	N		N (lip-reading)	
RP-1915	12/0446	c.4297delC	p.(Gln1433Serfs*116)	FERM 1/fs/t	c.4297delC	p.(Gln1433Serfs*116)	FERM 1/fs/t	Usher I	P	F		Y	24	N		N	
RP-0473	98/0492	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.3979G>A	p.(Glu1327Lys)	FERM 1_U/ms/ms	Usher I	P	M		Y					
RP-1229	08/0057	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.640G>A	p.(Gly214Arg)	MH/ms/ms	Usher I	P	F	10 neck control	Y	17	Y	26 months		
RP-2475	15/1774	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	c.5857-?_*544+?del	del ex 43-49	FERM 2/d/t	Usher I	P	M				Y	1		
RP-0367	97/0006	c.1478A>C	p.(Gln493Pro)	MH/ms/ms	c.4450C>T	p.(Leu1484Phe)	FERM 1/ms/ms	Usher I	P	M							
RP-0237	2322	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	P	F						N	
RP-0237	2323	c.395C>T	p.(Pro132Leu)	MH/ms/ms	c.395C>T	p.(Pro132Leu)	MH/ms/ms	Usher I	S	F							
RP-2934	18/1272	c.5944G>A	p.(Gly1982Arg)	FERM 2/fs-sp/t	c.5944G>A	p.(Val1953Glufs*12)	FERM 2/fs-sp/t	Usher I	P	M							
RP-2947	18/1411	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	c.4114G>A	p.(Val1372Met)	FERM 1/ms/ms	Usher II / RP+HL	P	M				N (hearing aids) 9yr			
RP-1808	11/1123	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F						N	
RP-0284	17/3679	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	c.6052-2A>G	p.?	FERM 2/sp/t	Usher I	P	M		Y		Y	39yr	N	
RP-1218	07/0994	c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3G>A	p.(Met1Ile)	MD/ms/t	Atypical Usher	P	M				N (hearing aids)→Y	42yr		
RP-1710	10/1915	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	P	F							

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
RP-1710	10/1968	c.397C>T	p.(His133Tyr)	MD/ms/ms	c.397C>T	p.(His133Tyr)	MD/ms/ms	Usher I	S	F							
RP-1716	10/2009	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I	P	M		Y	36	Y	2 and 8yr		
RP-0026	1357	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	P	M						N	
RP-0026	1358	c.6025delG	p.(Ala2009fs*)	FERM 2/fs/t	c.6025DelG	p.(Ala2009fs*)	FERM 2/fs/t	Usher I	B	M				Y	43yr	N	
RP-0279	2737	c.2171delC	p.(Thr724fs*)	MH/fs/t	c.2171delC	p.(Thr724fs*)	MD/fs/t	Usher I	P	M							
RP-0393	97/0465	c.4039_4053del	p.(Ala1347_Phe1351del)	FERM 1/lin-fid/t	c.4117C>T	p.(Arg1373*)	FERM 1/fs/t	Usher I	P	M		Y		N			
RP-0848	04/0188	c.2283-1G>T	p.?	IQ1/sp/t	c.2283-1G>T	p.?	IQ1/sp/t	Usher I	P	F							
RP-0868	04/0972	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	Usher I	P	F							
RP-1966	12/1049	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	c.5648G>A	p.(Arg1883Gln)	MyTH4 2/ms/ms	Usher I	P	F							
RP-2781	17/2194	c.133-2A>G	p.?	MD 5'/sp/t	c.3979G>A	p.(Glu1327Lys)	FERM 1/ms/ms	Usher I	P	M						Y	
RP-0594	00/0369	c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.5856G>A	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I	P	F			18			N	
RP-2401	15/0075	c.397C>T	p.(His133Tyr)	MH/ms/ms	c.397C>T	p.(His133Tyr)	MH/ms/ms	Usher I	P	F							
RP-2632	16/2173	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	c.5886_5889del	p.(Phe1962Leufs*7)	FERM 2/fs/t	Usher I	P	F							
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M				N			
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M							
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		F			18	N		N	
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.2461C>T	p.(Gln821*)	IQ4/ns/t	Usher I		M							
FRP-1		c.2461C>T	p.(Gln821*)	IQ4/ns/t	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	MD/fs/t	Usher I		M		Y	36	N		N	
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F				N		N	
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		M		Y	4 yr	N		N	
FRP-11		c.2461C>T	p.(Gln821*)	IQ4ns/t	c.4740C>G	p.(Tyr1580*)	FERM 1/ns/t	Usher I		F				N		N	
FRP-159		c.3610C>A	p.(Pro1204Thr)	MyTH4 1/ms/ms	c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	Usher II		M		Y	18				
FRP-175		c.448C>T	p.(Arg150*)	MD/ns/t	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs-sp/t	Usher I		M		Y	>20	N		N	
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2/sp/t	Usher I		F							
FRP-23		c.1190C>A	p.(Ala397Asp)	MD/ms/ms	c.5856G>A (UV4)	p.(Ala1915_Lys1952del)	FERM 2sp/t	Usher I		M							
FRP-246		c.3764delA	p.(Lys1255Argfs*8)	MyTH4 1_FERM1/fs/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F		Y	18	Y	26yr	N	
FRP-286		c.6025delG	p.(Ala2009Profs)	FERM 2/fs/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F							
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		M		Y	19	N		N	

Family	DNA	Nucleotide (Allele1)	Amino acid (Allele1)	Prot Domain (A1)/ Type of variant-A /B (A1)	Nucleotide (Allele2)	Amino acid (Allele2)	Prot Domain (A2))/ Type of variant-A /B (A2)	Usher type*	K	G	Diag # yr	NB onset # yr	VF loss onset # yr	VA loss onset # yr	Ocular Fundus	Cataract (Y/N, type, #)	ERG and VEP # examination
FRP-3		c.3238A>T	p.(Lys1080*)	MyTH4 1/ns/t	c.3508G>A	p.(Glu1170Lys)	MyTH4 1/ms/ms	Usher I		F		Y	19	Y	17yr	N	2-3 y speech therapy and hearing aids
FRP-342		c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	c.5581C>T	p.(Arg1861*)	MyTH4 2/ns/t	Usher I		F		Y		N		N	
FRP-451		c.4544_4551delAGATCATGinsCA (UV4)	p.(Glu1515_Met1517delinsAla)	FERM 1/in-f d/t	c.6025delG	p.(Ala2009Profs*32)	FERM 2/fs/t	Usher I		F		Y	21	Y	1 and 5yr	Y	Normal
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		F							
FRP-530		c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	c.494C>T	p.(Thr165Met)	MD ATP/ms/ms	Usher I		M							
FRP-550		c.1935G>A	p.(Met645Ilefs*67)	MD/fs-sp/t	c.1691-1G>A	p.?	MD/sp/t	Usher I		M		Y	22	Y	0	Y	
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M						N	
FRP-77		c.640G>A	p.(Gly214Arg)	MH/ms/ms	c.1342_1343 delAG (UV4)	p.(Asn443_Glu450del)	MD/sp/t	Usher I		M						N	
FRP-78		c.470G>A	p.(Ser157Asn)	MH/fs-sp/t	c.1454delT	p.(Leu485Argfs*14)	MD/fs/t	Usher I		M							
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		M			16	N (hearing aids)	2	Y(lip-reading)	
FRP-290		c.5516T>C	p.(Leu1839Pro)	MyTH4 2/ms/ms	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59)	MyTH4 1/fs/sp	Usher I		F			16	N (hearing aids)	1	Y(lip-reading)	

Abbreviations;*: Presumed diagnosis. #: age at. K: Kin. G: Gender. P: Proband. B: Brother. S: Sister. DOB: Date of Birth. N: No. Y: Yes. Yr: years. Diag: diagnosis. BE: Both Eyes. OD: Oculus Dexter. OS: Oculus Sinister. BCVA: Best Corrected Visual Acuity. dec: decimal. NB: Night Blindness. VA: Visual Acuity. VF: Visual Field. Prot. Protein. FERM 1: FERM 1 (1258-1602). FERM 2: FERM 2 (1902-2205). IQ1: IQ 1 (745-765). IQ4: IQ 4 (814-834). MD: Motor domain (1-729). MD Ac: Motor domain (1-729) actin binding site (632-639). MD ATP: Motor domain (1-729) ATP binding site (158-165). MH: Myosin head.motor domain (P-loop containing nucleoside triphosphate hydrolase). MyTH4 1: MyTH4 1 (1017-1253). MyTH4 2: MyTH4 2 (1747-1896). MyTH4 1_FERM1: between MyTH4 1 and FERM1 domains. MD 5': MD upstream. d: deletion. fs: frameshift. fs-sp: frameshift-splicing. in f –d: in frame deletion. in f –id: in frame indel. ms: missense. ns: non-sense. sp: splicing. t: truncating. A RP: Advanced Retinitis Pigmentosa. T RP: Typical Retinitis Pigmentosa. At V: Attenuated vessels. S: Spicules. M: Macula/Macular. RPE: Retinal Pigment epithelium. ON: Optic Nerve. HL: Hearing Loss. ERG: electroretinogram. Sco: Scotopic. Pho: Photopic. NR: Non-recordable. VEP: Visual Evoked Potentials. Ab: abnormal. HM: Hand movement. CF: Counts Fingers. LP: Light Perception. OCT: Optical Coherence Tomography. IOP: intraocular pressure. FAF: fundus autofluorescence. RE: Right Ear. LE: Left Ear. Mod: moderate. Sev: severe. Prof: Profound.

Table S3. Mutations found in the cohort studied in this work.

Family	ADN	Nucleotide (Allele1)	Amino acid (Allele1)	Reference (Allele 1)	Nucleotide (Allele2)	Amino acid (Allele2)	Reference (Allele 2)	Usher type (initial diagnosis)
RP-2552	16/0469	c.1344-1G>A	p.?	This study	c.1996C>T	p.(Arg666*)	(Janecke et al., 1999)	Usher I
FRP-477		c.1623dupC	p.(Lys542Glnfs*5)	(Bharadwaj, Kasztejna, Huq, Berson, & Dryja, 2000)	c.6232A>T	p.(Lys2078*)	(Fuster-García et al., 2018)	Usher I
FRP-463		c.1996C>T	p.(Arg666*)	(Janecke et al., 1999)	c.1996C>T	p.Arg666*	(Janecke et al., 1999)	Usher I
RP-2982	18/1956	c.4441+2delT	p.?	This study	c.5886_5889delCTT T	(p.Phe1962Leufs*7)	(Gerber et al., 2006)	Usher I
RP-1072	06/0494	c.3719G>A	p.(Arg1240Gln)	(Pennings et al., 2006)	c.3724C>T	p.(Gln1242*)	(Jaijo et al., 2010)	Usher I
RP-0207	1869	c.1884C>A	p.(Cys628*)	(Cuevas et al., 1998)	c.1884C>A	p.(Cys628*)	(Cuevas et al., 1998)	Usher I
RP-0207	1899	c.1884C>A	p.(Cys628*)	(Cuevas et al., 1998)	c.1884C>A	p.(Cys628*)	(Cuevas et al., 1998)	Usher I
RP-0207	1897	c.1884C>A	p.(Cys628*)	(Cuevas et al., 1998)	c.1884C>A	p.(Cys628*)	(Cuevas et al., 1998)	Usher I
RP-0405	97/0574	c.1190C>A	p.(Ala397Asp)	(Adato et al., 1997)	c.1190C>A	p.(Ala397Asp)	(Adato et al., 1997)	Usher I
RP-1915	12/0446	c.4297delC	p.(Gln1433Serfs*116)	(Roux et al., 2006)	c.4297delC	p.(Gln1433Serfs*116)	(Roux et al., 2006)	Usher I
RP-0473	98/0492	c.640G>A	p.(Gly214Arg)	(Adato et al., 1997)	c.3979G>A	p.(Glu1327Lys)	(Nájera et al., 2002)	Usher I
RP-2475	15/1774	c.5857-?_*544+?del	del ex 43-49	This study	c.5857-?_*544+?del	del ex 43-49	This study	Usher I
RP-0367	97/0006	c.1478A>C	p.(Gln493Pro)	(Jaijo et al., 2006)	c.4450C>T	p.(Leu1484Phe)	(Jaijo et al., 2006)	Usher I
RP-0237	2322	c.395C>T	p.(Pro132Leu)	(Jaijo et al., 2007)	c.395C>T	p.(Pro132Leu)	(Jaijo et al., 2007)	Usher I
RP-0237	2323	c.395C>T	p.(Pro132Leu)	(Jaijo et al., 2007)	c.395C>T	p.(Pro132Leu)	(Jaijo et al., 2007)	Usher I
RP-2934	18/1272	c.5944G>A	p.(Val1953Glufs*12) (skipping of exon 43)	(Riazuddin et al., 2008)	c.5944G>A	p.(Val1953Glufs*12) (skipping of exon 43)	(Riazuddin et al., 2008)	Usher I
RP-2947	18/1411	c.4114G>A	p.(Val1372Met)	This study	c.4114G>A	p.(Val1372Met)	This study	Usher II vs RP+hearing loss
RP-1808	11/1123	c.397C>T	p.(His133Tyr)	(Roux et al., 2011)	c.397C>T	p.(His133Tyr)	(Roux et al., 2011)	Usher I
RP-0284	17/3679	c.3508G>A	p.(Glu1170Lys)	(Cuevas et al., 1999)	c.6052-2A>G	p.?	This study	Usher I
RP-1218	07/0994	c.5516T>C	p.(Leu1839Pro)	(Aparisi et al., 2014)	c.3G>A	p.(Met1Ile)	(Fuster-García et al., 2018)	Atypical Usher

Family	ADN	Nucleotide (Allele1)	Amino acid (Allele1)	Reference (Allele 1)	Nucleotide (Allele2)	Amino acid (Allele2)	Reference (Allele 2)	Usher type (initial diagnosis)
RP-1710	10/1915	c.397C>T	p.(His133Tyr)	(Roux et al., 2011)	c.397C>T	p.(His133Tyr)	(Roux et al., 2011)	Usher I
RP-1710	10/1968	c.397C>T	p.(His133Tyr)	(Roux et al., 2011)	c.397C>T	p.(His133Tyr)	(Roux et al., 2011)	Usher I
RP-1716	oct-09	c.494C>T	p.(Thr165Met)	(Aparisi et al., 2014)	c.494C>T	p.(Thr165Met)	(Aparisi et al., 2014)	Usher I
RP-0026	1357	c.6025delG	p.(Ala2009fs*32)	(Nájera et al., 2002)	c.6025delG	p.(Ala2009fs*32)	(Nájera et al., 2002)	Usher I
RP-0026	1358	c.6025delG	p.(Ala2009fs*32)	(Nájera et al., 2002)	c.6025delG	p.(Ala2009fs*32)	(Nájera et al., 2002)	Usher I
RP-0279	2737	c.2171delC	p.(Thr724fs*)	(Fuster-García et al., 2018)	c.2171delC	p.(Thr724fs*)	(Fuster-García et al., 2018)	Usher I
RP-0393	97/0465	c.4039_4053del	p.(Ala1347_Phe1351del)	(Nájera et al., 2002)	c.4117C>T	p.(Arg1373*)	(Jaijo et al., 2006)	Usher I
RP-0848	04/0188	c.2283-1G>T	p.?	(Roux et al., 2006)	c.2283-1G>T	p?	(Roux et al., 2006)	Usher I
RP-0868	04/0972	c.3764delA	p.(Lys1255Argfs*8)	(Jaijo et al., 2007)	c.3764delA	p.(Lys1255Argfs*8)	(Jaijo et al., 2007)	Usher I
RP-1229	08/0057	c.640G>A	p.(Gly214Arg)	(Adato et al., 1997)	c.640G>A	p.(Gly214Arg)	(Adato et al., 1997)	Usher I
RP-1966	12/1049	c.5648G>A	p.(Arg1883Gln)	(Le Quesne Stabej et al., 2012)	c.5648G>A	p.(Arg1883Gln)	(Le Quesne Stabej et al., 2012)	Usher I
RP-2781	17/2194	c.133-2A>G	p.?	(Nájera et al., 2002)	c.3979G>A	p.(Glu1327Lys)	(Nájera et al., 2002)	Usher I
RP-0594	00/0369	c.640G>A	p.(Gly214Arg)	(Adato et al., 1997)	c.5856G>A	p.(Ala1915_Lys1952de)	(Jaijo et al., 2006)	Usher I
RP-2401	15/0075	c.397C>T	p.(His133Tyr)	(Le Guédard-Méreuze et al., 2010)	c.397C>T	p.(His133Tyr)	(Le Guédard-Méreuze et al., 2010)	Usher I
RP-2632	16/2173	c.5886_5889del	p.(Phe1962Leufs*7)	(Gerber et al., 2006)	c.5886_5889del	p.(Phe1962Leufs*7)	(Gerber et al., 2006)	Usher I
FRP-1		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	(Jaijo et al., 2006)	Usher I
FRP-1		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	Usher I
FRP-1		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	Usher I
FRP-1		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	Usher I
FRP-1		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.6_9dup (UV4)	p.(Leu4Aspfs*39)	(Jaijo et al., 2006)	Usher I
FRP-11		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.4740C>G	p.(Tyr1580*)	(Nájera et al., 2002)	Usher I
FRP-11		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.4740C>G	p.(Tyr1580*)	(Nájera et al., 2002)	Usher I
FRP-11		c.2461C>T	p.(Gln821*)	(Nájera et al., 2002)	c.4740C>G	p.(Tyr1580*)	(Nájera et al., 2002)	Usher I
FRP-159		c.3610C>A	p.(Pro1204Thr)	(Besnard et al., 2014)	c.3764delA	p.(Lys1255Argfs*8)	(Jaijo et al., 2007)	Usher II
FRP-175		c.448C>T	p.(Arg150*)	(Lévy et al., 1997)	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59) (skipping of exon 27)	(Jaijo et al., 2006)	Usher I

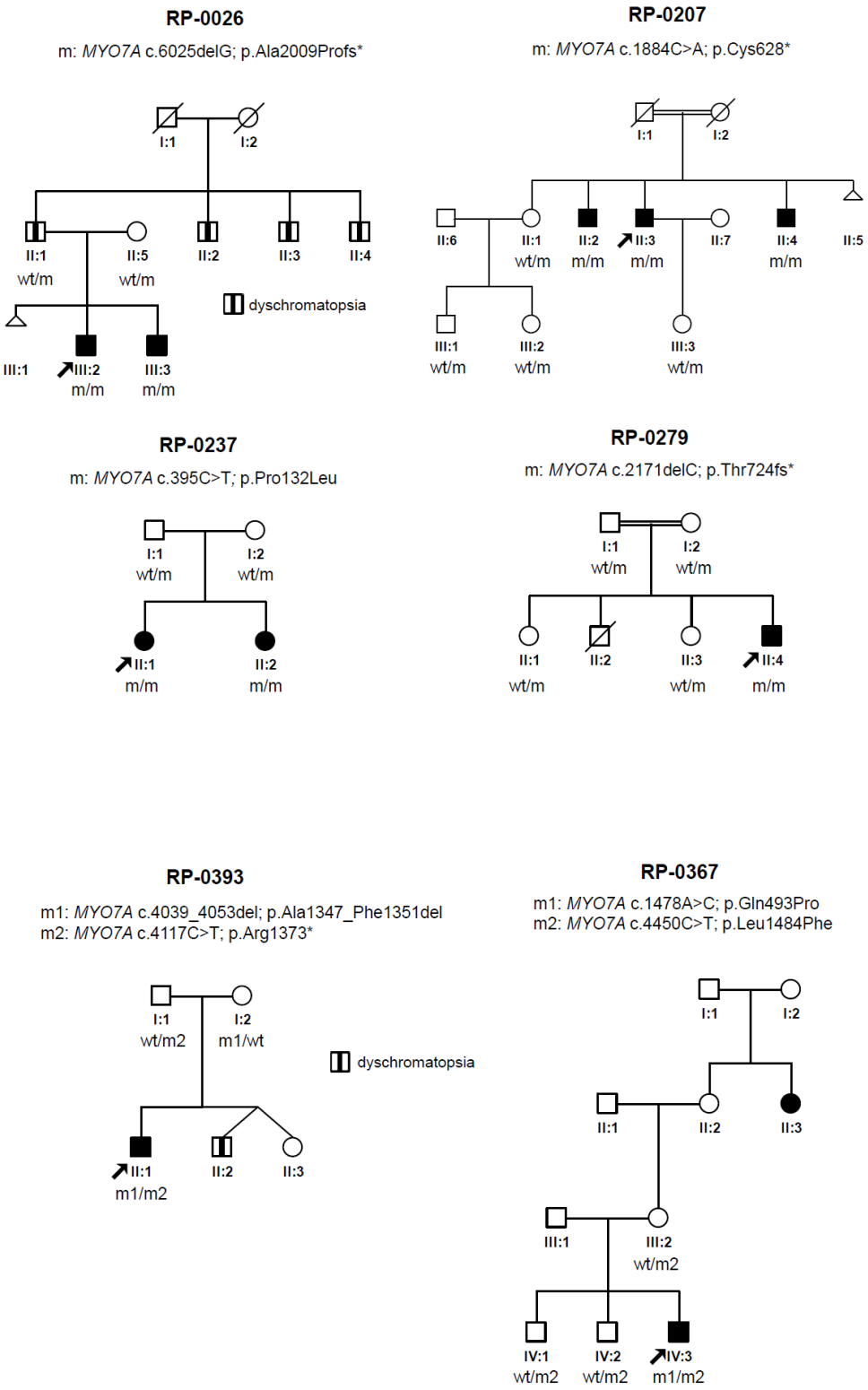
Family	ADN	Nucleotide (Allele1)	Amino acid (Allele1)	Reference (Allele 1)	Nucleotide (Allele2)	Amino acid (Allele2)	Reference (Allele 2)	Usher type (initial diagnosis)
FRP-23		c.1190C>A	p.(Ala397Asp)	(Adato et al., 1997)	c.5856G>A	p.(Ala1915_Lys1952del) (skipping of exon 42)	(Jaijo et al., 2006)	Usher I
FRP-23		c.1190C>A	p.(Ala397Asp)	(Adato et al., 1997)	c.5856G>A	p.(Ala1915_Lys1952del) (skipping of exon 42)	(Jaijo et al., 2006)	Usher I
FRP-246		c.3764delA	p.(Lys1255Argfs*8)	(Jaijo et al., 2007)	c.5581C>T	p.(Arg1861*)	(Adato et al., 1997)	Usher I
FRP-3		c.3238A>T	p.(Lys1080*)	(Cuevas et al., 1998)	c.3508G>A	p.(Glu1170Lys)	(Cuevas et al., 1998)	Usher I
FRP-3		c.3238A>T	p.(Lys1080*)	(Cuevas et al., 1998)	c.3508G>A	p.(Glu1170Lys)	(Cuevas et al., 1998)	Usher I
FRP-342		c.5581C>T	p.(Arg1861*)	(Adato et al., 1997)	c.5581C>T	p.(Arg1861*)	(Adato et al., 1997)	Usher I
FRP-286		c.6025delG	p.(Ala2009Profs*32)	(Nájera et al., 2002)	c.6025delG	p.(Ala2009Profs*32)	(Nájera et al., 2002)	Usher I
FRP-451		c.4544_4551delAGATCATGinsCA	p.(Glu1515_Met1517delinsAla)	(Cremers et al., 2007)	c.6025delG	p.(Ala2009Profs*32)	(Nájera et al., 2002)	Usher I
FRP-530		c.494C>T	p.(Thr165Met)	(Aparisi et al., 2014)	c.494C>T	p.(Thr165Met)	(Aparisi et al., 2014)	Usher I
FRP-530		c.494C>T	p.(Thr165Met)	(Aparisi et al., 2014)	c.494C>T	p.(Thr165Met)	(Aparisi et al., 2014)	Usher I
FRP-550		c.1935G>A	p.(Met645Ilefs*67) (skipping of exon 6)	(Ben Rebeh et al., 2010)	c.1691-1G>A	p.?	(Fuster-García et al., 2018)	Usher I
FRP-575		c.5392C>T	p.(Gln1798*)	(Janecke et al., 1999)	c.5516T>C	p.(Leu1839Pro)	(Aparisi et al., 2014)	Usher I
FRP-77		c.640G>A	p.(Gly214Arg)	(Adato et al., 1997)	c.1342_1343delAG	p.(Asn443_Glu450del)	(Jaijo et al., 2006)	Usher I
FRP-77		c.640G>A	p.(Gly214Arg)	(Adato et al., 1997)	c.1342_1343delAG	p.(Asn443_Glu450del)	(Jaijo et al., 2006)	Usher I
FRP-78		c.470G>A	p.(Thr96Trpfs*29) (skipping of exon 5)	(Jaijo et al., 2006)	c.1454delT	p.(Leu485Argfs*14)	(Jaijo et al., 2006)	Usher I
FRP-290		c.5516T>C	p.(Leu1839Pro)	(Aparisi et al., 2014)	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59) (skipping of exon 27)	(Aparisi et al., 2014)	Usher I
FRP-290		c.5516T>C	p.(Leu1839Pro)	(Aparisi et al., 2014)	c.3503G>C	p.(Arg1168Pro, Val1126Glyfs*59) (skipping of exon 27)	(Jaijo et al., 2006)	Usher I

Table S4: Novel mutations found in this study and their possible effect on the protein

Domain	mutation	Amino acid change	Possible effect on the protein	Allele frequency
Motor domain	c.1344-1G>A		Splicing of exon 12	0.00 (a, b, c, d, e)
Tail 1	c.4114G>A	p.(Val1372Met)	Modification of the hydrophobic interaction network	0.00 (a, b, c, d, e)
Tail 1	c.4441+2delT		Splicing of intron 33	0.00 (a, b, c, d, e)
Tail 2	delE43-E49	c.5857-?_*544+?del	Truncation of $MyTH4_1$-$FERM_2$	--
Tail 2	c.6052-2A>G		Splicing of intron 44	0.0 (a, b, c, d, e)

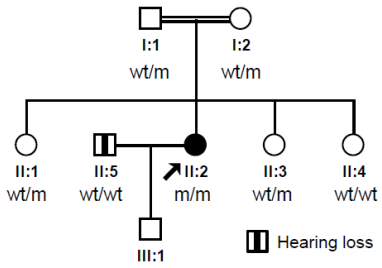
a: ExaC; b: GnomAD; c: CIBERER Exome Server; d: Exome Variant Server; e: 1000 Genomes.

Supplementary Figure 1. Family trees of the 29 families in which segregation analysis of the mutations found could be carried out.



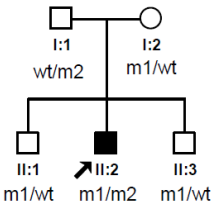
RP-0405

m: *MYO7A* c.1190C>A; p.Ala397Asp



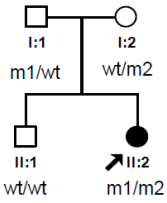
RP-0473

m1: *MYO7A* c.640G>A; p.Gly214Arg
m2: *MYO7A* c.3979G>A; p.Glu1327Lys



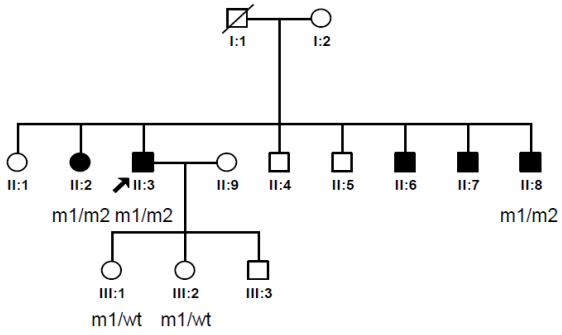
RP-0594

m1: *MYO7A* c.640G>A; p.Gly214Arg
m2: *MYO7A* c.5856G>A; p.Ala1915_Lys1952del



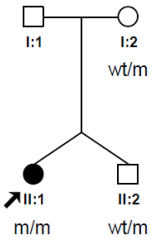
RP-1072

m1: *MYO7A* c.3719G>A ; p.Arg1240Gln
m2: *MYO7A* c.3724C>T; p.Gln1242*



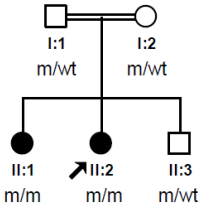
RP-1229

m: *MYO7A* c.640G>A p.Gly214Arg



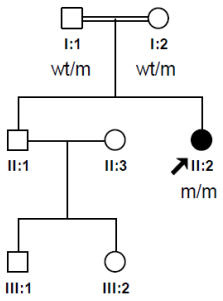
RP-1710

m: *MYO7A* c.397C>T; p.His133Tyr



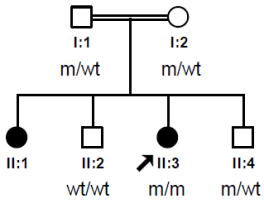
RP-1915

m: *MYO7A* c.4297delC; p.Gln1433Serfs*116



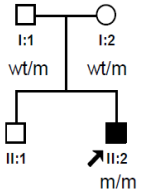
RP-1808

m: *MYO7A* c.397C>T; p.His133Tyr



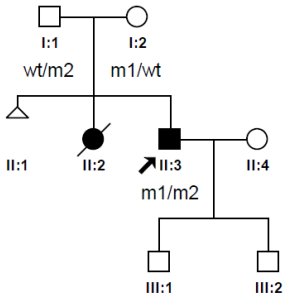
RP-2475

m: *MYO7A* c.5857-?_*544+?del; p?



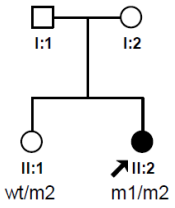
RP-1218

m1: *MYO7A* c.5516T>C; p.Leu1839Pro
m2: *MYO7A* c.3G>A; p.Met1Ile



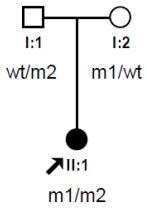
RP-2982

m1: *MYO7A* c.4441+2delT; p.?
m2: *MYO7A* c.5886_5889delCTTT; p.Phe1962Leufs*7



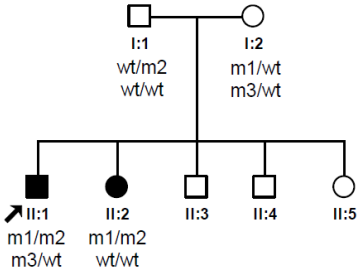
FRP-451

m1: *MYO7A* c.4544_4551delAGATCATGinsCA; p.Glu1515_Met1517delinsAla
m2: *MYO7A* c.6025delG; p.Ala2009Profs*32



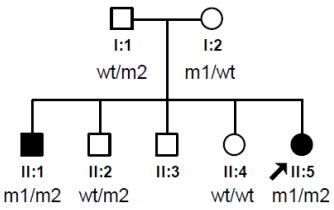
FRP-3

m1: *MYO7A* c.3238A>T; p.Lys1080*
m2: *MYO7A* c.3508G>A; p.Glu1170Lys
m3: *USH2A* c.2276G>T; p.Cys759Phe



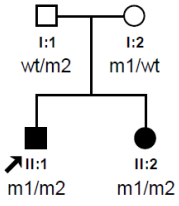
FRP-23

m1: *MYO7A* c.1190C>A; p.Ala397Asp
m2: *MYO7A* c.5856G>A; p.Lys1952Lys



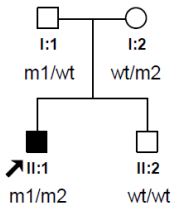
FRP-290

m1: *MYO7A* c.5516T>C; p.Leu1839Pro
m2: *MYO7A* c.3503G>C; p.Arg1168Pro



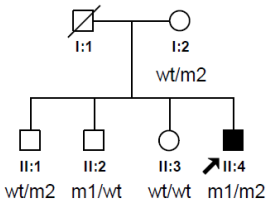
FRP-175

m1: *MYO7A* c.448C>T; p.Arg150*
m2: *MYO7A* c.3503G>C; p.Arg1168Pro



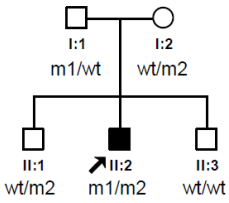
FRP-78

m1: *MYO7A* c.470G>A; p.Ser157Asn
m2: *MYO7A* c.1454delT; p.Leu485Argfs*14



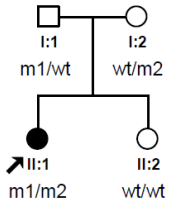
FRP-159

m1: *MYO7A* c.3610C>A; p.Pro1204Thr
m2: *MYO7A* c.3764delA; p.Lys1255Argfs*8



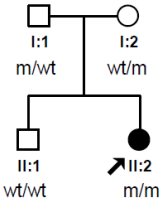
FRP-246

m1: *MYO7A* c.3764delA; p.Lys1255Argfs*8
m2: *MYO7A* c.5581C>T; p.Arg1861*



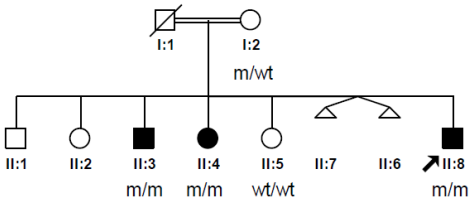
FRP-530

m: *MYO7A* c.494C>T; p.Thr165Met



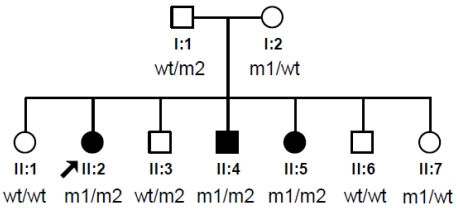
FRP-1

m: *MYO7A* c.2461C>T; p.Gln821*



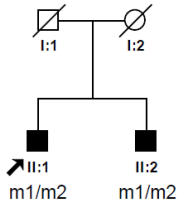
FRP-11

m1: *MYO7A* c.2461C>T; p.Gln821*
m2: *MYO7A* c.4740C>G; p.Tyr1580*

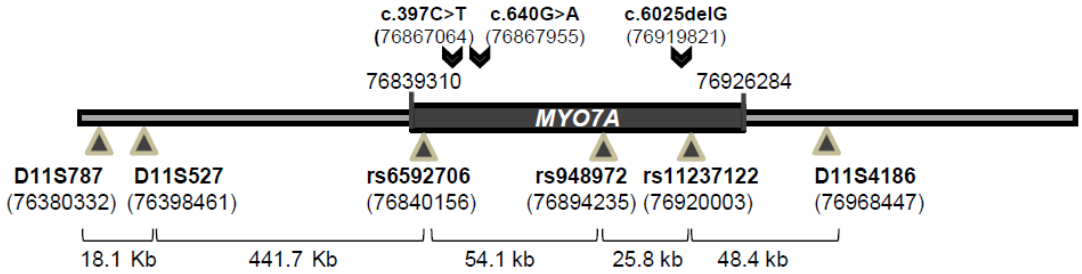


FRP-77

m1: *MYO7A* c.640G>A ; p.Gly214Arg
m2: *MYO7A* c.1342_1343delAG; p.Ser448Leufs*2

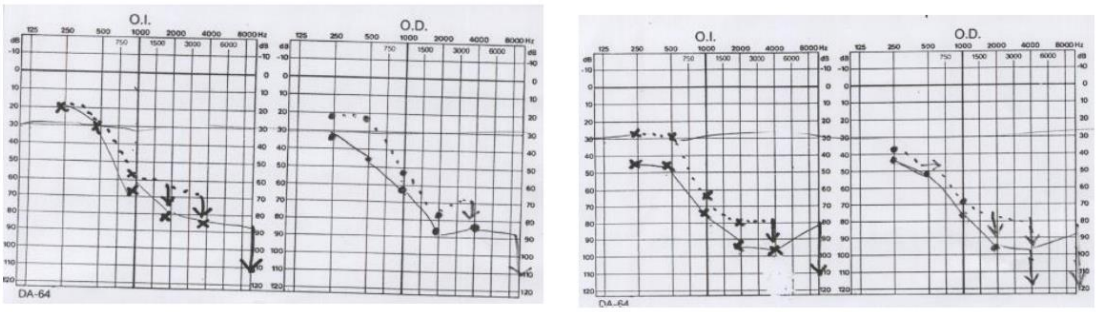


Supplementary Figure 2. Diagram representing *MYO7A* gene and surrounding regions, and the relative position of the markers selected for haplotyping. Localizations of the recurrent mutations are also shown by an arrow. Numbers in brackets indicate chromosomal position in chromosome 11.



Supplementary Figure 3. Audiograms from the patient from RP-1218 family diagnosed of atypical USH.

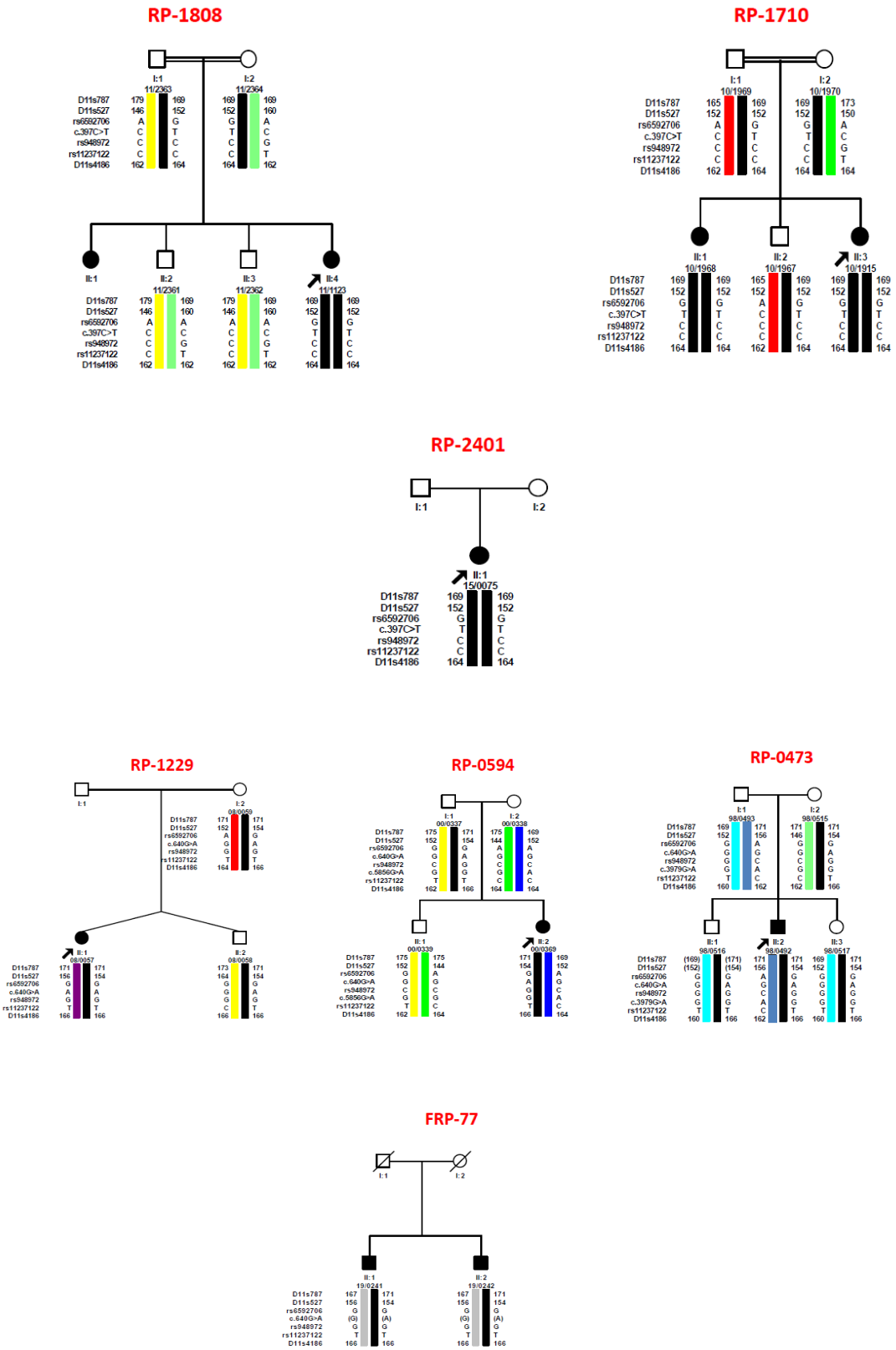
RP-1218



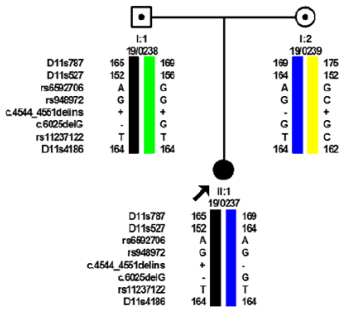
2002 (30 y)

2005 (33 y)

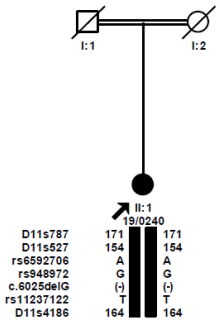
Supplementary Figure 4. Haplotype analysis of the three most recurrent *MYO7A* mutations found in this study.



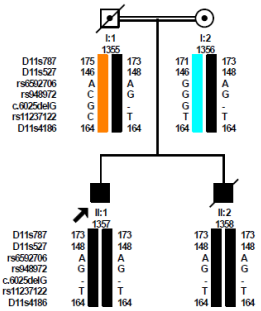
FRP-451



FRP-286



RP-0026



References of the Supplementary Material

1. Adato A, Weil D, Kalinski H, Pel-Or Y, Ayadi H, Petit C, Korostishevsky M & Bonne-Tamir B (1997): Mutation profile of all 49 exons of the human myosin VIIA gene, and haplotype analysis, in Usher 1B families from diverse origins. Am J Hum Genet 61:813–821.
2. Aparisi MJ, Aller E, Fuster-García C et al. (2014): Targeted next generation sequencing for molecular diagnosis of Usher syndrome. Orphanet J Rare Dis 9: 168.
3. Ben Rebeh I, Morinière M, Ayadi L et al. (2010): Reinforcement of a minor alternative splicing event in MYO7A due to a missense mutation results in a mild form of retinopathy and deafness. Mol Vis 16: 1898-1906.
4. Besnard T, García-García G, Baux D et al. (2014): Experience of targeted Usher exome sequencing as a clinical test. Mol Genet Genom Med 2: 30–43.
5. Bharadwaj A K, Kasztejna JP, Huq S, Berson EL & Dryja TP (2000): Evaluation of the myosin VIIA gene and visual function in patients with Usher syndrome type I. Exp Eye Res 71: 173-181.
6. Cremers FP, Kimberling WJ, Külm M et al. (2007): Development of a genotyping microarray for Usher syndrome. J Med Genet 44: 153-160.
7. Cuevas JM, Espinós C, Millán JM, Sánchez F, Trujillo MJ, Ayuso C, Beneyto M & Nájera C (1999): Identification of three novel mutations in the MYO7A gene. Hum Mutat 14: 181.
8. Cuevas JM, Espinós C, Millán JM, Sánchez F, Trujillo MJ, García-Sandoval B, Ayuso C, Nájera C & Beneyto M (1998): Detection of a novel Cys628STOP mutation of the myosin VIIA gene in Usher syndrome type Ib. Mol Cell Probes 12: 417-420.
9. Fuster-García C, García-García G, Jaijo T, Fornés N, Ayuso C, Fernández-Burriel M, Sánchez-De la Morena A, Aller E & Millán JM (2018): High-throughput sequencing for the molecular diagnosis of Usher syndrome reveals 42 novel mutations and consolidates CEP250 as Usher-like disease causative. Scientific Reports 8: 17113.
10. Gerber S, Bonneau D, Gilbert B, Munnich A, Dufier JL, Rozet JM, & Kaplan J (2006): USH1A: Chronicle of a slow death. Am J Hum Genet 78: 357-359.
11. Jaijo T, Aller E, Beneyto M et al. (2007): MYO7A mutation screening in Usher syndrome type I patients from diverse origins. J Med Genetv 44: e71.
12. Jaijo T, Aller E, Oltra S et al. (2006): Mutation profile of the MYO7A gene in Spanish patients with Usher syndrome type I. Human Mutat 27: 290-291.
13. Jaijo T, Aller E, García-García G et al. (2010): Microarray-based mutation analysis of 183 Spanish families with Usher syndrome. Invest Ophthalmol Vis Sci 51: 1311–1317.
14. Janecke AR, Meins M, Sadeghi M, Grundmann K, Apfelstedt-Sylla E, Zrenner E, Rosenberg T & Gal A (1999): Twelve novel myosin VIIA mutations in 34 patients with Usher syndrome type I: Confirmation of genetic heterogeneity. Human Mutat 13: 133-140.
15. Le Guédard-Méreuze S, Vaché C, Baux D et al. (2010): Ex vivo splicing assays of mutations at noncanonical positions of splice sites in USHER genes. Human Mutat, 31: 347–55.
16. Le Quesne Stabej P, Saihan Z, Rangesh N, et al. (2012): Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study. J Med Genet 49: 27–36.
17. Lévy G, Levi-Acobas F, Blanchard S et al. (1997): Myosin VIIA gene: Heterogeneity of the mutations responsible for Usher syndrome type IB. Hum Mol Genet 6: 111-116.
18. Nájera C, Beneyto M, Blanca J, Aller E, Fontcuberta A, Millán JM & Ayuso C (2002): Mutations in myosin VIIA (MYO7A) and usherin (USH2A) in Spanish patients with Usher syndrome types I and II, respectively. Hum Mutat 20: 76-77.

19. Pennings RJE, Damen GWJA, Snik AFM, Hoefsloot L, Cremers CWRJ, & Mylanus EAM (2006): Audiologic performance and benefit of cochlear implantation in Usher syndrome type I. The Laryngoscope, 116: 717-722.
20. Riazuddin S, Nazli S, Ahmed ZM. (2008): Mutation spectrum of MYO7A and evaluation of a novel nonsyndromic deafness DFNB2 allele with residual function. Hum Mutat 29: 502–511.
21. Roux AF, Fougère V, Le Guédard S, et al. (2006): Survey of the frequency of USH1 gene mutations in a cohort of Usher patients shows the importance of cadherin 23 and protocadherin 15 genes and establishes a detection rate of above 90%. J Med Genet 43:763–768.
22. Roux AF, Faugère V, Vaché C et al. (2011): Four-year follow-up of diagnostic service in USH1 patients. Invest Ophthalmol Vis Sci 52: 4063–4071.

DISCUSIÓN GENERAL

El objetivo principal de esta Tesis Doctoral ha sido refinar y caracterizar molecularmente distintas subcohortes de pacientes con EHR para su posible inclusión en estudios observacionales o de intervención. Con el fin de profundizar en el conocimiento de las bases moleculares y genéticas de estas enfermedades tan heterogéneas, se han utilizado diversos abordajes de secuenciación masiva, incluyendo tanto análisis de SNVs como de CNVs, ontología fenotípica estructurada y otras herramientas *in silico*. Estos estudios han servido para mejorar el diagnóstico clínico y molecular de pacientes con ERS, identificando genes y mutaciones responsables de enfermedad y detectando potenciales combinaciones oligogénicas y alelos modificadores del fenotipo, así como para establecer correlaciones genotipo-fenotipo que permitan dirigir el diagnóstico y que tengan utilidad en la evaluación de los ensayos clínicos.

1. Prevalencia de las EHR en España

En el primer trabajo de esta Tesis Doctoral se presentó el mayor estudio a nivel nacional publicado hasta la fecha que aborda la prevalencia y la epidemiología de las EHR de forma global en la población española, si bien hay estudios parciales publicados previamente [248–252]. Estas enfermedades se encuentran entre las principales causas de ceguera en el mundo [253,254]. Identificar las alteraciones genéticas que causan la enfermedad retiniana es imprescindible para facilitar el diagnóstico clínico, establecer correlaciones genotipo-fenotipo, descubrir nuevos genes, realizar un consejo genético adecuado y determinar la elegibilidad de los pacientes que podrían beneficiarse de terapias dirigidas a genes y variantes específicas [255,256].

La cohorte estudiada engloba a 6089 pacientes procedentes de 4403 familias no emparentadas entre ellas, reclutadas hasta 2019, en un único centro a lo largo de 30 años. Basándonos en los datos generales de prevalencia obtenidos (1:7673), la prevalencia mundial estimada para las EHR (1:1000-1:4000) y los datos del padrón de la población española a fecha de 2018 (46 722 980 habitantes), esta cohorte recogería entre el 20% y el 53% de los pacientes que se estiman que están diagnosticados en nuestro país. Gracias a una mayor accesibilidad a los estudios genéticos mediante NGS, la caracterización clínico-genética de las DHR se está realizando cada vez en más centros de nuestro país. Sin embargo, dado que aún no existe un registro nacional de pacientes de EHR en España, esta cohorte representa el mayor esfuerzo de reclutamiento y estudio de las DHR realizado hasta el momento. Nuestra cohorte presenta algunos sesgos en la distribución poblacional, con un enriquecimiento de pacientes procedentes de la Comunidad de Madrid y regiones aledañas, reflejo de que nuestro hospital ha sido centro de referencia para la zona centro. No obstante, gracias al gran tamaño muestral, la aletoriedad de los pacientes que componen la cohorte y los análisis moleculares realizados a lo largo del tiempo, considerando las características genéticas globales de la población española [257], podemos extrapolar los genes y variantes más frecuentemente encontradas.

A lo largo del tiempo se han ido publicando todo tipo de estudios sobre la prevalencia y distribución de las EHR a nivel mundial, que varían en el subtipo de patología y tamaño de la cohorte estudiada, entre otras variables. De esta manera, podemos encontrar cohortes afectas de EHR de manera general [254,258,259] o cohortes con afectaciones visuales concretas (p. ej. DM [260] o USH [261]), así como trabajos que engloban desde pocos casos [32,260,262–267] hasta grandes cohortes de hasta 6000 pacientes [34,156,268–271], así como metaanálisis [272] publicados en los últimos años.

2. Estudio genético en pacientes con EHR

Las pruebas genómicas son herramientas esenciales para alcanzar o confirmar el diagnóstico en las enfermedades raras visuales. Existen numerosas pruebas de los beneficios del diagnóstico para los pacientes y sus familias, ya que a menudo los pacientes con enfermedades raras sufren una prolongada y costosa odisea diagnóstica, con la carga emocional y socio-sanitaria añadida que ello conlleva [273]. El estudio genético facilita el proceso del diagnóstico, haciéndolo más rápido y preciso [274].

Los datos genómicos deben ser un componente dinámico en la atención clínica, integrándose en la historia clínica electrónica de los pacientes para facilitar la reevaluación de los datos, informar diagnósticos y permitir la medicina de precisión [275].

Con la aparición de la NGS a principios de la última década se revolucionó el análisis de las EHR y se mejoró significativamente su diagnóstico molecular. Desde entonces, la NGS ha superado rápidamente las limitaciones de la secuenciación tradicional Sanger y su uso se ha generalizado para la identificación de genes causales de enfermedad [276]. Sin embargo, el desarrollo de estas pruebas genómicas ha requerido también un desarrollo en paralelo de métodos bioinformáticos para la priorización de SNVs y CNVs para determinar e interpretar la importancia clínica de las variantes encontradas [277,278].

2.1. Rendimiento diagnóstico en EHR

La tasa de caracterización varía considerablemente según la enfermedad, la población, el tipo de prueba genética usada, los genes incluidos en el estudio o los criterios de inclusión [256,278]. Gracias a los avances en las técnicas de NGS, el rendimiento diagnóstico general de las EHR ha aumentado hasta el 50%-70% [185,263,270,279], similar al 53% que fue obtenido globalmente en el análisis de nuestra cohorte en agosto de 2019 (**Capítulo 1**). A día de hoy, la tasa de caracterización general de las EHR en el Departamento de Genética del HUFJD ha superado el 60%, lo cual puede deberse al incremento en el uso de la NGS durante este tiempo en nuestra cohorte (+12% aproximadamente; datos sin publicar), ya que actualmente la NGS es tanto el primer método elegido para el cribado de los casos prospectivos como la tecnología escogida en el estudio adicional de los casos retrospectivos en nuestro laboratorio.

Cuando consideramos los casos agrupados bajo distintas categorías fenotípicas generales (p. ej. presentaciones dominadas por una afectación de conos (“NO-RP”), presentaciones dominadas por una afectación de bastones (“RP”) o formas sindrómicas), un 2,5% de los casos (53/2100) fueron sometidos a una reevaluación y reclasificación clínica tras la obtención de un resultado concluyente en las pruebas genéticas, lo cual puede deberse a que el fenotipo no se había manifestado completamente en el momento del diagnóstico inicial, a la existencia de formas intermedias y solapamientos clínicos, a que el fenotipo esté muy evolucionado o a la falta de datos clínicos en el momento de la canalización, entre otras muchas razones.

En nuestra cohorte se identificaron un total de 1549 variantes patogénicas y probablemente patogénicas diferentes en 142 genes como causantes de EHR, lo que pone de manifiesto la naturaleza extremadamente heterogénea de estas patologías. Cada subgrupo de la cohorte presentaba un enriquecimiento de casos caracterizados en genes específicos, p. ej. *ABCA4* (MIM *601691) en AR-“NO-RP”, *USH2A* en AR-“RP” o USH2, *MYO7A* en USH1 o *BBS1* en BBS (incluido en el subgrupo de “otras sindrómicas”). Adicionalmente, teniendo en cuenta el número total de familias caracterizadas, los 10 genes más frecuentemente implicados fueron: *ABCA4* en 1 de cada 4 pacientes, *USH2A* en casi el 13% y los genes *RS1* (MIM *300839), *CRB1* (MIM *604210),

RPGR, *RHO* (MIM *300023), *PRPH2* (MIM *179605), *CHM* (2.7%), *BEST1* (MIM *607854) y *RP1* (MIM *603937), que explican cada uno entre el 2%-4% de los pacientes. Estos genes estaban implicados en el 61% de todas las familias caracterizadas molecularmente. En otras publicaciones que reportan grandes cohortes de pacientes con EHR [156,270,271] se describen unos hallazgos similares a los encontrados en nuestro trabajo: tasas de caracterización de en torno al 55%-65%, entre 129-135 genes implicados con la ayuda de distintas técnicas moleculares y entre el 48%-68% de la cohorte explicada por los genes más frecuentes asociados a EHR.

Las dos variantes patogénicas más frecuentes se encontraron en *ABCA4* y *USH2A*. La variante p.(Arg1129Leu) en *ABCA4* representa el 5% del total de alelos patogénicos identificados en nuestra cohorte y está presente en el 21,5% de las familias "NO-RP" caracterizadas. Esta variante se ha identificado casi prácticamente en pacientes españoles "NO-RP" [252,280,281], siendo probablemente una mutación fundadora de origen español [280,282]. En la distribución de esta variante en la Península Ibérica, se observa un gradiente norte-sur, con una frecuencia que varía desde el 19,4% de todos los alelos encontrados en País Vasco al 11,3% en la Región de Murcia.

Por otro lado, la variante p.(Cys759Phe) en *USH2A* supone el 3,8% del total de alelos patogénicos encontrados y está presente en el 8,4% de las familias "RP" y en el 9,7% de las familias sindrómicas caracterizadas. A pesar de esta elevada frecuencia, no es exclusiva de la población española, habiéndose descrito como una variante *Hot Spot* en otras poblaciones [269,283,284]. Esta variante también muestra un gradiente, pero en este caso de este a oeste, con una frecuencia del 13,3% de los alelos identificados en Murcia y del 5,7% en Extremadura.

Además, otras dos variantes parecen estar sobrerrepresentadas, como ocurre con la variante p.(Arg311Gln) en *NR2E3* (MIM *604485), con una frecuencia de 9,6% en las Islas Canarias, y la variante p.(Arg22*) en *PRCD* (MIM *610598), con una frecuencia de 7,2% en la Región de Murcia. Esta última variante normalmente se ha descrito en individuos procedentes de países del Mediterráneo Oriental y de Oriente Medio [264,270], por lo que su frecuencia en Murcia podría deberse al asentamiento de poblaciones musulmanas a lo largo de varios siglos durante la Edad Media [285].

2.2. Datos generales del estudio de una cohorte de pacientes con enfermedades hereditarias de la retina sindrómicas mediante NGS

En enero de 2018, previamente al desarrollo de esta Tesis Doctoral, el número de casos índice que habían sido estudiados con sospecha de retinopatía sindrómica de tipo no Usher ascendía a 183, de los cuales 77 habían sido caracterizados molecularmente (42%) y 106 presentaban resultados no concluyentes tras análisis mediante *microarrays* comerciales de genotipado, secuenciación Sanger y NGS con un panel de 121 genes [163]. A principios de 2022, hay un total de 216 familias registradas en nuestra base de datos. Gracias a los estudios desarrollados en este trabajo de Tesis, junto con la implementación simultánea de la NGS en el Servicio de Genética del HUFJD para el estudio genético de rutina de los casos desde 2018, se han caracterizado 66 familias adicionales. En conjunto, se alcanza una tasa de caracterización del 66% (143/216) en esta subcohorte (datos sin publicar).

En el **Capítulo 2** se describe la caracterización genética de 52 de esos 66 pacientes, ya que los 14 restantes han sido diagnosticados molecularmente tras la publicación del artículo que describe los resultados de este trabajo en 2020 (Anexo 3).

En este trabajo se profundizó en el estudio clínico-genético de una subcohorte de 100 casos prospectivos y retrospectivos no caracterizados con sospecha de distintas formas de ERS, de tipo no Usher, los cuales fueron recogidos hasta octubre de 2020. En estos pacientes, hemos conseguido una tasa de caracterización del 52%, utilizando principalmente un análisis de CES con paneles virtuales específicos, pero también mediante WES. Dicha tasa es similar a la observada en otra cohorte de características parecidas, compuesta también por una miscelánea de casos con ERS [286,287]. Sin embargo, la tasa de caracterización es mayor en dos grupos de pacientes: los casos prospectivos y aquellos con sospecha de síndromes concretos. En los 18 casos prospectivos, los cuales están enriquecidos en fenotipos asociados a formas de ciliopatías, la tasa de diagnóstico aumentó hasta el 83% gracias al uso de CES como primer abordaje para el estudio molecular. Mientras que en los pacientes que presentaban síndromes reconocibles, el rendimiento se incrementó hasta el 65%, incluso cuando en el análisis de CES se usaron paneles de genes de menor tamaño dirigidos al estudio de fenotipos concretos. Este aumento en la tasa de caracterización se ha descrito en cohortes formadas exclusivamente por familias consanguíneas con ciliopatías, donde la tasa de diagnóstico alcanzó un 85% [288].

Las tasas de caracterización obtenidas en los pacientes con presentaciones sistémicas menos específicas son del 45% e indican la utilidad del enfoque empleado para identificar las causas genéticas y para el diagnóstico diferencial. Este el caso de los pacientes con DR acompañada por un síntoma extraocular aislado, en los que finalmente la retinopatía viene explicada por defectos en un gen no sindrómico en el 27% de ellos (3/11). En estos 3 pacientes, caracterizados con *EYS*, *RHO* y *RDH12*, el síntoma extraocular aislado (hipoacusia unilateral leve, diabetes mellitus de tipo 1 y obesidad) no parece estar asociado con la alteración visual y probablemente se explique por una causa no genética. Adicionalmente, la mitad de los casos sin diagnóstico clínico *a priori* pudieron ser clasificados a alguna categoría clínica concreta tras las pruebas moleculares. Por lo tanto, este tipo de enfoque basado en NGS ayuda al diagnóstico clínico, y con ello, a mejorar el manejo de los pacientes y, en algunos casos, su pronóstico, pues permite establecer seguimientos periódicos para la detección precoz de complicaciones asociadas al síndrome específico.

En el total de esta subcohorte de casos con formas sindrómicas estudiadas en el **Capítulo 2**, la tasa de reclasificación clínica aumenta significativamente ($p < 0,00001$) hasta el 27% (12/44), en comparación a la cohorte de EHR general en España (**Capítulo 1**), ya que este tipo de casos son más difíciles de clasificar bajo entidades clínicas específicas, debido al solapamiento clínico entre las distintas formas sindrómicas, el alto grado de variabilidad fenotípica o las presentaciones atípicas [27,131]. Esto ocurre sobre todo con los pacientes que presentan síntomas extraoculares inespecíficos, donde el estudio genético permite clasificar clínicamente el 42%.

Esto pone de manifiesto la importancia de la realización de un fenotipado detallado para describir los casos sindrómicos y del uso de términos HPO como una herramienta adecuada para la descripción clínica de los pacientes con formas sindrómicas. Además, según nuestra experiencia, es altamente recomendable que este fenotipado esté acompañado del seguimiento de criterios clínicos establecidos para cada síndrome específico (**Capítulo 2**). Todo ello, combinado con los hallazgos moleculares, permite llegar a un diagnóstico adecuado y constituiría la base de la medicina personalizada en las ERS.

Los métodos *in silico* basados en el fenotipo para la priorización de variantes mejoran el rendimiento de los algoritmos bioinformáticos a la hora de identificar genes causantes de enfermedad a partir de los datos de NGS [157,289]. Estos métodos de priorización pueden dividirse en dos tipos según los datos iniciales que necesitan, los que solo usan términos HPO o

los que utilizan datos fenotípicos y archivos *vcf*. Estos últimos son los que muestran el mejor rendimiento [290], concretamente dos métodos de priorización, AMELIE [291] y LIRICAL [292]. Se ha visto que el uso combinado de estos dos métodos podría ayudar en la mejora diagnóstica [290], por lo que serían buenas herramientas para usar en el estudio de nuevos datos de NGS o en el reanálisis de los datos antiguos en los casos con ERS que aún hoy siguen sin caracterizarse.

Durante el estudio, se identificaron un total de 113 alelos, que correspondían a 82 variantes únicas (75 SNVs y 7 CNVs) en 47 genes distintos. Solo 6 de estos genes estaban implicados en más de un caso: *BBS1* (4 pacientes), *AHI1* (MIM *608894) (n=3), *MKKS* (n=3), *C8orf37* (MIM *614477) (n=2), *RDH12* (MIM *608830) (n=2) y *VPS13B* (n=2), remarcando la heterogeneidad genética de estas enfermedades. Estos 6 genes explican el 31% del total de casos caracterizados. Asimismo, gracias al desarrollo de este trabajo (**Capítulo 2**), se han identificado 45 SNVs (60%) que no habían sido descritas anteriormente. Solo 4 de ellas se encontraron en población del sur de Europa en la base de datos de frecuencia gnomAD: p.(Arg767Cys) en *ACO2* (frecuencia = 1,74E-04), p.(Arg577Cys) en *PEX1* (8,69E-05), p.(Phe97Ile) en *TMEM216* (1,74E-04) y p.(Arg4120His) en *USH2A* (8,73E-05).

De las 82 variantes únicas, 80 se observaron en una única familia, mientras que solo dos aparecieron en más de una, p.(Met390Arg) en *BBS1* y p.(Arg189*) en *BBS2*.

El alelo más prevalente fue p.(Met390Arg) en *BBS1,* con una frecuencia alélica del 6% (7/113), se identificó en 4 pacientes caracterizados, apareciendo 3 veces en homocigosis y una en heterocigosis compuesta. El segundo más frecuente fue p.(Arg189*) en *BBS2*, que representaba el 2,7% de los alelos totales (3/113). Este se encontró en homocigosis en un caso caracterizado y en heterocigosis en un caso monoalélico sin caracterizar. Adicionalmente, 23 alelos fueron encontrados dos veces, apareciendo todos en homocigosis en un solo caso.

Aunque la mayoría de casos con ERS están causados por SNVs, existe un 9% que se diagnostican de manera total o parcial por la presencia de CNVs causales. Dicho dato va en consonancia con el obtenido por Zampaglione *et al.* (2020) [293] en una serie de pacientes con EHR. Como se ha visto en el porcentaje de familias que se caracterizan por CNVs, es fundamental la detección de CNVs usando algoritmos basados en NGS dentro del análisis genético rutinario.

2.2.1. Aproximación experimental y bioinformática en el estudio de las ERS

El estudio de WES presenta una menor sensibilidad y rendimiento que el uso de estrategias de captura de paneles dirigidos, debido a que los datos generados mediante WES se suelen obtener a una menor profundidad de lectura (x100), lo que puede conducir a una menor cobertura en ciertas zonas del genoma. Además, la WES presenta una menor flexibilidad para su personalización, ya que no incluye regiones no codificantes (UTRs e intrónicas) o regiones difíciles de secuenciar [294,295]. Sin embargo, el WES se ha establecido como el mejor enfoque para el estudio de los casos con ERS, debido a su eficacia en la identificación de nuevas variantes [164,296], genes causantes [297] y correlaciones genotipo-fenotipo [298].

Tal como ha sido descrito en distintos estudios, el reanálisis periódico de los datos de NGS permite identificar variantes patogénicas ocultas en genes ya relacionados previamente con la enfermedad en estudio [299–302]. En este sentido, la aproximación de WES, permite un reanálisis periódico de los casos utilizando bien paneles virtuales actualizados con la inclusión de genes que hayan sido asociados a la patología tras el análisis inicial, bien mediante una priorización de variantes sin hipótesis, lo que permite la identificación de nuevos genes, así como el uso de nuevas herramientas bioinformáticas, bases de datos actualizadas y/o nuevas *pipelines*

[184,303]. Sin embargo, las estrategias genómicas basadas en el uso de paneles de captura específicos no pueden reanalizarse para considerar genes adicionales sin llevar a cabo nuevas pruebas de laboratorio [304]. De hecho, nuestros datos en la caracterización de nuestra cohorte de pacientes con ERS evidencian que el uso de paneles virtuales bien diseñados y curados, formados por genes conocidos, conduce a buenas tasas de caracterización, independientemente del tipo de aproximación para el estudio del exoma empleada, CES (36%) o WES (30%).

El reanálisis de los datos de NGS puede aumentar la tasa de diagnóstico hasta el 30% en los pacientes no resueltos, según el espectro de trastornos genéticos reanalizados, el tipo de NGS, el modo de reanálisis o el período de tiempo transcurrido desde el primer análisis [302,305–308]. El reanálisis de los datos de WES puede permitir la caracterización de un 30% adicional de los pacientes con WES sin necesidad de hacer un WGS [305]. En nuestra cohorte, se pudieron caracterizar 5 pacientes adicionales (19%) gracias al reanálisis de los datos de WES, mediante la priorización de variantes sin hipótesis, la descripción de una nueva asociación en el gen *HK1* (MIM *142600) que no era conocida en el primer análisis [309] o la recopilación de nuevos datos tras una reevaluación clínica del paciente. Esto hizo que aumentase la tasa de caracterización general del 47% al 52%.

Por todo ello, y de acuerdo con la rutina clínica seguida en el Departamento de Genética del HUFJD, se propone llevar a cabo el reanálisis periódico de los datos de NGS en los casos con ERS cuando exista nueva información clínica o familiar o cuando se reporte en la literatura un nuevo gen candidato que tenga relación con la presentación clínica de algún caso en concreto. El momento del reanálisis es un dato en el que no hay un consenso establecido, ya que hay trabajos que la sitúan de 6 a 12 meses después del primer análisis [306,310], mientras que otros la ponen a los 2 años o incluso más [184,307,311].

En nuestra cohorte, en 4 casos se encontró una VUS monoalélica en un gen recesivo relacionado con la presentación fenotípica. Uno de los grandes problemas relacionados con el análisis de datos de NGS con grandes paneles de genes o mediante enfoques basados en la priorización de variantes sin hipótesis es la interpretación de las VUSes identificadas. Se debe reevaluar periódicamente la importancia clínica de las VUSes, mediante su seguimiento y eventual reclasificación a deletéreas o benignas [312].

Del mismo modo, otros 6 pacientes mostraron variantes monoalélicas de clase 4 o 5 en genes recesivos. En estos casos monoalélicos, la posterior realización de una estrategia de WGS permitiría estudiar la presencia de posibles segundos alelos en el gen en regiones no codificantes, como en los UTR, promotor, elementos reguladores o regiones intrónicas profundas [278,313]. Además, esta aproximación de estudio genómico permite mejorar la tasa de detección de variantes estructurales, algunas de las cuales no pueden ser detectadas mediante los sistemas de captura [33]. Se ha determinado la utilidad clínica de un flujo de trabajo basado en la realización de WES y WGS al caso índice de forma consecutiva, así como en la interpretación con un algoritmo de priorización basado en el fenotipo y el interactoma para descubrir nuevos fenotipos y genes candidatos [314]. El 98% de nuestros pacientes no resueltos fueron estudiados mediante paneles dirigidos de genes, pero solo el 42% de ellos tenía WES. Por lo que en todos esos pacientes sin resolver, especialmente aquellos que ya han sido estudiados por WES, la WGS sería la mejor opción para identificar posibles variantes/genes candidatos, mutaciones no codificantes o variantes estructurales [33,313,315].

2.3. Asociaciones nuevas o raras en casos con ERS

Como hemos tratado, el uso de la NGS ha revolucionado el estudio de las ERS, ya que ha contribuido en gran medida a mejorar su tasa de diagnóstico molecular mediante la identificación de variantes causales en un gen ya conocido o el descubrimiento de nuevos genes causantes de enfermedad, así como a la ampliación del espectro clínico de estas patologías [164,260,316]. En el caso de las nuevas asociaciones, estas no solo ayudan a delinear el espectro fenotípico y mejorar el diagnóstico molecular, sino que también revelan la importancia de realizar previamente un análisis clínico inicial detallado [164,317].

Como se aprecia en los resultados mostrados en el **Capítulo 2**, no es raro encontrar presentaciones clínicas atípicas en síndromes ya conocidos, en concreto en el 17% de los casos completamente caracterizados. En estos casos, las características clínicas de los pacientes no encajan perfectamente con el fenotipo previamente descrito y asociado al gen causante. Un ejemplo es el caso RP-2995, en el que, en base a los datos clínicos disponibles, inicialmente se sospechó un ALMS-*like*. Tras los estudios moleculares, se encontró en el gen *C8orf37* una variante homocigota de cambio en el marco de lectura, p.(Asp10Lysfs*12). Está descrito que dicho gen provoca BBS, otra ciliopatía solapante con el síndrome ALMS [318]. Por tanto, se concluyó que este caso presentaba un BBS atípico con hipoacusia neurosensorial, la cual es un hallazgo raro en este síndrome, ya que las complicaciones auditivas suelen ser producidas por otitis media crónica [82]. Anteriormente, la pérdida auditiva neurosensorial fue descrita solo una vez en un paciente con BBS genéticamente no caracterizado [319], pero en el **Capítulo 2** es la primera vez que se caracteriza molecularmente al paciente con esta presentación adicional.

Asimismo, pueden existir casos con ERS que presenten síntomas más leves que los ya descritos para una ciliopatía concreta, los cuales pasan desapercibidos antes del diagnóstico genético, como es el caso del paciente RP-1372 que presentaba alteraciones visuales con obesidad y alteraciones endocrinas leves, en el cual se llegó a un diagnóstico genético final de BBS. Esto también sucede en el paciente RP-2876, que fue caracterizado con dos variantes causales en el gen *AHI1*, c.1913-2A>G y p.(Gln925*), el cual provoca JBTS [320]. A este caso se le diagnosticó un JBTS atípico con problemas importantes en el equilibrio y la coordinación. Dicho retraso en el desarrollo motor se puede ver en un modelo de ratón *AHI1*$^{-/-}$ [321].

Existen ciliopatías complejas, como la ciliopatía esquelética inusual que presenta la RP-0132, en la que se descubrió la mutación sin sentido p.(Gln657*) en homocigosis en el gen *ITF81* (MIM *605489), cuya proteína es crucial en el transporte intraflagelar. Ha sido relacionado con fenotipos de ciliopatías complejas [322,323] y a la espermiogénesis y fertilidad en un modelo de ratón [324], todo lo cual podría explicar la sintomatología de este paciente, que presentaba RP, hipoacusia, diabetes insípida y alteraciones psiquiátricas y esqueléticas.

Más allá de las ciliopatías, existen casos no definidos clínicamente y que están caracterizados genéticamente, pero en los que se ven discrepancias entre la sus síntomas y las asociaciones genotipo-fenotipo ya conocidas del gen causal encontrado. El caso RP-0094 presentaba RP, atrofia óptica, cataratas y pérdida auditiva. Tras el estudio molecular, se encontraron variantes bialélicas en *ACO2*, p.(Cys592Tyr) y p.(Arg767Cys). Generalmente, las mutaciones en esta aconitasa mitocondrial producen alteraciones del neurodesarrollo acompañadas de atrofia óptica y degeneración retiniana, pero se ha descrito un amplio rango de fenotipos asociados, desde casos no sindrómicos [325] hasta otras presentaciones más atípicas [326]. Sin embargo, la pérdida auditiva es una asociación muy poco común.

La RP-2032 presentaba alteraciones visuales, entre las que incluye distrofia corneal, pérdida de audición, discapacidad intelectual moderada y niveles anormales de hierro en el suero y, en este caso, se detectó la variante c.878-1G>A en homocigosis en el gen *MCOLN1* (MIM *605248), el cual codificaba para la proteína TRPM1. Por lo general, la ML manifiesta distrofia corneal, deficiencia de hierro y discapacidad intelectual, pero hasta la fecha no se ha informado ninguna relación con la pérdida auditiva de TRPM1 [327].

Normalmente, el gen *ARL13B* (MIM *608922) se ha asociado con el JBTS y el espectro de las ciliopatías. La RP-2310 tenía DCB y síndrome de Asperger, el cual forma parte de los trastornos del espectro autista. Este paciente fue caracterizado molecularmente con la variante c.57_59+16dup en homocigosis. Se ha demostrado que la pérdida selectiva de ARL13B ciliar en las interneuronas altera su morfología y conectividad sináptica, lo que conduce a la alteración del equilibrio de la actividad de excitación/inhibición, que es uno de los mecanismos subyacentes a los trastornos del neurodesarrollo, como es el trastorno del espectro autista [328].

En trabajos publicados previamente, las variantes dominantes en *OTX2* (MIM *600037) se relacionaron de forma habitual con RP y alteraciones neuroendocrinas [163], así como con malformaciones oculares congénitas [329]. Sin embargo, hemos descrito un caso (RP-1691) sin problemas neuroendocrinos ni anomalías del desarrollo ocular, sino que presentaba LCA, malformaciones mandibulares y del conducto auditivo, mala coordinación motora e hipotonía.

Finalmente, en la RP-3018, que solo exhibió degeneración retiniana y pérdida de audición neurosensorial, se encontraron dos variantes en heterocigosis compuesta en el gen *PEX6* (MIM *601498), p.(Glu439Glyfs*3) y p.(Arg876Trp), el cual está comúnmente asociado con los PBD. No obstante, se han descrito variantes causales bialélicas en pacientes con formas leves del espectro fenotípico de los PBD y que comenzaron solo con degeneración retiniana y pérdida de audición [330]. Actualmente, el paciente RP-3018 aún es joven, por lo que sería obligatoria una reevaluación clínica en unos años.

3. Modos de herencia en las EHR

Los estudios moleculares permitieron la identificación de los genes asociados a la patología y la reclasificación del tipo de herencia en algunos casos. El modo de herencia más común para las EHR es el AR [185,270,272], seguido de AD y XL. Este hecho puede verse en el **Capítulo 1** para los casos no sindrómicos (AR 68%, 1210/1792; AD 18%, 328/1792; XL 14%, 254/1792). El USH fue la forma sindrómica más frecuente y, concretamente, el USH2, que representa casi la mitad del total de familias con ERS. El USH siempre presenta una herencia AR.

Los estudios moleculares no solo permiten identificar el gen responsable de la enfermedad, sino también poder reclasificar el tipo de herencia. Tras la detección de las variantes causales en un gen con un patrón de herencia concreto, reclasificamos genéticamente al 8,2% de los pacientes. Además, en todos los casos esporádicos caracterizados se pudo realizar una clasificación y un asesoramiento genético más preciso [251], ya que, aunque como se esperaba, en la mayoría de ellos se confirmó una herencia AR [270], hubo 79 casos (4%) que terminaron siendo AD o XL.

Dentro del grupo con ERS no Usher (**Capítulo 2**), la distribución de los modos de herencia fue AR (79%, 41/52) y, en los subgrupos restantes, AD (9%, 5/52), XL (2%, 1/52), patrones de transmisión no mendelianos (6%, mitocondrial 1/52 y herencia trialélica 2/52) y diagnósticos genéticos duales (4%, 2/52).

3.1. Mecanismos complejos de herencia

En las EHR, el modo de herencia puede asumirse incorrectamente debido a la existencia de pseudodominancia, penetrancia incompleta, herencia oligogénica o presencia de más de una causa genética en la misma familia [251,331–333]. Durante el desarrollo de esta Tesis Doctoral, se han estudiado distintos tipos de herencia oligogénica, como serían los diagnósticos genéticos duales (**Capítulo 2**) o la herencia trialélica en el BBS (**Capítulo 3**).

3.1.1. Causas genéticas duales

La presencia de hallazgos extraoculares junto con una enfermedad visual no siempre se debe a una única causa genética. En nuestra cohorte, presentada en el **Capítulo 2**, hay casos con forma sindrómicas explicados bien por una EHR no sindrómica más una enfermedad no genética, o bien por dos enfermedades genéticas distintas. Hasta la fecha, muchos trabajos han destacado la coexistencia de dos enfermedades de base genética en un mismo paciente, aunque la mayoría describen casos con enfermedades no oculares [334–336]. Este tema es importante de cara al manejo y seguimiento de los casos, ya que el diagnóstico real podría haberse pasado por alto debido al diagnóstico clínico satisfactorio de un síndrome ya conocido o a que no parecía haber relación entre los distintos fenotipos (casos sindrómicos no definidos) y, al final, solo las pruebas genéticas son las que pueden revelar la presencia de dos o más fenotipos distintos [337].

La incidencia reportada para el diagnóstico genético dual en enfermedades monogénicas está entre el 4,6% y el 4,9% [333,338], similar al valor obtenido de 4,3% en el estudio de la cohorte de 100 casos con formas sindrómicas (**Capítulo 2**).

En dos casos se encontró la combinación de un reordenamiento genómico junto con una enfermedad monogénica producida por un gen de EHR no sindrómica. Estos hallazgos se suman al caso con un diagnóstico genético dual de USH2 con síndrome de Koolen de Vries previamente reportado por nuestro grupo [163]. No obstante, en el estudio de Posey *et al.* (2017) [333], que incluía todo tipo de pacientes con sospecha de enfermedades genéticas excepto cáncer, la combinación de una CNV junto con una SNV solo se encontró en el 12% de los pacientes con un diagnóstico molecular dual. La tasa dependerá de si la aproximación experimental permite la detección de CNVs o no.

En nuestra cohorte de ERS, la tasa de diagnóstico genético dual podría estar subestimada debido a que encontramos 6 pacientes con un diagnóstico parcial, los cuales no estaban caracterizados por completo debido a la posibilidad de que existiese una segunda enfermedad no descubierta, siendo esta genética o no. Si considerásemos todos estos pacientes reportados en el **Capítulo 2** de esta Tesis, la tasa de diagnóstico genético dual podría llegar hasta el 15%. Esto puede deberse a que los casos de nuestra cohorte presentan unos fenotipos bastante complejos.

Dados estos resultados, en los casos con afectación sindrómica y que están parcialmente caracterizados con genes asociados a EHR, se deberían estudiar los reordenamientos genómicos. Mientras que en aquellos casos en los que solo se hayan analizado los genes relacionados con ERS, se deberían también evaluar los relacionados con EHR no sindrómicas, así como grandes CNVs.

3.1.2. Herencia oligogénica en el BBS

Generalmente, el BBS tiene una herencia AR, pero en algunos casos se ha propuesto una herencia oligogénica, en forma de trialelismo o presencia de alelos modificadores [93,100]. Aunque la existencia del trialelismo es controvertida [98,99], existen más evidencias de la

posible existencia de un tercer alelo que actúe como modificador [102,339]. Estos datos van en consonancia con los resultados obtenidos en el **Capítulo 3**, en los que no hemos encontrado pruebas de la existencia de trialelismo en ninguna de las 77 familias con diagnóstico molecular de BBS incluidas en esta serie, pero sí de la presencia de posibles alelos modificadores.

Se ha estimado que menos del 10% de las familias con BBS presentan una herencia oligogénica [332]. Este valor se confirma con la distribución trialélica encontrada, donde obtenemos una tasa del 13% en las familias trialélicas informativas (6/45), que presentaban diferencias intrafamiliares en la penetrancia de las principales características del BBS y/o la gravedad en los individuos afectos. Dicha tasa aumentó hasta el 51% cuando se incluyeron todas las familias con sospecha de alelos modificadores (23/45). No obstante, no es posible establecer si el tercer alelo produce un efecto modificador en la mayoría de las familias, o bien porque solo hubiera un afecto en la familia (13 familias), porque los dos hermanos presentasen el mismo genotipo-fenotipo (3 familias) o porque no se dispusiera de información clínica (1 familia).

Un 87% (20/23) de las variantes en el gen secundario no se predicen directamente como patogénicas, sino que serían VUSes, ya que estas también pueden ser alelos hipomorfos o comunes con altas frecuencias poblacionales. Por lo tanto, es necesario realizar ensayos funcionales para estudiar la relevancia biológica de esas variantes genéticas concretas. Normalmente, se utilizan estrategias *in vitro* [209] e *in vivo* [102,340]. Sin embargo, existen limitaciones y no siempre es posible realizar estudios funcionales. En estas situaciones, las herramientas *in silico* pueden ayudar a descubrir y predecir combinaciones candidatas de variantes que pueden estar afectando al fenotipo de los pacientes [189,190]. Para ello, hemos usado la plataforma ORVAL en 19 de las 23 familias en las que se identificó un tercer alelo además de las variantes bialélicas, y el predictor DiGePred en 22 de las 23. DiGePred tiene una tasa 4 veces menor de falsos positivos que ORVAL [190]. Este último incluye el efecto de las variantes como una variable en su predicción, por lo que si uno de los genes del par porta una variante predicha como patogénica, sería muy probable que se prediga como oligogénico [190]. La combinación de los resultados obtenidos de ORVAL (confianza del 95%) y DiGePred (umbral de confianza más alto) respaldaban la posible existencia de herencia oligogénica en el 44% de las familias estudiadas (10/23), donde se incluye a 5 de las 6 familias informativas con expresividad variable entre hermanos. Además, se predice al 91% de las familias cuando se consideró un solo método de forma aislada (4 solo con DiGePred y 8 solo con ORVAL).

La novedad de nuestro estudio es el descubrimiento de 23 nuevas combinaciones trialélicas relacionadas con BBS detectadas que no están actualmente reportadas en la base de datos DIDA [195], la cual recoge actualmente 43 combinaciones (Anexo 4). Además, en DIDA solo aparecen 2 de las 20 combinaciones de genes identificadas (*BBS1_MKKS* y *BBS1_BBS7*). Encontramos 11 nuevos genes relacionados de algún modo en una posible herencia oligogénica (*ALMS1, C8orf37, CEP290, IFT172*, *MKS1, NPHP4, PDE6B, SDCCAG8, TRIM32* y *WDPCP*), ya sea como gen principal o como gen modificador. Estos genes serían buenos candidatos para realizar futuros estudios funcionales para dilucidar su posible efecto modificador en el fenotipo de BBS, bien porque ya están relacionados con el BBS (p. ej. *IFT172*, *TRIM32* o *WDPCP*), bien porque han sido previamente informados como posibles candidatos o modificadores de BBS (p. ej. *ALMS1*, *CORO2B*, *NPHP4* o *PDE6B*).

Por ello, los datos obtenidos sobre posibles casos trialélicos de nuestra cohorte guardarían especial relevancia, ya que servirían, por ejemplo, para engrosar la base de datos pública de DIDA, incrementando el conocimiento sobre este mecanismo complejo de herencia, para el desarrollo de nuevos predictores que se basen en el aprendizaje automático (p. ej. DiGePred)

[190] o la realización de estudios funcionales. Además, la presencia de alelos modificadores tendría repercusiones en el asesoramiento genético y manejo clínico.

4. Análisis de asociaciones genotipo-fenotipo

La mayor precisión del diagnóstico molecular, en combinación con la accesibilidad de la retina, su privilegio inmunológico y la existencia de criterios de valoración anatómicos y funcionales medibles, han fomentado el desarrollo de terapias para tratar las EHR [341]. Todo ello es muy importante en el camino hacia la medicina personalizada, porque permite la identificación de pacientes que podrían beneficiarse de estrategias terapéuticas basadas en terapia génica [342], ya que muchas se sustentan en un diagnóstico molecular confirmado [343]. Las terapias basadas en genes se están convirtiendo en una realidad. De hecho, hay numerosos estudios observacionales y ensayos clínicos en marcha para distintos tipos de EHR y ERS [344–347]. Recientemente, se ha aprobado y comercializado el primer fármaco de esta clase para el tratamiento de la LCA o RP producidas por variantes bialélicas en el gen *RPE65* (MIM *180069) (voretigene neparvovec, *Luxturna*) [348].

4.1. Correlación genotipo-fenotipo en una cohorte de pacientes con USH1 producido por mutaciones bialélicas en *MYO7A*

A pesar de los numerosos esfuerzos por establecer correlaciones específicas entre la heterogeneidad genotípica y la variabilidad fenotípica en las EHR, estas relaciones han resultado difíciles de alcanzar para la mayoría de los genes involucrados [349]. Los resultados expuestos en el **Capítulo 4** indican que no existe una correlación directa entre la gravedad del fenotipo del USH1 y el tipo de mutación encontrada en *MYO7A*, aunque inicialmente se esperaba que en este gen ocurriese como en el gen *USH2A* [30].

No se detectaron diferencias estadísticamente significativas para 5 de las 6 variables analizadas (edad de diagnóstico, edad de inicio de la ceguera nocturna, edad de inicio de la constricción del campo visual, edad de inicio de la reducción de la agudeza visual y edad de inicio para caminar sin ayuda), aunque existe una ligera diferencia en el inicio de los distintos síntomas visuales para los portadores de dos mutaciones no sinónimas, en los que el inicio parece ser más temprano que en aquellos con dos mutaciones truncantes. No obstante, la falta de una correlación genotipo-fenotipo, podría deberse a una potencia estadística insuficiente o a la ausencia de relación genotipo-fenotipo para *MYO7A*.

La única variable significativa fue la edad de inicio de la pérdida de audición (p-valor = 0,008). La pérdida auditiva del USH1 se define por ser neurosensorial, profunda, estable y congénita o muy temprana [47]. En la serie de casos presentada, la hipoacusia se detectó en la mayoría de los pacientes en el primer año de vida. Sin embargo, las diferencias observadas en la cohorte se deben a 3 casos concretos diagnosticados de UHS atípico o USH2: FRP-159, RP-2947 y RP-1218, principalmente a este último, donde la hipoacusia no comenzó hasta los 15 años de edad. Mutaciones en *MYO7A* normalmente conducen a USH1, sin embargo el rango fenotípico de este gen es amplio y también ha sido asociado a USH2 [350], formas atípicas de USH [71] y pérdida auditiva no sindrómica [70]. Por lo que el estudio de *MYO7A* debe considerarse en pacientes con fenotipo USH2 que no presenten variantes patogénicas en los genes relacionados con USH2.

Otro sesgo a tener en consideración es que la toma de algunas variables del estudio se basó en cierta medida en datos autorreportados, con el consiguiente sesgo subjetivo de los pacientes, lo cual podría reducir las posibilidades de detectar diferencias reales, especialmente si estas son pequeñas.

Los datos extraídos de este trabajo podrían ser útiles para la evaluación de la eficacia en ensayos clínicos, ya que proporciona una descripción detallada del fenotipo de *MYO7A* y sugiere que el inicio, la gravedad y la progresión de la afectación visual no están determinadas por el tipo de mutación en *MYO7A* o su ubicación. De hecho, este estudio ha ayudado a identificar y seleccionar casos de nuestra cohorte de pacientes con USH caracterizados genéticamente con variantes bialélicas en *MYO7A*, para su inclusión en un estudio observacional (NCT03814499) y el subsiguiente ensayo clínico de terapia génica, en el que se planea evaluar la seguridad y la eficacia de la administración subretiniana de vectores duales AAV-*MYO7A* para tratar la RP.

CONCLUSIONES, *CONCLUSIONS*

1. La cohorte registrada en el HUFJD representa el 20%-53% de los pacientes con EHR en España, presentando una tasa de diagnóstico general del 53%, con un total de 142 genes y 1549 variantes patogénicas y probablemente patogénicas diferentes identificadas. Las variantes más prevalentes en la población española son la p.Arg1129Leu de *ABCA4* (variante específica en población española) y la p.Cys759Phe de *USH2A* (representada en distintas poblaciones).

2. El modo de herencia más común es el AR, tanto para las EHR no sindrómicas como para las formas sindrómicas. Adicionalmente, tras la detección de las variantes causales en un gen con un patrón de herencia concreto, se pudo reclasificar genéticamente al 8,2% de los pacientes y clasificar a los casos esporádicos, refinando su asesoramiento genético.

3. El fenotipado detallado, usando términos HPO y aplicando criterios clínicos establecidos para describir los casos con sospecha de ERS de tipo no Usher, previo a los estudios moleculares, permite llegar a un diagnóstico adecuado en el 52% de los casos.

4. La tasa de caracterización general aumenta para los casos prospectivos (83%) y los que presentaban síndromes reconocibles (62%), incluso cuando se usaron paneles de genes más pequeños en CES. El uso de paneles virtuales bien diseñados conduce a buenas tasas de caracterización, independientemente del tipo de aproximación para el estudio del exoma empleada (36% en CES vs 30% en WES).

5. El análisis bioinformático de los datos de NGS debe incluir la evaluación de CNVs (3%; 3/97) y reanálisis periódicos (19%; 5/26). Estos enfoques aumentan el rendimiento diagnóstico en el estudio de las enfermedades hereditarias de la retina sindrómicas del 44% al 52%.

6. Tras la identificación del gen causante, el 27% de los casos con ERS de tipo no Usher completamente caracterizados fueron reclasificados clínicamente (12/44) y en un 17% se encontraron asociaciones nuevas o raras (8/46). Asimismo, se identificaron pacientes con afectaciones duales, ya sea porque tienen una combinación de una EHR no sindrómica con una enfermedad no genética (6%) o porque presentan dos enfermedades genéticas distintas (gen EHR no sindrómico + reordenamiento genómico; 4%). Por lo que no solo se deben considerar los genes relacionados con ERS, sino que también se deben evaluar los genes EHR y los reordenamientos genéticos.

7. No se han encontrado evidencias de la existencia de trialelismo en BBS en nuestra serie, pero sí de la presencia de potenciales alelos modificadores del fenotipo en un 13% de las familias con diagnóstico genético final de BBS (6/45). En ellas, había diferencias intrafamiliares en la penetrancia de las principales características fenotípicas y/o en la gravedad en los individuos afectos. Esta tasa aumenta hasta el 51% (23/45) cuando se incluyen todas las familias con sospecha de alelos modificadores.

8. Las herramientas *in silico*, como ORVAL y DiGePred, son una buena alternativa cuando no se puede realizar estudios funcionales para determinar la relevancia biológica de las variantes genéticas modificadoras. La combinación de estos predictores respalda la posible existencia de herencia oligogénica en el 44% de las familias de BBS trialélicas, donde se incluye a 5 de las 6 familias con diferencias fenotípicas entre hermanos.

9. No se pudo encontrar una correlación genotipo-fenotipo para *MYO7A*. Además, el tipo de variante en *MYO7A* no se asocia significativamente al inicio, la gravedad o la evolución de la enfermedad visual.

10. Aunque las mutaciones en *MYO7A* conducen en la mayoría de los casos a USH1, el rango fenotípico de *MYO7A* es amplio. Por lo que el estudio de este gen debe considerarse en aquellos pacientes afectos de USH2 que no presenten mutaciones en los genes comúnmente relacionados con USH2, ya que estos pacientes también podrían incluirse y beneficiarse de las actuales y futuras acciones terapéuticas basadas en terapia génica.

1. The cohort registered in the HUFJD represents 20%-53% of patients with IRDs in Spain, presenting a general diagnostic rate of 53%, with a total of 142 genes and 1549 different pathogenic and likely pathogenic variants identified. The most prevalent variants in the Spanish population are p.(Arg1129Leu) in *ABCA4* (specific variant in the Spanish population) and p.(Cys759Phe) in *USH2A* (represented in different populations).

2. The most common inheritance mode is AR, both for non-syndromic IRDs and for syndromic forms. Additionally, after the detection of the causal variants in a gene with a specific inheritance pattern, it was possible to genetically classify 8.2% of the patients and classify the sporadic cases, refining their genetic counselling.

3. Detailed phenotyping, using HPO terms and applying established clinical criteria to describe cases with suspected non-Usher SRDs, prior to molecular studies, allows an adequate diagnosis in 52% of cases.

4. The overall characterization rate increases for prospective cases (83%) and those with well-recognizable syndromes (62%), even when smaller gene panels were used in CES. The use of well-designed virtual panels leads to good characterization rates, regardless of the type of approach for the study of the exome used (36% in CES vs 30% in WES).

5. Bioinformatic analysis of NGS data should include evaluation of CNVs (3%; 3/97) and periodic reanalysis (19%; 5/26). These approaches increment the diagnostic yield in the study of SRD cases from 44% to 52%.

6. After the identification of the causative gene, 27% of the fully characterized cases with non-Usher SRD were clinically reclassified (12/44) and new or rare clinical associations were found in 17% (8/46). Besides, patients with dual affectations were identified, either because they have a combination of non-syndromic IRD with a non-genetic disease (6%) or because they have two different genetic diseases (non-syndromic IRD gene + genomic rearrangement; 4%). Therefore, not only SRD-related genes should be considered in the analysis, but also IRD genes and genetic rearrangements should be evaluated.

7. No evidence has been found of the existence of triallelism in BBS in our series, but there is evidence of the presence of potential phenotype modifier alleles in 13% of studied families with final genetic diagnosis of BBS (6/45). In them, there were intra-familial differences in the penetrance of the main phenotypic characteristics and/or in the severity of the affected individuals. This rate increases to 51% (23/45) when all families with suspected modifiers alleles were included.

8. *In silico* tools, such as ORVAL and DiGePred, are a good alternative when functional studies cannot be carried out to study the biological relevance of modifying genetic variants. The combination of these predictors supports the possible existence of oligogenic inheritance in 44% of triallelic BBS families, including 5 of the 6 families with phenotypic differences between siblings.

9. No genotype-phenotype correlation could be found for *MYO7A*. In addition, the type of variant in *MYO7A* is not significantly associated with the onset, severity, or progression of the visual disease.

10. Although mutations in *MYO7A* lead in most cases to USH1, the phenotypic range of *MYO7A* is wide. Therefore, the study of this gene should be considered in those patients affected by USH2, who do not present mutations in the genes commonly related to USH2, since these patients could also be included and benefit from current and future therapeutic actions based on gene therapy.

BIBLIOGRAFÍA

1. Fuhrmann S. Eye morphogenesis and patterning of the optic vesicle. *Curr Top Dev Biol* 2010;**93**:61–84.

2. Kels BD, Grzybowski A, Grant-Kels JM. Human ocular anatomy. *Clin Dermatol* 2015;**33**:140–6.

3. Tsang SH, Sharma T. Retinal Histology and Anatomical Landmarks. *Adv Exp Med Biol* 2018;**1085**:3–5.

4. Ramón y Cajal S. *Notas Preventivas Sobre La Retina y Gran Simpático de Los Mamíferos*. Barcelona: Imprenta de la Casa Provincial de Caridad, 1891.

5. Kolb H. Simple Anatomy of the Retina. In: Kolb H, Fernandez E, Nelson R (eds.). *Webvision: The Organization of the Retina and Visual System*. Salt Lake City (UT): University of Utah Health Sciences Center, 1995.

6. Kennedy B, Malicki J. What drives cell morphogenesis: a look inside the vertebrate photoreceptor. *Dev Dyn* 2009;**238**:2115–38.

7. Fei Y. Development of the cone photoreceptor mosaic in the mouse retina revealed by fluorescent cones in transgenic mice. *Mol Vis* 2003;**9**:31–42.

8. Masland RH. The fundamental plan of the retina. *Nat Neurosci* 2001;**4**:877–86.

9. Molday RS, Moritz OL. Photoreceptors at a glance. *J Cell Sci* 2015;**128**:4039–45.

10. Wright AF, Chakarova CF, Abd El-Aziz MM *et al.* Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. *Nat Rev Genet* 2010;**11**:273–84.

11. Klapper SD, Swiersy A, Bamberg E *et al.* Biophysical Properties of Optogenetic Tools and Their Application for Vision Restoration Approaches. *Front Syst Neurosci* 2016;**10**:74.

12. Lamb TD, Pugh EN. Phototransduction, dark adaptation, and rhodopsin regeneration the proctor lecture. *Invest Ophthalmol Vis Sci* 2006;**47**:5137–52.

13. Luo D-G, Xue T, Yau K-W. How vision begins: an odyssey. *Proc Natl Acad Sci U S A* 2008;**105**:9855–62.

14. Arshavsky VY, Burns ME. Photoreceptor signaling: supporting vision across a wide range of light intensities. *J Biol Chem* 2012;**287**:1620–6.

15. Ayuso C, Millan JM. Retinitis pigmentosa and allied conditions today: a paradigm of translational research. *Genome Med* 2010;**2**:34.

16. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet* 2006;**368**:1795–809.

17. Berger W, Kloeckener-Gruissem B, Neidhardt J. The molecular basis of human retinal and vitreoretinal diseases. *Prog Retin Eye Res* 2010;**29**:335–75.

18. Rivolta C, Sharon D, DeAngelis MM *et al.* Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. *Hum Mol Genet* 2002;**11**:1219–27.

19. Nangia V, Jonas JB, Khare A *et al.* Prevalence of retinitis pigmentosa in India: the Central India Eye and Medical Study. *Acta Ophthalmol* 2012;**90**:e649-650.

20. Na K-H, Kim HJ, Kim KH *et al.* Prevalence, Age at Diagnosis, Mortality, and Cause of Death in Retinitis Pigmentosa in Korea-A Nationwide Population-based Study. *Am J Ophthalmol* 2017;**176**:157–65.

21. Zeitz C, Robson AG, Audo I. Congenital stationary night blindness: an analysis and update of genotype-phenotype correlations and pathogenic mechanisms. *Prog Retin Eye Res* 2015;**45**:58–110.

22. Hamel C. Retinitis pigmentosa. *Orphanet J Rare Dis* 2006;**1**:40.

23. Michaelides M, Hardcastle AJ, Hunt DM *et al.* Progressive cone and cone-rod dystrophies: phenotypes and underlying molecular genetic basis. *Surv Ophthalmol* 2006;**51**:232–58.

24. Aboshiha J, Dubis AM, Carroll J *et al.* The cone dysfunction syndromes. *Br J Ophthalmol* 2016;**100**:115–21.

25. den Hollander AI, Roepman R, Koenekoop RK *et al.* Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog Retin Eye Res* 2008;**27**:391–419.

26. Verbakel SK, van Huet RAC, Boon CJF *et al.* Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res* 2018;**66**:157–86.

27. Tatour Y, Ben-Yosef T. Syndromic Inherited Retinal Diseases: Genetic, Clinical and Diagnostic Aspects. *Diagnostics (Basel)* 2020;**10**, DOI: 10.3390/diagnostics10100779.

28. Chiang JP-W, Lamey T, McLaren T *et al.* Progress and prospects of next-generation sequencing testing for inherited retinal dystrophy. *Expert Rev Mol Diagn* 2015;**15**:1269–75.

29. Ehrenberg M, Weiss S, Orenstein N *et al.* The co-occurrence of rare non-ocular phenotypes in patients with inherited retinal degenerations. *Mol Vis* 2019;**25**:691–702.

30. Pérez-Carro R, Blanco-Kelly F, Galbis-Martínez L *et al.* Unravelling the pathogenic role and genotype-phenotype correlation of the USH2A p.(Cys759Phe) variant among Spanish families. *PLoS One* 2018;**13**:e0199048.

31. Estrada-Cuzcano A, Koenekoop RK, Senechal A *et al.* BBS1 mutations in a wide spectrum of phenotypes ranging from nonsyndromic retinitis pigmentosa to Bardet-Biedl syndrome. *Arch Ophthalmol* 2012;**130**:1425–32.

32. Eisenberger T, Neuhaus C, Khan AO *et al.* Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. *PLoS One* 2013;**8**:e78496.

33. Ellingford JM, Barton S, Bhaskar S *et al.* Molecular findings from 537 individuals with inherited retinal disease. *J Med Genet* 2016;**53**:761–7.

34. Motta FL, Martin RP, Filippelli-Silva R *et al.* Relative frequency of inherited retinal dystrophies in Brazil. *Sci Rep* 2018;**8**:15939.

35. Focșa IO, Budișteanu M, Bălgrădean M. Clinical and genetic heterogeneity of primary ciliopathies (Review). *Int J Mol Med* 2021;**48**:176.

36. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol* 2017;**18**:533–47.

37. Carvalho-Santos Z, Azimzadeh J, Pereira-Leal JB *et al.* Evolution: Tracing the origins of centrioles, cilia, and flagella. *J Cell Biol* 2011;**194**:165–75.

38. Marshall WF, Nonaka S. Cilia: tuning in to the cell's antenna. *Curr Biol* 2006;**16**:R604-614.

39. Mitchison HM, Valente EM. Motile and non-motile cilia in human pathology: from function to phenotypes. *J Pathol* 2017;**241**:294–309.

40. May-Simera H, Nagel-Wolfrum K, Wolfrum U. Cilia - The sensory antennae in the eye. *Prog Retin Eye Res* 2017;**60**:144–80.

41. Mirvis M, Stearns T, James Nelson W. Cilium structure, assembly, and disassembly regulated by the cytoskeleton. *Biochem J* 2018;**475**:2329–53.

42. Gherman A, Davis EE, Katsanis N. The ciliary proteome database: an integrated community resource for the genetic and functional dissection of cilia. *Nat Genet* 2006;**38**:961–2.

43. Mick DU, Rodrigues RB, Leib RD *et al.* Proteomics of Primary Cilia by Proximity Labeling. *Dev Cell* 2015;**35**:497–512.

44. Boldt K, van Reeuwijk J, Lu Q *et al.* An organelle-specific protein landscape identifies novel diseases and molecular mechanisms. *Nat Commun* 2016;**7**:11491.

45. Mockel A, Perdomo Y, Stutzmann F *et al.* Retinal dystrophy in Bardet-Biedl syndrome and related syndromic ciliopathies. *Prog Retin Eye Res* 2011;**30**:258–74.

46. Waters AM, Beales PL. Ciliopathies: an expanding disease spectrum. *Pediatr Nephrol* 2011;**26**:1039–56.

47. Fuster-García C, García-Bohórquez B, Rodríguez-Muñoz A *et al.* Usher Syndrome: Genetics of a Human Ciliopathy. *Int J Mol Sci* 2021;**22**:6723.

48. von Gräfe A. Exceptionelles Verhalten des Gesichtsfeldes bei Pigmententartung der Netzhaut. *Archiv Für Ophthalmologie*. Vol 4. 1858, 250–3.

49. Usher CH. On a few hereditary eye affections. *Transactions of the Ophthalmological Societies of the United Kingdom*. Vol 55. 1935, 164–245.

50. Grøndahl J. Estimation of prognosis and prevalence of retinitis pigmentosa and Usher syndrome in Norway. *Clin Genet* 1987;**31**:255–64.

51. Tamayo ML, Bernal JE, Tamayo GE *et al.* Usher syndrome: results of a screening program in Colombia. *Clin Genet* 1991;**40**:304–11.

52. Hope CI, Bundey S, Proops D *et al.* Usher syndrome in the city of Birmingham--prevalence and clinical classification. *Br J Ophthalmol* 1997;**81**:46–53.

53. Kimberling WJ, Hildebrand MS, Shearer AE *et al.* Frequency of Usher syndrome in two pediatric populations: Implications for genetic screening of deaf and hard of hearing children. *Genet Med* 2010;**12**:512–6.

54. Yan D, Liu XZ. Genetics and pathological mechanisms of Usher syndrome. *J Hum Genet* 2010;**55**:327–35.

55. Bujakowska KM, Liu Q, Pierce EA. Photoreceptor Cilia and Retinal Ciliopathies. *Cold Spring Harb Perspect Biol* 2017;**9**:a028274.

56. Khalaileh A, Abu-Diab A, Ben-Yosef T *et al.* The Genetics of Usher Syndrome in the Israeli and Palestinian Populations. *Invest Ophthalmol Vis Sci* 2018;**59**:1095–104.

57. Sun T, Xu K, Ren Y *et al.* Comprehensive Molecular Screening in Chinese Usher Syndrome Patients. *Invest Ophthalmol Vis Sci* 2018;**59**:1229–37.

58. Bonnet C, Riahi Z, Chantot-Bastaraud S *et al.* An innovative strategy for the molecular diagnosis of Usher syndrome identifies causal biallelic mutations in 93% of European patients. *Eur J Hum Genet* 2016;**24**:1730–8.

59. Aparisi MJ, Aller E, Fuster-García C *et al.* Targeted next generation sequencing for molecular diagnosis of Usher syndrome. *Orphanet J Rare Dis* 2014;**9**:168.

60. Fuster-García C, García-García G, Jaijo T *et al.* High-throughput sequencing for the molecular diagnosis of Usher syndrome reveals 42 novel mutations and consolidates CEP250 as Usher-like disease causative. *Sci Rep* 2018;**8**:17113.

61. Nolen RM, Hufnagel RB, Friedman TB *et al.* Atypical and ultra-rare Usher syndrome: a review. *Ophthalmic Genet* 2020;**41**:401–12.

62. Weil D, Blanchard S, Kaplan J *et al.* Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* 1995;**374**:60–1.

63. Kelley PM, Weston MD, Chen ZY *et al.* The genomic structure of the gene defective in Usher syndrome type Ib (MYO7A). *Genomics* 1997;**40**:73–9.

64. Reiners J, Nagel-Wolfrum K, Jürgens K *et al.* Molecular basis of human Usher syndrome: deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease. *Exp Eye Res* 2006;**83**:97–119.

65. Roccio M, Perny M, Ealy M *et al.* Molecular characterization and prospective isolation of human fetal cochlear hair cell progenitors. *Nat Commun* 2018;**9**:4027.

66. Liu X, Udovichenko IP, Brown SD *et al.* Myosin VIIa participates in opsin transport through the photoreceptor cilium. *J Neurosci* 1999;**19**:6267–74.

67. Gibbs D, Kitamoto J, Williams DS. Abnormal phagocytosis by retinal pigmented epithelium that lacks myosin VIIa, the Usher syndrome 1B protein. *Proc Natl Acad Sci U S A* 2003;**100**:6481–6.

68. Williams DS, Lopes VS. The many different cellular functions of MYO7A in the retina. *Biochem Soc Trans* 2011;**39**:1207–10.

69. Jouret G, Poirsier C, Spodenkiewicz M *et al.* Genetics of Usher Syndrome: New Insights From a Meta-analysis. *Otol Neurotol* 2019;**40**:121–9.

70. Del Castillo I, Morín M, Domínguez-Ruiz M *et al.* Genetic etiology of non-syndromic hearing loss in Europe. *Hum Genet* 2022, DOI: 10.1007/s00439-021-02425-6.

71. Ben Rebeh I, Morinière M, Ayadi L *et al.* Reinforcement of a minor alternative splicing event in MYO7A due to a missense mutation results in a mild form of retinopathy and deafness. *Mol Vis* 2010;**16**:1898–906.

72. Liu XZ, Angeli SI, Rajput K *et al.* Cochlear implantation in individuals with Usher type 1 syndrome. *Int J Pediatr Otorhinolaryngol* 2008;**72**:841–7.

73. Toms M, Pagarkar W, Moosajee M. Usher syndrome: clinical features, molecular genetics and advancing therapeutics. *Ther Adv Ophthalmol* 2020;**12**:2515841420952194.

74. Trapani I, Colella P, Sommella A *et al.* Effective delivery of large genes to the retina by dual AAV vectors. *EMBO Mol Med* 2014;**6**:194–211.

75. Lopes VS, Diemer T, Williams DS. Assessment of different virus-mediated approaches for retinal gene therapy of Usher 1B. *Adv Exp Med Biol* 2014;**801**:725–31.

76. Riise R, Andréasson S, Borgaström MK *et al.* Intrafamilial variation of the phenotype in Bardet-Biedl syndrome. *Br J Ophthalmol* 1997;**81**:378–85.

77. M'hamdi O, Ouertani I, Chaabouni-Bouhamed H. Update on the genetics of bardet-biedl syndrome. *Mol Syndromol* 2014;**5**:51–6.

78. Klein D, Ammann F. The syndrome of Laurence-Moon-Bardet-Biedl and allied diseases in Switzerland. Clinical, genetic and epidemiological studies. *J Neurol Sci* 1969;**9**:479–513.

79. Farag TI, Teebi AS. High incidence of Bardet Biedl syndrome among the Bedouin. *Clin Genet* 1989;**36**:463–4.

80. Moore SJ, Green JS, Fan Y *et al.* Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. *Am J Med Genet A* 2005;**132A**:352–60.

81. Florea L, Caba L, Gorduza EV. Bardet-Biedl Syndrome-Multiple Kaleidoscope Images: Insight into Mechanisms of Genotype-Phenotype Correlations. *Genes (Basel)* 2021;**12**:1353.

82. Forsythe E, Beales PL. Bardet-Biedl syndrome. *Eur J Hum Genet* 2013;**21**:8–13.

83. Slavotinek AM, Stone EM, Mykytyn K *et al.* Mutations in MKKS cause Bardet-Biedl syndrome. *Nat Genet* 2000;**26**:15–6.

84. Katsanis N, Beales PL, Woods MO *et al.* Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. *Nat Genet* 2000;**26**:67–70.

85. Chandra B, Tung ML, Hsu Y *et al.* Retinal ciliopathies through the lens of Bardet-Biedl Syndrome: Past, present and future. *Prog Retin Eye Res* 2021:101035.

86. Forsyth R, Gunay-Aygun M. Bardet-Biedl Syndrome Overview. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds.). *GeneReviews®*. Seattle (WA): University of Washington, Seattle, 1993.

87. Scheidecker S, Etard C, Pierce NW *et al.* Exome sequencing of Bardet-Biedl syndrome patient identifies a null mutation in the BBSome subunit BBIP1 (BBS18). *J Med Genet* 2014;**51**:132–6.

88. Aldahmesh MA, Li Y, Alhashem A *et al.* IFT27, encoding a small GTPase component of IFT particles, is mutated in a consanguineous family with Bardet-Biedl syndrome. *Hum Mol Genet* 2014;**23**:3307–15.

89. Kim SK, Shindo A, Park TJ *et al.* Planar cell polarity acts through septins to control collective cell movement and ciliogenesis. *Science* 2010;**329**:1337–40.

90. Zaghloul NA, Katsanis N. Mechanistic insights into Bardet-Biedl syndrome, a model ciliopathy. *J Clin Invest* 2009;**119**:428–37.

91. Sathya Priya C, Sen P, Umashankar V *et al.* Mutation spectrum in BBS genes guided by homozygosity mapping in an Indian cohort. *Clin Genet* 2015;**87**:161–6.

92. Lindstrand A, Frangakis S, Carvalho CMB *et al.* Copy-Number Variation Contributes to the Mutational Load of Bardet-Biedl Syndrome. *Am J Hum Genet* 2016;**99**:318–36.

93. Katsanis N, Ansley SJ, Badano JL *et al.* Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science* 2001;**293**:2256–9.

94. Beales PL, Badano JL, Ross AJ *et al.* Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. *Am J Hum Genet* 2003;**72**:1187–99.

95. Fauser S, Munz M, Besch D. Further support for digenic inheritance in Bardet-Biedl syndrome. *J Med Genet* 2003;**40**:e104.

96. Katsanis N. The oligogenic properties of Bardet-Biedl syndrome. *Hum Mol Genet* 2004;**13 Spec No 1**:R65-71.

97. Delvallée C, Nicaise S, Antin M *et al.* A BBS1 SVA F retrotransposon insertion is a frequent cause of Bardet-Biedl syndrome. *Clin Genet* 2021;**99**:318–24.

98. Smaoui N, Chaabouni M, Sergeev YV *et al.* Screening of the eight BBS genes in Tunisian families: no evidence of triallelism. *Invest Ophthalmol Vis Sci* 2006;**47**:3487–95.

99. Abu-Safieh L, Al-Anazi S, Al-Abdi L *et al.* In search of triallelism in Bardet-Biedl syndrome. *Eur J Hum Genet* 2012;**20**:420–7.

100. Badano JL, Kim JC, Hoskins BE *et al.* Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. *Hum Mol Genet* 2003;**12**:1651–9.

101. Badano JL, Leitch CC, Ansley SJ *et al.* Dissection of epistasis in oligogenic Bardet-Biedl syndrome. *Nature* 2006;**439**:326–30.

102. Zaghloul NA, Liu Y, Gerdes JM *et al.* Functional analyses of variants reveal a significant role for dominant negative and common alleles in oligogenic Bardet-Biedl syndrome. *Proc Natl Acad Sci U S A* 2010;**107**:10602–7.

103. Kenny J, Forsythe E, Beales P *et al.* Toward personalized medicine in Bardet-Biedl syndrome. *Per Med* 2017;**14**:447–56.

104. Forsythe E, Kenny J, Bacchelli C *et al.* Managing Bardet-Biedl Syndrome-Now and in the Future. *Front Pediatr* 2018;**6**:23.

105. Seo S, Mullins RF, Dumitrescu AV *et al.* Subretinal gene therapy of mice with Bardet-Biedl syndrome type 1. *Invest Ophthalmol Vis Sci* 2013;**54**:6118–32.

106. Haws RM, Gordon G, Han JC *et al.* The efficacy and safety of setmelanotide in individuals with Bardet-Biedl syndrome or Alström syndrome: Phase 3 trial design. *Contemp Clin Trials Commun* 2021;**22**:100780.

107. Minton J a. L, Owen KR, Ricketts CJ *et al.* Syndromic obesity and diabetes: changes in body composition with age and mutation analysis of ALMS1 in 12 United Kingdom kindreds with Alstrom syndrome. *J Clin Endocrinol Metab* 2006;**91**:3110–6.

108. Marshall JD, Maffei P, Collin GB *et al.* Alström syndrome: genetics and clinical overview. *Curr Genomics* 2011;**12**:225–35.

109. Marshall JD, Beck S, Maffei P *et al.* Alström syndrome. *Eur J Hum Genet* 2007;**15**:1193–202.

110. Choudhury AR, Munonye I, Sanu KP *et al.* A review of Alström syndrome: a rare monogenic ciliopathy. *Intractable Rare Dis Res* 2021;**10**:257–62.

111. Collin GB, Marshall JD, Ikeda A *et al.* Mutations in ALMS1 cause obesity, type 2 diabetes and neurosensory degeneration in Alström syndrome. *Nat Genet* 2002;**31**:74–8.

112. Ozantürk A, Marshall JD, Collin GB *et al.* The phenotypic and molecular genetic spectrum of Alström syndrome in 44 Turkish kindreds and a literature review of Alström syndrome in Turkey. *J Hum Genet* 2015;**60**:1–9.

113. Rethanavelu K, Fung JLF, Chau JFT *et al.* Phenotypic and mutational spectrum of 21 Chinese patients with Alström syndrome. *Am J Med Genet A* 2020;**182**:279–88.

114. Knorz VJ, Spalluto C, Lessard M *et al.* Centriolar association of ALMS1 and likely centrosomal functions of the ALMS motif-containing proteins C10orf90 and KIAA1731. *Mol Biol Cell* 2010;**21**:3617–29.

115. Álvarez-Satta M, Castro-Sánchez S, Valverde D. Alström syndrome: current perspectives. *Appl Clin Genet* 2015;**8**:171–9.

116. Hampshire DJ, Ayub M, Springell K *et al.* MORM syndrome (mental retardation, truncal obesity, retinal dystrophy and micropenis), a new autosomal recessive disorder, links to 9q34. *Eur J Hum Genet* 2006;**14**:543–8.

117. Jacoby M, Cox JJ, Gayral S *et al.* INPP5E mutations cause primary cilium signaling defects, ciliary instability and ciliopathies in human and mouse. *Nat Genet* 2009;**41**:1027–31.

118. Bielas SL, Silhavy JL, Brancati F *et al.* Mutations in INPP5E, encoding inositol polyphosphate-5-phosphatase E, link phosphatidyl inositol signaling to the ciliopathies. *Nat Genet* 2009;**41**:1032–6.

119. de Goede C, Yue WW, Yan G *et al.* Role of reverse phenotyping in interpretation of next generation sequencing data and a review of INPP5E related disorders. *Eur J Paediatr Neurol* 2016;**20**:286–95.

120. Drole Torkar A, Avbelj Stefanija M, Bertok S *et al.* Novel Insights Into Monogenic Obesity Syndrome Due to INPP5E Gene Variant: A Case Report of a Female Patient. *Front Endocrinol (Lausanne)* 2021;**12**:581134.

121. Chávez M, Ena S, Van Sande J *et al.* Modulation of Ciliary Phosphoinositide Content Regulates Trafficking and Sonic Hedgehog Signaling Output. *Dev Cell* 2015;**34**:338–50.

122. Parisi MA, Doherty D, Chance PF *et al.* Joubert syndrome (and related disorders) (OMIM 213300). *Eur J Hum Genet* 2007;**15**:511–21.

123. Bachmann-Gagescu R, Dempsey JC, Bulgheroni S *et al.* Healthcare recommendations for Joubert syndrome. *Am J Med Genet A* 2020;**182**:229–49.

124. Brancati F, Dallapiccola B, Valente EM. Joubert Syndrome and related disorders. *Orphanet J Rare Dis* 2010;**5**:20.

125. Radha Rama Devi A, Naushad SM, Lingappa L. Clinical and Molecular Diagnosis of Joubert Syndrome and Related Disorders. *Pediatr Neurol* 2020;**106**:43–9.

126. Parisi MA. The molecular genetics of Joubert syndrome and related ciliopathies: The challenges of genetic and phenotypic heterogeneity. *Transl Sci Rare Dis* 2019;**4**:25–49.

127. Bachmann-Gagescu R, Dempsey JC, Phelps IG *et al.* Joubert syndrome: a model for untangling recessive disorders with extreme genetic heterogeneity. *J Med Genet* 2015;**52**:514–22.

128. Adamiok-Ostrowska A, Piekiełko-Witkowska A. Ciliary Genes in Renal Cystic Diseases. *Cells* 2020;**9**:E907.

129. Otto EA, Loeys B, Khanna H *et al.* Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. *Nat Genet* 2005;**37**:282–8.

130. Ronquillo CC, Bernstein PS, Baehr W. Senior-Løken syndrome: a syndromic form of retinal dystrophy associated with nephronophthisis. *Vision Res* 2012;**75**:88–97.

131. Werdich XQ, Place EM, Pierce EA. Systemic diseases associated with retinal dystrophies. *Semin Ophthalmol* 2014;**29**:319–28.

132. Ferreira CR, van Karnebeek CDM. Inborn errors of metabolism. *Handb Clin Neurol* 2019;**162**:449–81.

133. Leroy JG, Cathey SS, Friez MJ. Mucolipidosis III Alpha/Beta – RETIRED CHAPTER, FOR HISTORICAL REFERENCE ONLY. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds.). *GeneReviews®*. Seattle (WA): University of Washington, Seattle, 1993.

134. Dogterom EJ, Wagenmakers MAEM, Wilke M *et al.* Mucolipidosis type II and type III: a systematic review of 843 published cases. *Genet Med* 2021;**23**:2047–56.

135. Khan SA, Tomatsu SC. Mucolipidoses Overview: Past, Present, and Future. *Int J Mol Sci* 2020;**21**:E6812.

136. Levy M, Feingold J. Estimating prevalence in single-gene kidney diseases progressing to renal failure. *Kidney Int* 2000;**58**:925–43.

137. Pedrosa AL, Bitencourt L, Paranhos RM *et al.* Alport Syndrome: A Comprehensive Review on Genetics, Pathophysiology, Histology, Clinical and Therapeutic Perspectives. *Curr Med Chem* 2021;**28**:5602–24.

138. Specchio N, Ferretti A, Trivisano M *et al.* Neuronal Ceroid Lipofuscinosis: Potential for Targeted Therapy. *Drugs* 2021;**81**:101–23.

139. Cárcel-Trullols J, Kovács AD, Pearce DA. Cell biology of the NCL proteins: What they do and don't do. *Biochim Biophys Acta* 2015;**1852**:2242–55.

140. Wang H, Falk MJ, Wensel C *et al.* Cohen Syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds.). *GeneReviews®*. Seattle (WA): University of Washington, Seattle, 1993.

141. Duplomb L, Duvet S, Picot D *et al.* Cohen syndrome is associated with major glycosylation defects. *Hum Mol Genet* 2014;**23**:2391–9.

142. Barrett TG, Bundey SE, Macleod AF. Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. *Lancet* 1995;**346**:1458–63.

143. Kinsley BT, Swift M, Dumont RH *et al.* Morbidity and mortality in the Wolfram syndrome. *Diabetes Care* 1995;**18**:1566–70.

144. Abreu D, Urano F. Current Landscape of Treatments for Wolfram Syndrome. *Trends Pharmacol Sci* 2019;**40**:711–4.

145. Steinberg SJ, Dodt G, Raymond GV *et al.* Peroxisome biogenesis disorders. *Biochim Biophys Acta* 2006;**1763**:1733–48.

146. Shimozawa N, Nagase T, Takemoto Y *et al.* Genetic heterogeneity of peroxisome biogenesis disorders among Japanese patients: evidence for a founder haplotype for the most common PEX10 gene mutation. *Am J Med Genet A* 2003;**120A**:40–3.

147. Braverman NE, D'Agostino MD, Maclean GE. Peroxisome biogenesis disorders: Biological, clinical and pathophysiological perspectives. *Dev Disabil Res Rev* 2013;**17**:187–96.

148. Wang F, Wang H, Tuan H-F *et al.* Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. *Hum Genet* 2014;**133**:331–45.

149. Ku CA, Pennesi ME. The new landscape of retinal gene therapy. *Am J Med Genet C Semin Med Genet* 2020;**184**:846–59.

150. Köhler S, Doelken SC, Mungall CJ *et al.* The Human Phenotype Ontology project: linking molecular biology and disease through phenotype data. *Nucleic Acids Res* 2014;**42**:D966-974.

151. Köhler S, Vasilevsky NA, Engelstad M *et al.* The Human Phenotype Ontology in 2017. *Nucleic Acids Res* 2017;**45**:D865–76.

152. Köhler S, Gargano M, Matentzoglu N *et al.* The Human Phenotype Ontology in 2021. *Nucleic Acids Res* 2021;**49**:D1207–17.

153. Robinson PN, Köhler S, Bauer S *et al.* The Human Phenotype Ontology: a tool for annotating and analyzing human hereditary disease. *Am J Hum Genet* 2008;**83**:610–5.

154. Trujillano D, Bertoli-Avella AM, Kumar Kandaswamy K *et al.* Clinical exome sequencing: results from 2819 samples reflecting 1000 families. *Eur J Hum Genet* 2017;**25**:176–82.

155. Fang H, Wu Y, Yang H *et al.* Whole genome sequencing of one complex pedigree illustrates challenges with genomic medicine. *BMC Med Genomics* 2017;**10**:10.

156. Pontikos N, Murphy C, Moghul I *et al.* Phenogenon: Gene to phenotype associations for rare genetic diseases. *PLoS One* 2020;**15**:e0230587.

157. Jezela-Stanek A, Ciara E, Jurkiewicz D *et al.* The phenotype-driven computational analysis yields clinical diagnosis for patients with atypical manifestations of known intellectual disability syndromes. *Mol Genet Genomic Med* 2020;**8**:e1263.

158. Haimel M, Pazmandi J, Heredia RJ *et al.* Curation and expansion of Human Phenotype Ontology for defined groups of inborn errors of immunity. *J Allergy Clin Immunol* 2022;**149**:369–78.

159. Köhler S, Carmody L, Vasilevsky N *et al.* Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. *Nucleic Acids Res* 2019;**47**:D1018–27.

160. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. *Nat Rev Genet* 2016;**17**:333–51.

161. Yohe S, Thyagarajan B. Review of Clinical Next-Generation Sequencing. *Arch Pathol Lab Med* 2017;**141**:1544–57.

162. Wright CF, Fitzgerald TW, Jones WD *et al.* Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. *Lancet* 2015;**385**:1305–14.

163. Sanchez-Navarro I, R J da Silva L, Blanco-Kelly F *et al.* Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. *Sci Rep* 2018;**8**:5285.

164. Abu Diab A, AlTalbishi A, Rosin B *et al.* The combination of whole-exome sequencing and clinical analysis allows better diagnosis of rare syndromic retinal dystrophies. *Acta Ophthalmol* 2019;**97**:e877–86.

165. Boycott KM, Vanstone MR, Bulman DE *et al.* Rare-disease genetics in the era of next-generation sequencing: discovery to translation. *Nat Rev Genet* 2013;**14**:681–91.

166. Stenton SL, Prokisch H. Advancing genomic approaches to the molecular diagnosis of mitochondrial disease. *Essays Biochem* 2018;**62**:399–408.

167. Kozarewa I, Armisen J, Gardner AF *et al.* Overview of Target Enrichment Strategies. *Curr Protoc Mol Biol* 2015;**112**:7.21.1-7.21.23.

168. Bao R, Huang L, Andrade J *et al.* Review of current methods, applications, and data management for the bioinformatics analysis of whole exome sequencing. *Cancer Inform* 2014;**13**:67–82.

169. Morbidoni V, Agolini E, Slep KC *et al.* Biallelic mutations in the TOGARAM1 gene cause a novel primary ciliopathy. *J Med Genet* 2021;**58**:526–33.

170. Petersen B-S, Fredrich B, Hoeppner MP *et al.* Opportunities and challenges of whole-genome and -exome sequencing. *BMC Genet* 2017;**18**:14.

171. Wheway G, Mitchison HM, Genomics England Research Consortium. Opportunities and Challenges for Molecular Understanding of Ciliopathies-The 100,000 Genomes Project. *Front Genet* 2019;**10**:127.

172. Méjécase C, Malka S, Guan Z *et al.* Practical guide to genetic screening for inherited eye diseases. *Ther Adv Ophthalmol* 2020;**12**:2515841420954592.

173. Altmann A, Weber P, Bader D *et al.* A beginners guide to SNP calling from high-throughput DNA-sequencing data. *Hum Genet* 2012;**131**:1541–54.

174. MacArthur DG, Manolio TA, Dimmock DP *et al.* Guidelines for investigating causality of sequence variants in human disease. *Nature* 2014;**508**:469–76.

175. Richards S, Aziz N, Bale S *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;**17**:405–24.

176. Matthijs G, Souche E, Alders M *et al.* Guidelines for diagnostic next-generation sequencing. *Eur J Hum Genet* 2016;**24**:2–5.

177. Macé A, Kutalik Z, Valsesia A. Copy Number Variation. *Methods Mol Biol* 2018;**1793**:231–58.

178. Plagnol V, Curtis J, Epstein M *et al.* A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics* 2012;**28**:2747–54.

179. Johansson LF, van Dijk F, de Boer EN *et al.* CoNVaDING: Single Exon Variation Detection in Targeted NGS Data. *Hum Mutat* 2016;**37**:457–64.

180. Brett M, McPherson J, Zang ZJ *et al.* Massively parallel sequencing of patients with intellectual disability, congenital anomalies and/or autism spectrum disorders with a targeted gene panel. *PLoS One* 2014;**9**:e93409.

181. Marshall CR, Chowdhury S, Taft RJ *et al.* Best practices for the analytical validation of clinical whole-genome sequencing intended for the diagnosis of germline disease. *NPJ Genom Med* 2020;**5**:47.

182. Goh G, Choi M. Application of whole exome sequencing to identify disease-causing variants in inherited human diseases. *Genomics Inform* 2012;**10**:214–9.

183. Lee H, Deignan JL, Dorrani N *et al.* Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA* 2014;**312**:1880–7.

184. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. *Nat Rev Genet* 2018;**19**:253–68.

185. Carss KJ, Arno G, Erwood M *et al.* Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease. *Am J Hum Genet* 2017;**100**:75–90.

186. Gilissen C, Hoischen A, Brunner HG *et al.* Disease gene identification strategies for exome sequencing. *Eur J Hum Genet* 2012;**20**:490–7.

187. Warr A, Robert C, Hume D *et al.* Exome Sequencing: Current and Future Perspectives. *G3 (Bethesda)* 2015;**5**:1543–50.

188. Quinodoz M, Peter VG, Bedoni N *et al.* AutoMap is a high performance homozygosity mapping tool using next-generation sequencing data. *Nat Commun* 2021;**12**:518.

189. Renaux A, Papadimitriou S, Versbraegen N *et al.* ORVAL: a novel platform for the prediction and exploration of disease-causing oligogenic variant combinations. *Nucleic Acids Res* 2019;**47**:W93–8.

190. Mukherjee S, Cogan JD, Newman JH *et al.* Identifying digenic disease genes via machine learning in the Undiagnosed Diseases Network. *Am J Hum Genet* 2021;**108**:1946–63.

191. Wakeling MN, Laver TW, Wright CF *et al.* Homozygosity mapping provides supporting evidence of pathogenicity in recessive Mendelian disease. *Genet Med* 2019;**21**:982–6.

192. Kar A, Phadke SR, Das Bhowmik A *et al.* Whole exome sequencing reveals a mutation in ARMC9 as a cause of mental retardation, ptosis, and polydactyly. *Am J Med Genet A* 2018;**176**:34–40.

193. Zhang D, Dai L, Zhou Z *et al.* Homozygosity mapping and whole exome sequencing reveal a novel ERCC8 mutation in a Chinese consanguineous family with unique cerebellar ataxia. *Clin Chim Acta* 2019;**494**:64–70.

194. Papadimitriou S, Gazzo A, Versbraegen N *et al.* Predicting disease-causing variant combinations. *Proc Natl Acad Sci U S A* 2019;**116**:11878–87.

195. Gazzo AM, Daneels D, Cilia E *et al.* DIDA: A curated and annotated digenic diseases database. *Nucleic Acids Res* 2016;**44**:D900-907.

196. Mkaouar R, Abdallah LCB, Naouali C *et al.* Oligogenic Inheritance Underlying Incomplete Penetrance of PROKR2 Mutations in Hypogonadotropic Hypogonadism. *Front Genet* 2021;**12**:665174.

197. Dallali H, Kheriji N, Kammoun W *et al.* Multiallelic Rare Variants in BBS Genes Support an Oligogenic Ciliopathy in a Non-obese Juvenile-Onset Syndromic Diabetic Patient: A Case Report. *Front Genet* 2021;**12**:664963.

198. Forsythe E, Beales PL. Bardet-Biedl syndrome. *Eur J Hum Genet* 2013;**21**:8–13.

199. M'hamdi O, Ouertani I, Chaabouni-Bouhamed H. Update on the genetics of bardet-biedl syndrome. *Mol Syndromol* 2014;**5**:51–6.

200. Klein D, Ammann F. The syndrome of Laurence-Moon-Bardet-Biedl and allied diseases in Switzerland. Clinical, genetic and epidemiological studies. *J Neurol Sci* 1969;**9**:479–513.

201. Katsanis N, Ansley SJ, Badano JL *et al.* Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science* 2001;**293**:2256–9.

202. Smaoui N, Chaabouni M, Sergeev YV *et al.* Screening of the eight BBS genes in Tunisian families: no evidence of triallelism. *Invest Ophthalmol Vis Sci* 2006;**47**:3487–95.

203. Abu-Safieh L, Al-Anazi S, Al-Abdi L *et al.* In search of triallelism in Bardet-Biedl syndrome. *Eur J Hum Genet* 2012;**20**:420–7.

204. Katsanis N, Eichers ER, Ansley SJ *et al.* BBS4 is a minor contributor to Bardet-Biedl syndrome and may also participate in triallelic inheritance. *Am J Hum Genet* 2002;**71**:22–9.

205. Beales PL, Badano JL, Ross AJ *et al.* Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. *Am J Hum Genet* 2003;**72**:1187–99.

206. Badano JL, Kim JC, Hoskins BE *et al.* Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. *Hum Mol Genet* 2003;**12**:1651–9.

207. Bin J, Madhavan J, Ferrini W *et al.* BBS7 and TTC8 (BBS8) mutations play a minor role in the mutational load of Bardet-Biedl syndrome in a multiethnic population. *Hum Mutat* 2009;**30**:E737-746.

208. Katsanis N. The oligogenic properties of Bardet-Biedl syndrome. *Hum Mol Genet* 2004;**13 Spec No 1**:R65-71.

209. Woodsmith J, Apelt L, Casado-Medrano V *et al.* Protein interaction perturbation profiling at amino-acid resolution. *Nat Methods* 2017;**14**:1213–21.

210. Zaghloul NA, Liu Y, Gerdes JM *et al.* Functional analyses of variants reveal a significant role for dominant negative and common alleles in oligogenic Bardet-Biedl syndrome. *Proc Natl Acad Sci U S A* 2010;**107**:10602–7.

211. Renaux A, Papadimitriou S, Versbraegen N *et al.* ORVAL: a novel platform for the prediction and exploration of disease-causing oligogenic variant combinations. *Nucleic Acids Res* 2019;**47**:W93–8.

212. Perea-Romero I, Gordo G, Iancu IF *et al.* Genetic landscape of 6089 inherited retinal dystrophies affected cases in Spain and their therapeutic and extended epidemiological implications. *Sci Rep* 2021;**11**:1526.

213. Beales PL, Elcioglu N, Woolf AS *et al.* New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. *J Med Genet* 1999;**36**:437–46.

214. Castro-Sánchez S, Álvarez-Satta M, Cortón M *et al.* Exploring genotype-phenotype relationships in Bardet-Biedl syndrome families. *J Med Genet* 2015;**52**:503–13.

215. Perea-Romero I, Blanco-Kelly F, Sanchez-Navarro I *et al.* NGS and phenotypic ontology-based approaches increase the diagnostic yield in syndromic retinal diseases. *Hum Genet* 2021;**140**:1665–78.

216. Pereiro I, Valverde D, Piñeiro-Gallego T *et al.* New mutations in BBS genes in small consanguineous families with Bardet-Biedl syndrome: detection of candidate regions by homozygosity mapping. *Mol Vis* 2010;**16**:137–43.

217. Pereiro I, Hoskins BE, Marshall JD *et al.* Arrayed primer extension technology simplifies mutation detection in Bardet-Biedl and Alström syndrome. *Eur J Hum Genet* 2011;**19**:485–8.

218. Álvarez-Satta M, Castro-Sánchez S, Pereiro I *et al.* Overview of Bardet-Biedl syndrome in Spain: identification of novel mutations in BBS1, BBS10 and BBS12 genes. *Clin Genet* 2014;**86**:601–2.

219. Castro-Sánchez S, Álvarez-Satta M, Tohamy MA *et al.* Whole exome sequencing as a diagnostic tool for patients with ciliopathy-like phenotypes. *PLoS One* 2017;**12**:e0183081.

220. Sanchez-Navarro I, R J da Silva L, Blanco-Kelly F *et al.* Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. *Sci Rep* 2018;**8**:5285.

221. Richards S, Aziz N, Bale S *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;**17**:405–24.

222. Riise R, Andréasson S, Borgaström MK *et al.* Intrafamilial variation of the phenotype in Bardet-Biedl syndrome. *Br J Ophthalmol* 1997;**81**:378–85.

223. Eichers ER, Lewis RA, Katsanis N *et al.* Triallelic inheritance: a bridge between Mendelian and multifactorial traits. *Ann Med* 2004;**36**:262–72.

224. Rooryck C, Lacombe D. [Bardet-Biedl syndrome]. *Ann Endocrinol (Paris)* 2008;**69**:463–71.

225. Forsythe E, Kenny J, Bacchelli C *et al.* Managing Bardet-Biedl Syndrome-Now and in the Future. *Front Pediatr* 2018;**6**:23.

226. Jeziorny K, Antosik K, Jakiel P *et al.* Next-Generation Sequencing in the Diagnosis of Patients with Bardet-Biedl Syndrome-New Variants and Relationship with Hyperglycemia and Insulin Resistance. *Genes (Basel)* 2020;**11**:E1283.

227. Castro-Sánchez S, Álvarez-Satta M, Pereiro I *et al.* [Algorithm for the molecular analysis of Bardet-Biedl syndrome in Spain]. *Med Clin (Barc)* 2015;**145**:147–52.

228. Marion V, Stutzmann F, Gérard M *et al.* Exome sequencing identifies mutations in LZTFL1, a BBSome and smoothened trafficking regulator, in a family with Bardet--Biedl syndrome with situs inversus and insertional polydactyly. *J Med Genet* 2012;**49**:317–21.

229. Ajmal M, Khan MI, Neveling K *et al.* Exome sequencing identifies a novel and a recurrent BBS1 mutation in Pakistani families with Bardet-Biedl syndrome. *Mol Vis* 2013;**19**:644–53.

230. Khan SA, Muhammad N, Khan MA *et al.* Genetics of human Bardet-Biedl syndrome, an updates. *Clin Genet* 2016;**90**:3–15.

231. Mykytyn K, Nishimura DY, Searby CC *et al.* Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (BBS1). *Am J Hum Genet* 2003;**72**:429–37.

232. Forsythe E, Sparks K, Best S *et al.* Risk Factors for Severe Renal Disease in Bardet-Biedl Syndrome. *J Am Soc Nephrol* 2017;**28**:963–70.

233. Focșa IO, Budișteanu M, Burloiu C *et al.* A case of Bardet-Biedl syndrome caused by a recurrent variant in BBS12: A case report. *Biomed Rep* 2021;**15**:103.

234. Suspitsin EN, Imyanitov EN. Bardet-Biedl Syndrome. *Mol Syndromol* 2016;**7**:62–71.

235. Niederlova V, Modrak M, Tsyklauri O *et al.* Meta-analysis of genotype-phenotype associations in Bardet-Biedl syndrome uncovers differences among causative genes. *Hum Mutat* 2019;**40**:2068–87.

236. Nachury MV, Loktev AV, Zhang Q *et al.* A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* 2007;**129**:1201–13.

237. Tory K, Lacoste T, Burglen L *et al.* High NPHP1 and NPHP6 mutation rate in patients with Joubert syndrome and nephronophthisis: potential epistatic effect of NPHP6 and AHI1 mutations in patients with NPHP1 mutations. *J Am Soc Nephrol* 2007;**18**:1566–75.

238. Khanna H, Davis EE, Murga-Zamalloa CA *et al.* A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat Genet* 2009;**41**:739–45.

239. Louie CM, Caridi G, Lopes VS *et al.* AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. *Nat Genet* 2010;**42**:175–80.

240. Marshall JD, Beck S, Maffei P *et al.* Alström syndrome. *Eur J Hum Genet* 2007;**15**:1193–202.

241. Dassie F, Favaretto F, Bettini S *et al.* Alström syndrome: an ultra-rare monogenic disorder as a model for insulin resistance, type 2 diabetes mellitus and obesity. *Endocrine* 2021;**71**:618–25.

242. Cho NH, Shaw JE, Karuranga S *et al.* IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018;**138**:271–81.

243. González-Del Pozo M, Méndez-Vidal C, Santoyo-Lopez J *et al.* Deciphering intrafamilial phenotypic variability by exome sequencing in a Bardet-Biedl family. *Mol Genet Genomic Med* 2014;**2**:124–33.

244. Lindstrand A, Frangakis S, Carvalho CMB *et al.* Copy-Number Variation Contributes to the Mutational Load of Bardet-Biedl Syndrome. *Am J Hum Genet* 2016;**99**:318–36.

245. Chakrabarty S, Savantre SB, Ramachandra Bhat C *et al.* Multiple genetic mutations implicate spectrum of phenotypes in Bardet-Biedl syndrome. *Gene* 2020;**725**:144164.

246. Hostelley TL, Lodh S, Zaghloul NA. Whole organism transcriptome analysis of zebrafish models of Bardet-Biedl Syndrome and Alström Syndrome provides mechanistic insight into shared and divergent phenotypes. *BMC Genomics* 2016;**17**:318.

247. Kenny J, Forsythe E, Beales P *et al.* Toward personalized medicine in Bardet-Biedl syndrome. *Per Med* 2017;**14**:447–56.

248. Blanco-Kelly F, Jaijo T, Aller E *et al.* Clinical aspects of Usher syndrome and the USH2A gene in a cohort of 433 patients. *JAMA Ophthalmol* 2015;**133**:157–64.

249. Castro-Sánchez S, Álvarez-Satta M, Cortón M *et al.* Exploring genotype-phenotype relationships in Bardet-Biedl syndrome families. *J Med Genet* 2015;**52**:503–13.

250. Bravo-Gil N, González-Del Pozo M, Martín-Sánchez M *et al.* Unravelling the genetic basis of simplex Retinitis Pigmentosa cases. *Sci Rep* 2017;**7**:41937.

251. Martin-Merida I, Avila-Fernandez A, Del Pozo-Valero M *et al.* Genomic Landscape of Sporadic Retinitis Pigmentosa: Findings from 877 Spanish Cases. *Ophthalmology* 2019;**126**:1181–8.

252. Del Pozo-Valero M, Riveiro-Alvarez R, Martin-Merida I *et al.* Impact of Next Generation Sequencing in Unraveling the Genetics of 1036 Spanish Families With Inherited Macular Dystrophies. *Invest Ophthalmol Vis Sci* 2022;**63**:11.

253. Sahel J-A, Bennett J, Roska B. Depicting brighter possibilities for treating blindness. *Sci Transl Med* 2019;**11**:eaax2324.

254. Heath Jeffery RC, Mukhtar SA, McAllister IL *et al.* Inherited retinal diseases are the most common cause of blindness in the working-age population in Australia. *Ophthalmic Genet* 2021;**42**:431–9.

255. Scholl HPN, Strauss RW, Singh MS *et al.* Emerging therapies for inherited retinal degeneration. *Sci Transl Med* 2016;**8**:368rv6.

256. Burdon KP. The utility of genomic testing in the ophthalmology clinic: A review. *Clin Exp Ophthalmol* 2021;**49**:615–25.

257. Bycroft C, Fernandez-Rozadilla C, Ruiz-Ponte C *et al.* Patterns of genetic differentiation and the footprints of historical migrations in the Iberian Peninsula. *Nat Commun* 2019;**10**:551.

258. Thorsteinsson DA, Stefansdottir V, Eysteinsson T *et al.* Molecular genetics of inherited retinal degenerations in Icelandic patients. *Clin Genet* 2021;**100**:156–67.

259. Tracewska AM, Kocyła-Karczmarewicz B, Rafalska A *et al.* Non-syndromic inherited retinal diseases in Poland: Genes, mutations, and phenotypes. *Mol Vis* 2021;**27**:457–65.

260. Birtel J, Eisenberger T, Gliem M *et al.* Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. *Sci Rep* 2018;**8**:4824.

261. Yoshimura H, Nishio S-Y, Isaka Y *et al.* A nationwide epidemiologic, clinical, genetic study of Usher syndrome in Japan. *Acta Otolaryngol* 2021;**141**:841–6.

262. Ziviello C, Simonelli F, Testa F *et al.* Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): a comprehensive study of 43 Italian families. *J Med Genet* 2005;**42**:e47.

263. Huang X-F, Huang F, Wu K-C *et al.* Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet Med* 2015;**17**:271–8.

264. Beheshtian M, Saee Rad S, Babanejad M *et al.* Impact of whole exome sequencing among Iranian patients with autosomal recessive retinitis pigmentosa. *Arch Iran Med* 2015;**18**:776–85.

265. Tiwari A, Bahr A, Bähr L *et al.* Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. *Sci Rep* 2016;**6**:28755.

266. Maeda A, Yoshida A, Kawai K *et al.* Development of a molecular diagnostic test for Retinitis Pigmentosa in the Japanese population. *Jpn J Ophthalmol* 2018;**62**:451–7.

267. Kim MS, Joo K, Seong MW *et al.* Genetic Mutation Profiles in Korean Patients with Inherited Retinal Diseases. *J Korean Med Sci* 2019;**34**:e161.

268. Bertelsen M, Jensen H, Bregnhøj JF *et al.* Prevalence of generalized retinal dystrophy in Denmark. *Ophthalmic Epidemiol* 2014;**21**:217–23.

269. Holtan JP, Selmer KK, Heimdal KR *et al.* Inherited retinal disease in Norway - a characterization of current clinical and genetic knowledge. *Acta Ophthalmol* 2020;**98**:286–95.

270. Sharon D, Ben-Yosef T, Goldenberg-Cohen N *et al.* A nationwide genetic analysis of inherited retinal diseases in Israel as assessed by the Israeli inherited retinal disease consortium (IIRDC). *Hum Mutat* 2020;**41**:140–9.

271. Goetz KE, Reeves MJ, Gagadam S *et al.* Genetic testing for inherited eye conditions in over 6,000 individuals through the eyeGENE network. *Am J Med Genet C Semin Med Genet* 2020;**184**:828–37.

272. Jaffal L, Joumaa WH, Assi A *et al.* Next Generation Sequencing Identifies Five Novel Mutations in Lebanese Patients with Bardet-Biedl and Usher Syndromes. *Genes (Basel)* 2019;**10**, DOI: 10.3390/genes10121047.

273. Marshall DA, MacDonald KV, Heidenreich S *et al.* The value of diagnostic testing for parents of children with rare genetic diseases. *Genet Med* 2019;**21**:2798–806.

274. Stark Z, Schofield D, Alam K *et al.* Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. *Genet Med* 2017;**19**:867–74.

275. Boycott KM, Innes AM. When One Diagnosis Is Not Enough. *N Engl J Med* 2017;**376**:83–5.

276. Salmaninejad A, Motaee J, Farjami M *et al.* Next-generation sequencing and its application in diagnosis of retinitis pigmentosa. *Ophthalmic Genet* 2019;**40**:393–402.

277. Xue Y, Ankala A, Wilcox WR *et al.* Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: single-gene, gene panel, or exome/genome sequencing. *Genet Med* 2015;**17**:444–51.

278. de Bruijn SE, Fadaie Z, Cremers FPM *et al.* The Impact of Modern Technologies on Molecular Diagnostic Success Rates, with a Focus on Inherited Retinal Dystrophy and Hearing Loss. *Int J Mol Sci* 2021;**22**:2943.

279. Villanueva-Mendoza C, Tuson M, Apam-Garduño D *et al.* The Genetic Landscape of Inherited Retinal Diseases in a Mexican Cohort: Genes, Mutations and Phenotypes. *Genes (Basel)* 2021;**12**:1824.

280. Del Pozo-Valero M, Riveiro-Alvarez R, Blanco-Kelly F *et al.* Genotype-Phenotype Correlations in a Spanish Cohort of 506 Families With Biallelic ABCA4 Pathogenic Variants. *Am J Ophthalmol* 2020;**219**:195–204.

281. Riveiro-Alvarez R, Lopez-Martinez M-A, Zernant J *et al.* Outcome of ABCA4 disease-associated alleles in autosomal recessive retinal dystrophies: retrospective analysis in 420 Spanish families. *Ophthalmology* 2013;**120**:2332–7.

282. Cremers FPM, Lee W, Collin RWJ *et al.* Clinical spectrum, genetic complexity and therapeutic approaches for retinal disease caused by ABCA4 mutations. *Prog Retin Eye Res* 2020;**79**:100861.

283. Pennings RJE, Te Brinke H, Weston MD *et al.* USH2A mutation analysis in 70 Dutch families with Usher syndrome type II. *Hum Mutat* 2004;**24**:185.

284. Baux D, Blanchet C, Hamel C *et al.* Enrichment of LOVD-USHbases with 152 USH2A genotypes defines an extensive mutational spectrum and highlights missense hotspots. *Hum Mutat* 2014;**35**:1179–86.

285. Gayán J, Galan JJ, González-Pérez A *et al.* Genetic structure of the Spanish population. *BMC Genomics* 2010;**11**:326.

286. Manara E, Paolacci S, D'Esposito F *et al.* Mutation profile of BBS genes in patients with Bardet-Biedl syndrome: an Italian study. *Ital J Pediatr* 2019;**45**:72.

287. Jiman OA, Taylor RL, Lenassi E *et al.* Diagnostic yield of panel-based genetic testing in syndromic inherited retinal disease. *Eur J Hum Genet* 2020;**28**:576–86.

288. Shaheen R, Szymanska K, Basu B *et al.* Characterizing the morbid genome of ciliopathies. *Genome Biol* 2016;**17**:242.

289. Smedley D, Robinson PN. Phenotype-driven strategies for exome prioritization of human Mendelian disease genes. *Genome Med* 2015;**7**:81.

290. Yuan X, Wang J, Dai B *et al.* Evaluation of phenotype-driven gene prioritization methods for Mendelian diseases. *Brief Bioinform* 2022:bbac019.

291. Birgmeier J, Haeussler M, Deisseroth CA *et al.* AMELIE speeds Mendelian diagnosis by matching patient phenotype and genotype to primary literature. *Sci Transl Med* 2020;**12**:eaau9113.

292. Robinson PN, Ravanmehr V, Jacobsen JOB *et al.* Interpretable Clinical Genomics with a Likelihood Ratio Paradigm. *Am J Hum Genet* 2020;**107**:403–17.

293. Zampaglione E, Kinde B, Place EM *et al.* Copy-number variation contributes 9% of pathogenicity in the inherited retinal degenerations. *Genet Med* 2020;**22**:1079–87.

294. Consugar MB, Navarro-Gomez D, Place EM *et al.* Panel-based genetic diagnostic testing for inherited eye diseases is highly accurate and reproducible, and more sensitive for variant detection, than exome sequencing. *Genet Med* 2015;**17**:253–61.

295. Wang L, Zhang J, Chen N *et al.* Application of Whole Exome and Targeted Panel Sequencing in the Clinical Molecular Diagnosis of 319 Chinese Families with Inherited Retinal Dystrophy and Comparison Study. *Genes (Basel)* 2018;**9**:E360.

296. Gupta S, Chatterjee S, Mukherjee A *et al.* Whole exome sequencing: Uncovering causal genetic variants for ocular diseases. *Exp Eye Res* 2017;**164**:139–50.

297. Cogné B, Latypova X, Senaratne LDS *et al.* Mutations in the Kinesin-2 Motor KIF3B Cause an Autosomal-Dominant Ciliopathy. *Am J Hum Genet* 2020;**106**:893–904.

298. Chiu N, Lee W, Liu P-K *et al.* A homozygous in-frame duplication within the LRRCT consensus sequence of CFAP410 causes cone-rod dystrophy, macular staphyloma and short stature. *Ophthalmic Genet* 2021:1–7.

299. Scott HA, Larson A, Rong SS *et al.* A Hidden Structural Variation in a Known IRD Gene: A Cautionary Tale of Two New Disease Candidate Genes. *Cold Spring Harb Mol Case Stud* 2021:mcs.a006131.

300. Ma DJ, Lee H-S, Kim K *et al.* Whole-exome sequencing in 168 Korean patients with inherited retinal degeneration. *BMC Med Genomics* 2021;**14**:74.

301. Song F, Owczarek-Lipska M, Ahmels T *et al.* High-Throughput Sequencing to Identify Mutations Associated with Retinal Dystrophies. *Genes (Basel)* 2021;**12**:1269.

302. Romero R, de la Fuente L, Del Pozo-Valero M *et al.* An evaluation of pipelines for DNA variant detection can guide a reanalysis protocol to increase the diagnostic ratio of genetic diseases. *NPJ Genom Med* 2022;**7**:7.

303. Stephenson KAJ, Zhu J, Dockery A *et al.* Clinical and Genetic Re-Evaluation of Inherited Retinal Degeneration Pedigrees following Initial Negative Findings on Panel-Based Next Generation Sequencing. *Int J Mol Sci* 2022;**23**:995.

304. Haer-Wigman L, van Zelst-Stams WA, Pfundt R *et al.* Diagnostic exome sequencing in 266 Dutch patients with visual impairment. *Eur J Hum Genet* 2017;**25**:591–9.

305. Alfares A, Aloraini T, Subaie LA *et al.* Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. *Genet Med* 2018;**20**:1328–33.

306. Ewans LJ, Schofield D, Shrestha R *et al.* Whole-exome sequencing reanalysis at 12 months boosts diagnosis and is cost-effective when applied early in Mendelian disorders. *Genet Med* 2018;**20**:1564–74.

307. Liu P, Meng L, Normand EA *et al.* Reanalysis of Clinical Exome Sequencing Data. *N Engl J Med* 2019;**380**:2478–80.

308. Liu Y, Teng Y, Li Z *et al.* Increase in diagnostic yield achieved for 174 whole-exome sequencing cases reanalyzed 1-2 years after initial analysis. *Clin Chim Acta* 2021;**523**:163–8.

309. Okur V, Cho MT, van Wijk R *et al.* De novo variants in HK1 associated with neurodevelopmental abnormalities and visual impairment. *Eur J Hum Genet* 2019;**27**:1081–9.

310. Li J, Gao K, Yan H *et al.* Reanalysis of whole exome sequencing data in patients with epilepsy and intellectual disability/mental retardation. *Gene* 2019;**700**:168–75.

311. Jalkh N, Corbani S, Haidar Z *et al.* The added value of WES reanalysis in the field of genetic diagnosis: lessons learned from 200 exomes in the Lebanese population. *BMC Med Genomics* 2019;**12**:11.

312. Iancu I-F, Avila-Fernandez A, Arteche A *et al.* Prioritizing variants of uncertain significance for reclassification using a rule-based algorithm in inherited retinal dystrophies. *NPJ Genom Med* 2021;**6**:18.

313. Qian X, Wang J, Wang M *et al.* Identification of Deep-Intronic Splice Mutations in a Large Cohort of Patients With Inherited Retinal Diseases. *Front Genet* 2021;**12**:647400.

314. Schlüter A, Rodríguez-Palmero A, Verdura E *et al.* Diagnosis of Genetic White Matter Disorders by Singleton Whole-Exome and Genome Sequencing Using Interactome-Driven Prioritization. *Neurology* 2022;**98**:e912–23.

315. González-Del Pozo M, Fernández-Suárez E, Bravo-Gil N *et al.* A comprehensive WGS-based pipeline for the identification of new candidate genes in inherited retinal dystrophies. *NPJ Genom Med* 2022;**7**:17.

316. Jespersgaard C, Fang M, Bertelsen M *et al.* Molecular genetic analysis using targeted NGS analysis of 677 individuals with retinal dystrophy. *Sci Rep* 2019;**9**:1219.

317. Shamseldin HE, Shaheen R, Ewida N *et al.* The morbid genome of ciliopathies: an update. *Genet Med* 2020;**22**:1051–60.

318. Heon E, Kim G, Qin S *et al.* Mutations in C8ORF37 cause Bardet Biedl syndrome (BBS21). *Hum Mol Genet* 2016;**25**:2283–94.

319. Singh MK, Shrinkhal null, Pradhan S *et al.* Bardet Biedl Syndrome - A Report of Two Cases with Otolaryngologic Symptoms. *J Clin Diagn Res* 2017;**11**:ND01–2.

320. Valente EM, Brancati F, Silhavy JL *et al.* AHI1 gene mutations cause specific forms of Joubert syndrome-related disorders. *Ann Neurol* 2006;**59**:527–34.

321. Bourgeois JR, Ferland RJ. Loss of the neurodevelopmental Joubert syndrome causing protein, Ahi1, causes motor and muscle development delays independent of central nervous system involvement. *Dev Biol* 2019;**448**:36–47.

322. Ashraf T, Vaina C, Giri D *et al.* Expanding the phenotypic spectrum of IFT81: Associated ciliopathy syndrome. *Am J Med Genet A* 2020;**182**:2403–8.

323. Perrault I, Halbritter J, Porath JD *et al.* IFT81, encoding an IFT-B core protein, as a very rare cause of a ciliopathy phenotype. *J Med Genet* 2015;**52**:657–65.

324. Qu W, Yuan S, Quan C *et al.* The essential role of intraflagellar transport protein IFT81 in male mice spermiogenesis and fertility. *Am J Physiol Cell Physiol* 2020;**318**:C1092–106.

325. Gibson S, Azamian MS, Lalani SR *et al.* Recessive ACO2 variants as a cause of isolated ophthalmologic phenotypes. *Am J Med Genet A* 2020;**182**:1960–6.

326. Blackburn PR, Schultz MJ, Lahner CA *et al.* Expanding the clinical and phenotypic heterogeneity associated with biallelic variants in ACO2. *Ann Clin Transl Neurol* 2020;**7**:1013–28.

327. Wang W, Zhang X, Gao Q *et al.* TRPML1: an ion channel in the lysosome. *Handb Exp Pharmacol* 2014;**222**:631–45.

328. Guo J, Otis JM, Higginbotham H *et al.* Primary Cilia Signaling Shapes the Development of Interneuronal Connectivity. *Dev Cell* 2017;**42**:286-300.e4.

329. Schilter KF, Schneider A, Bardakjian T *et al.* OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype. *Clin Genet* 2011;**79**:158–68.

330. García-García G, Sanchez-Navarro I, Aller E *et al.* Exome sequencing identifies PEX6 mutations in three cases diagnosed with Retinitis Pigmentosa and hearing impairment. *Mol Vis* 2020;**26**:216–25.

331. Churchill JD, Bowne SJ, Sullivan LS *et al.* Mutations in the X-linked retinitis pigmentosa genes RPGR and RP2 found in 8.5% of families with a provisional diagnosis of autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2013;**54**:1411–6.

332. Khan SA, Muhammad N, Khan MA *et al.* Genetics of human Bardet-Biedl syndrome, an updates. *Clin Genet* 2016;**90**:3–15.

333. Posey JE, Harel T, Liu P *et al.* Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. *N Engl J Med* 2017;**376**:21–31.

334. Lal D, Neubauer BA, Toliat MR *et al.* Increased Probability of Co-Occurrence of Two Rare Diseases in Consanguineous Families and Resolution of a Complex Phenotype by Next Generation Sequencing. *PLoS One* 2016;**11**:e0146040.

335. He Y, Li D-Z. Co-occurrence of two rare genetic diseases: A potential pitfall for prenatal diagnosis in successive pregnancies. *Prenat Diagn* 2020;**40**:1606–9.

336. Tabolacci E, Pomponi MG, Remondini L *et al.* Co-Occurrence of Fragile X Syndrome with a Second Genetic Condition: Three Independent Cases of Double Diagnosis. *Genes (Basel)* 2021;**12**:1909.

337. Kurolap A, Orenstein N, Kedar I *et al.* Is one diagnosis the whole story? patients with double diagnoses. *Am J Med Genet A* 2016;**170**:2338–48.

338. Yang Y, Muzny DM, Xia F *et al.* Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA* 2014;**312**:1870–9.

339. Bin J, Madhavan J, Ferrini W *et al.* BBS7 and TTC8 (BBS8) mutations play a minor role in the mutational load of Bardet-Biedl syndrome in a multiethnic population. *Hum Mutat* 2009;**30**:E737-746.

340. Castro-Sánchez S, Suarez-Bregua P, Novas R *et al.* Functional analysis of new human Bardet-Biedl syndrome loci specific variants in the zebrafish model. *Sci Rep* 2019;**9**:12936.

341. Koulisis N, Nagiel A. Precision Therapy for Inherited Retinal Disease: At the Forefront of Genomic Medicine. *Clin Lab Med* 2020;**40**:189–204.

342. Ong T, Pennesi ME, Birch DG *et al.* Adeno-Associated Viral Gene Therapy for Inherited Retinal Disease. *Pharm Res* 2019;**36**:34.

343. Cehajic-Kapetanovic J, Xue K, Martinez-Fernandez de la Camara C *et al.* Initial results from a first-in-human gene therapy trial on X-linked retinitis pigmentosa caused by mutations in RPGR. *Nat Med* 2020;**26**:354–9.

344. Amato A, Arrigo A, Aragona E *et al.* Gene Therapy in Inherited Retinal Diseases: An Update on Current State of the Art. *Front Med (Lausanne)* 2021;**8**:750586.

345. Martinez Velazquez LA, Ballios BG. The Next Generation of Molecular and Cellular Therapeutics for Inherited Retinal Disease. *Int J Mol Sci* 2021;**22**:11542.

346. Britten-Jones AC, Jin R, Gocuk SA *et al.* The safety and efficacy of gene therapy treatment for monogenic retinal and optic nerve diseases: A systematic review. *Genet Med* 2021:S1098-3600(21)05365-X.

347. Botto C, Rucli M, Tekinsoy MD *et al.* Early and late stage gene therapy interventions for inherited retinal degenerations. *Prog Retin Eye Res* 2022;**86**:100975.

348. Deng C, Zhao PY, Branham K *et al.* Real-world outcomes of voretigene neparvovec treatment in pediatric patients with RPE65-associated Leber congenital amaurosis. *Graefes Arch Clin Exp Ophthalmol* 2022, DOI: 10.1007/s00417-021-05508-2.

349. Sorrentino FS, Gallenga CE, Bonifazzi C *et al.* A challenge to the striking genotypic heterogeneity of retinitis pigmentosa: a better understanding of the pathophysiology using the newest genetic strategies. *Eye (Lond)* 2016;**30**:1542–8.

350. Rong W, Chen X, Zhao K *et al.* Novel and recurrent MYO7A mutations in Usher syndrome type 1 and type 2. *PLoS One* 2014;**9**:e97808.

351. Parisi M, Glass I. Joubert Syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds.). *GeneReviews®*. Seattle (WA): University of Washington, Seattle, 1993.

352. Stokman M, Lilien M, Knoers N. Nephronophthisis. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds.). *GeneReviews®*. Seattle (WA): University of Washington, Seattle, 1993.

353. Mykytyn K, Nishimura DY, Searby CC *et al.* Identification of the gene (BBS1) most commonly involved in Bardet-Biedl syndrome, a complex human obesity syndrome. *Nat Genet* 2002;**31**:435–8.

354. Nishimura DY, Searby CC, Carmi R *et al.* Positional cloning of a novel gene on chromosome 16q causing Bardet-Biedl syndrome (BBS2). *Hum Mol Genet* 2001;**10**:865–74.

355. Chiang AP, Nishimura D, Searby C *et al.* Comparative genomic analysis identifies an ADP-ribosylation factor-like gene as the cause of Bardet-Biedl syndrome (BBS3). *Am J Hum Genet* 2004;**75**:475–84.

356. Mykytyn K, Braun T, Carmi R *et al.* Identification of the gene that, when mutated, causes the human obesity syndrome BBS4. *Nat Genet* 2001;**28**:188–91.

357. Li JB, Gerdes JM, Haycraft CJ *et al.* Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell* 2004;**117**:541–52.

358. Ansley SJ, Badano JL, Blacque OE *et al.* Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature* 2003;**425**:628–33.

359. Nishimura DY, Swiderski RE, Searby CC *et al.* Comparative genomics and gene expression analysis identifies BBS9, a new Bardet-Biedl syndrome gene. *Am J Hum Genet* 2005;**77**:1021–33.

360. Stoetzel C, Laurier V, Davis EE *et al.* BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. *Nat Genet* 2006;**38**:521–4.

361. Chiang AP, Beck JS, Yen H-J *et al.* Homozygosity mapping with SNP arrays identifies TRIM32, an E3 ubiquitin ligase, as a Bardet-Biedl syndrome gene (BBS11). *Proc Natl Acad Sci U S A* 2006;**103**:6287–92.

362. Leitch CC, Zaghloul NA, Davis EE *et al.* Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. *Nat Genet* 2008;**40**:443–8.

363. Otto EA, Hurd TW, Airik R *et al.* Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. *Nat Genet* 2010;**42**:840–50.

364. Marion V, Stutzmann F, Gérard M *et al.* Exome sequencing identifies mutations in LZTFL1, a BBSome and smoothened trafficking regulator, in a family with Bardet--Biedl syndrome with situs inversus and insertional polydactyly. *J Med Genet* 2012;**49**:317–21.

365. Schaefer E, Stoetzel C, Scheidecker S *et al.* Identification of a novel mutation confirms the implication of IFT172 (BBS20) in Bardet-Biedl syndrome. *J Hum Genet* 2016;**61**:447–50.

366. Lindstrand A, Davis EE, Carvalho CMB *et al.* Recurrent CNVs and SNVs at the NPHP1 locus contribute pathogenic alleles to Bardet-Biedl syndrome. *Am J Hum Genet* 2014;**94**:745–54.

367. Wormser O, Gradstein L, Yogev Y *et al.* SCAPER localizes to primary cilia and its mutation affects cilia length, causing Bardet-Biedl syndrome. *Eur J Hum Genet* 2019;**27**:928–40.

368. Edvardson S, Shaag A, Zenvirt S *et al.* Joubert syndrome 2 (JBTS2) in Ashkenazi Jews is associated with a TMEM216 mutation. *Am J Hum Genet* 2010;**86**:93–7.

369. Ferland RJ, Eyaid W, Collura RV *et al.* Abnormal cerebellar development and axonal decussation due to mutations in AHI1 in Joubert syndrome. *Nat Genet* 2004;**36**:1008–13.

370. Macferran KM, Buchmann RF, Ramakrishnaiah R *et al.* Pontine tegmental cap dysplasia with a 2q13 microdeletion involving the NPHP1 gene: insights into malformations of the mid-hindbrain. *Semin Pediatr Neurol* 2010;**17**:69–74.

371. Valente EM, Silhavy JL, Brancati F *et al.* Mutations in CEP290, which encodes a centrosomal protein, cause pleiotropic forms of Joubert syndrome. *Nat Genet* 2006;**38**:623–5.

372. Baala L, Romano S, Khaddour R *et al.* The Meckel-Gruber syndrome gene, MKS3, is mutated in Joubert syndrome. *Am J Hum Genet* 2007;**80**:186–94.

373. Delous M, Baala L, Salomon R *et al.* The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet* 2007;**39**:875–81.

374. Arts HH, Doherty D, van Beersum SEC *et al.* Mutations in the gene encoding the basal body protein RPGRIP1L, a nephrocystin-4 interactor, cause Joubert syndrome. *Nat Genet* 2007;**39**:882–8.

375. Cantagrel V, Silhavy JL, Bielas SL *et al.* Mutations in the cilia gene ARL13B lead to the classical form of Joubert syndrome. *Am J Hum Genet* 2008;**83**:170–9.

376. Gorden NT, Arts HH, Parisi MA *et al.* CC2D2A is mutated in Joubert syndrome and interacts with the ciliopathy-associated basal body protein CEP290. *Am J Hum Genet* 2008;**83**:559–71.

377. Coene KLM, Roepman R, Doherty D *et al.* OFD1 is mutated in X-linked Joubert syndrome and interacts with LCA5-encoded lebercilin. *Am J Hum Genet* 2009;**85**:465–81.

378. Davis EE, Zhang Q, Liu Q *et al.* TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat Genet* 2011;**43**:189–96.

379. Dafinger C, Liebau MC, Elsayed SM *et al.* Mutations in KIF7 link Joubert syndrome with Sonic Hedgehog signaling and microtubule dynamics. *J Clin Invest* 2011;**121**:2662–7.

380. Garcia-Gonzalo FR, Corbit KC, Sirerol-Piquer MS *et al.* A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nat Genet* 2011;**43**:776–84.

381. Huang L, Szymanska K, Jensen VL *et al.* TMEM237 is mutated in individuals with a Joubert syndrome related disorder and expands the role of the TMEM family at the ciliary transition zone. *Am J Hum Genet* 2011;**89**:713–30.

382. Lee JE, Silhavy JL, Zaki MS *et al.* CEP41 is mutated in Joubert syndrome and is required for tubulin glutamylation at the cilium. *Nat Genet* 2012;**44**:193–9.

383. Srour M, Schwartzentruber J, Hamdan FF *et al.* Mutations in C5ORF42 cause Joubert syndrome in the French Canadian population. *Am J Hum Genet* 2012;**90**:693–700.

384. Ben-Salem S, Al-Shamsi AM, Gleeson JG *et al.* Mutation spectrum of Joubert syndrome and related disorders among Arabs. *Hum Genome Var* 2014;**1**:14020.

385. Chaki M, Airik R, Ghosh AK *et al.* Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. *Cell* 2012;**150**:533–48.

386. Akizu N, Silhavy JL, Rosti RO *et al.* Mutations in CSPP1 lead to classical Joubert syndrome. *Am J Hum Genet* 2014;**94**:80–6.

387. Thomas S, Wright KJ, Le Corre S *et al.* A homozygous PDE6D mutation in Joubert syndrome impairs targeting of farnesylated INPP5E protein to the primary cilium. *Hum Mutat* 2014;**35**:137–46.

388. Sang L, Miller JJ, Corbit KC *et al.* Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. *Cell* 2011;**145**:513–28.

389. Srour M, Hamdan FF, McKnight D *et al.* Joubert Syndrome in French Canadians and Identification of Mutations in CEP104. *Am J Hum Genet* 2015;**97**:744–53.

390. Sanders AAWM, de Vrieze E, Alazami AM *et al.* KIAA0556 is a novel ciliary basal body component mutated in Joubert syndrome. *Genome Biol* 2015;**16**:293.

391. Romani M, Micalizzi A, Kraoua I *et al.* Mutations in B9D1 and MKS1 cause mild Joubert syndrome: expanding the genetic overlap with the lethal ciliopathy Meckel syndrome. *Orphanet J Rare Dis* 2014;**9**:72.

392. Lambacher NJ, Bruel A-L, van Dam TJP *et al.* TMEM107 recruits ciliopathy proteins to subdomains of the ciliary transition zone and causes Joubert syndrome. *Nat Cell Biol* 2016;**18**:122–31.

393. Van De Weghe JC, Rusterholz TDS, Latour B *et al.* Mutations in ARMC9, which Encodes a Basal Body Protein, Cause Joubert Syndrome in Humans and Ciliopathy Phenotypes in Zebrafish. *Am J Hum Genet* 2017;**101**:23–36.

394. Roosing S, Romani M, Isrie M *et al.* Mutations in CEP120 cause Joubert syndrome as well as complex ciliopathy phenotypes. *J Med Genet* 2016;**53**:608–15.

395. De Mori R, Romani M, D'Arrigo S *et al.* Hypomorphic Recessive Variants in SUFU Impair the Sonic Hedgehog Pathway and Cause Joubert Syndrome with Cranio-facial and Skeletal Defects. *Am J Hum Genet* 2017;**101**:552–63.

396. Wheway G, Schmidts M, Mans DA *et al.* An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes. *Nat Cell Biol* 2015;**17**:1074–87.

397. Alkanderi S, Molinari E, Shaheen R *et al.* ARL3 Mutations Cause Joubert Syndrome by Disrupting Ciliary Protein Composition. *Am J Hum Genet* 2018;**103**:612–20.

398. Shaheen R, Jiang N, Alzahrani F *et al.* Bi-allelic Mutations in FAM149B1 Cause Abnormal Primary Cilium and a Range of Ciliopathy Phenotypes in Humans. *Am J Hum Genet* 2019;**104**:731–7.

399. Latour BL, Van De Weghe JC, Rusterholz TD *et al.* Dysfunction of the ciliary ARMC9/TOGARAM1 protein module causes Joubert syndrome. *J Clin Invest* 2020;**130**:4423–39.

400. Stephen J, Vilboux T, Mian L *et al.* Mutations in KIAA0753 cause Joubert syndrome associated with growth hormone deficiency. *Hum Genet* 2017;**136**:399–408.

401. Van De Weghe JC, Giordano JL, Mathijssen IB *et al.* TMEM218 dysfunction causes ciliopathies, including Joubert and Meckel syndromes. *HGG Adv* 2021;**2**:100016.

402. Luo M, Lin Z, Zhu T *et al.* Disrupted intraflagellar transport due to IFT74 variants causes Joubert syndrome. *Genet Med* 2021;**23**:1041–9.

403. Halbritter J, Porath JD, Diaz KA *et al.* Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy. *Hum Genet* 2013;**132**:865–84.

404. O'Toole JF, Otto EA, Frishberg Y *et al.* Retinitis pigmentosa and renal failure in a patient with mutations in INVS. *Nephrol Dial Transplant* 2006;**21**:1989–91.

405. Halbritter J, Diaz K, Chaki M *et al.* High-throughput mutation analysis in patients with a nephronophthisis-associated ciliopathy applying multiplexed barcoded array-based PCR amplification and next-generation sequencing. *J Med Genet* 2012;**49**:756–67.

406. Otto EA, Ramaswami G, Janssen S *et al.* Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy. *J Med Genet* 2011;**48**:105–16.

407. Coussa RG, Otto EA, Gee H-Y *et al.* WDR19: an ancient, retrograde, intraflagellar ciliary protein is mutated in autosomal recessive retinitis pigmentosa and in Senior-Loken syndrome. *Clin Genet* 2013;**84**:150–9.

408. Bizet AA, Becker-Heck A, Ryan R *et al.* Mutations in TRAF3IP1/IFT54 reveal a new role for IFT proteins in microtubule stabilization. *Nat Commun* 2015;**6**:8666.

409. Xu M, Eblimit A, Wang J *et al.* ADIPOR1 Is Mutated in Syndromic Retinitis Pigmentosa. *Hum Mutat* 2016;**37**:246–9.

410. Boczek NJ, Hopp K, Benoit L *et al.* Characterization of three ciliopathy pedigrees expands the phenotype associated with biallelic C2CD3 variants. *Eur J Hum Genet* 2018;**26**:1797–809.

411. Badano JL, Leitch CC, Ansley SJ *et al.* Dissection of epistasis in oligogenic Bardet-Biedl syndrome. *Nature* 2006;**439**:326–30.

412. Vilboux T, Malicdan MCV, Roney JC *et al.* CELSR2, encoding a planar cell polarity protein, is a putative gene in Joubert syndrome with cortical heterotopia, microophthalmia, and growth hormone deficiency. *Am J Med Genet A* 2017;**173**:661–6.

413. Maria M, Lamers IJC, Schmidts M *et al.* Genetic and clinical characterization of Pakistani families with Bardet-Biedl syndrome extends the genetic and phenotypic spectrum. *Sci Rep* 2016;**6**:34764.

414. Yıldız Bölükbaşı E, Mumtaz S, Afzal M *et al.* Homozygous mutation in CEP19, a gene mutated in morbid obesity, in Bardet-Biedl syndrome with predominant postaxial polydactyly. *J Med Genet* 2018;**55**:189–97.

415. Dixon-Salazar TJ, Silhavy JL, Udpa N *et al.* Exome sequencing can improve diagnosis and alter patient management. *Sci Transl Med* 2012;**4**:138ra78.

416. Pal LR, Kundu K, Yin Y *et al.* CAGI4 SickKids clinical genomes challenge: A pipeline for identifying pathogenic variants. *Hum Mutat* 2017;**38**:1169–81.

417. Oka M, Shimojima K, Yamamoto T *et al.* A novel HYLS1 homozygous mutation in living siblings with Joubert syndrome. *Clin Genet* 2016;**89**:739–43.

418. Bachmann-Gagescu R, Phelps IG, Dempsey JC *et al.* KIAA0586 is Mutated in Joubert Syndrome. *Hum Mutat* 2015;**36**:831–5.

419. Moran J, G Sanderson K, Maynes J *et al.* IFT80 mutations cause a novel complex ciliopathy phenotype with retinal degeneration. *Clin Genet* 2018;**94**:368–72.

420. Khan S, Lin S, Harlalka GV *et al.* BBS5 and INPP5E mutations associated with ciliopathy disorders in families from Pakistan. *Ann Hum Genet* 2019;**83**:477–82.

421. Putoux A, Thomas S, Coene KLM *et al.* KIF7 mutations cause fetal hydrolethalus and acrocallosal syndromes. *Nat Genet* 2011;**43**:601–6.

422. Shamseldin HE, Shaheen R, Ewida N *et al.* The morbid genome of ciliopathies: an update. *Genet Med* 2020;**22**:1051–60.

423. Shaheen R, Szymanska K, Basu B *et al.* Characterizing the morbid genome of ciliopathies. *Genome Biol* 2016;**17**:242.

424. Beck BB, Phillips JB, Bartram MP *et al.* Mutation of POC1B in a severe syndromic retinal ciliopathy. *Hum Mutat* 2014;**35**:1153–62.

425. Morisada N, Hamada R, Miura K *et al.* Bardet-Biedl syndrome in two unrelated patients with identical compound heterozygous SCLT1 mutations. *CEN Case Rep* 2020;**9**:260–5.

426. Katagiri S, Hayashi T, Yoshitake K *et al.* Compound heterozygous splice site variants in the SCLT1 gene highlight an additional candidate locus for Senior-Løken syndrome. *Sci Rep* 2018;**8**:16733.

427. Leitch CC, Zaghloul NA, Davis EE *et al.* Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. *Nat Genet* 2008;**40**:443–8.

428. Halbritter J, Diaz K, Chaki M *et al.* High-throughput mutation analysis in patients with a nephronophthisis-associated ciliopathy applying multiplexed barcoded array-based PCR amplification and next-generation sequencing. *J Med Genet* 2012;**49**:756–67.

429. Davis EE, Zhang Q, Liu Q *et al.* TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat Genet* 2011;**43**:189–96.

ANEXOS

Anexo I. Genes relacionados con los síndromes de Bardet-Biedl (BBS), Joubert (JBTS) y Senior-Løken (SLSN).

La información relacionada con el rol de cada proteína en el cilio o su localización ha sido tomada de Uniprot (https://www.uniprot.org/; último acceso marzo de 2022).

Gen (Locus)	Número MIM	Locus	Casos BBS, JBTS o SLSN atribuidos al gen [86,351,352]	Asociado a otras ciliopatías	Rol en el cilio	Localización
Síndrome de Bardet-Biedl						
BBS1 **[353] (BBS1)**	*209901	11q13.2	23,4%	RP	Parte del BBSoma	Axonema, centrosoma, cilio, cuerpo basal, membrana ciliar
BBS2 **[354] (BBS2)**	*606151	16q13	9,6%	MKS, RP	Parte del BBSoma (core)	Centrosoma, cilio, cuerpo basal, núcleo
ARL6 **[355] (BBS3)**	*608845	3q11.2	5,1%	RP	GTPasa. Montaje y tráfico del BBSoma	Axonema, cilio, citoplasma, cuerpo basal
BBS4 **[356] (BBS4)**	*600374	15q24.1	5,3%	MKS	Parte del BBSoma. Recluta el BBSoma al cilio	Centrosoma, cilio, cuerpo basal, zona de transición
BBS5 **[357] (BBS5)**	*603650	2q31.1	3,7%	-	Parte del BBSoma	Axonema, cuerpo basal, citosol, membrana ciliar
MKKS **[83,84] (BBS6)**	*604896	20p12.2	6,3%	MKKS, MKS	Complejo chaperonina. Ensamblaje del BBSoma	Centrosoma, citosol, cuerpo basal, núcleo
BBS7 **[100] (BBS7)**	*607590	4q27	4,2%	-	Parte del BBSoma (core)	Axonema, centrosoma, cuerpo basal, membrana ciliar, núcleo
TTC8 **[358] (BBS8)**	*608132	14q31.3	2%	RP	Parte del BBSoma	Centrosoma, cilio, citosol, cuerpo ciliar, membrana ciliar
BBS9 **[359] (BBS9)**	*607968	7p14.3	3,4%	-	Parte del BBSoma (core)	Cilio, citosol, membrana ciliar, zona de transición
BBS10 **[360] (BBS10)**	*610148	12q21.2	14,5%	-	Complejo chaperonina. Ensamblaje del BBSoma	Centrosoma, cuerpo basal
TRIM32 **[361] (BBS11)**	*602290	9q33.1	<1%	-	Ubiquitinación BBS2	Citoplasma, núcleo
BBS12 **[360] (BBS12)**	*610683	4q27	6,4%	-	Complejo chaperonina. Ensamblaje del BBSoma	Centrosoma, citoplasma, cuerpo basal
MKS1 **[362] (BBS13)**	*609883	17q22	1%	JBTS, MKS	Parte del complejo MKS. Organización de la zona de transición	Centrosoma, citosol, cuerpo basal, zona de transición

Gen (Locus)	Número MIM	Locus	Casos BBS, JBTS o SLSN atribuidos al gen [86,351,352]	Asociado a otras ciliopatías	Rol en el cilio	Localización
CEP290 **[362] (BBS14)**	*610142	12q21.32	6,3%	JBTS, LCA, MKS, SLSN	Parte del complejo MKS. Organización de la zona de transición. Ensamblaje del BBSoma	Centrosoma, citosol, cuerpo basal, núcleo, zona de transición
WDPCP **[89] (BBS15)**	*613580	2p15	<1%	-	Ciliogénesis, reclutamiento de proteínas	Axonema, citoesqueleto, cuerpo basal, zona de transición
SDCCAG8 **[363] (BBS16)**	*613524	1q43-q44	4,3%	NPHP, SLSN	Ciliogénesis, localización centrosoma	Centrosoma, citosol, cuerpo basal, zona de transición
LZTFL1 **[364] (BBS17)**	*606568	3p21.31	<1%	-	Tráfico de los elementos del BBSoma	Citoplasma
BBIP1 **[87] (BBS18)**	*613605	10q25.2	<1%	-	Parte del BBSoma. Ensamblaje de BBSoma y cilio primario	Cilio, citosol
IFT27 **[88] (BBS19)**	*615870	22q12.3	<1%	-	Parte del complejo IFTB. Transporte anterógrado del BBSoma y proteínas	Centrosoma, cilio, citoplasma, punta ciliar
IFT172 **[365] (BBS20)**	*607386	2p23.3	1%	RP, SRTD	Parte del complejo IFTB. Mantenimiento y formación del cilio	Axonema, cilio, cuerpo basal, punta ciliar
C8orf37 **[318] (BBS21)**	*614477	8q22.1	1,6%	DCB, RP	Función del cilio primario, Morfogénesis fotorreceptores	Base ciliar, citoplasma, membrana plasmática
IFT74 **[92] (BBS22)**	*608040	9p21.2	<1%	JBTS	Parte del complejo IFTB. Ciliogénesis	Centrosoma, cilio, núcleo, punta ciliar
NPHP1 **[366] (-)**	*607100	2q13	Desconocido	JBTS, NPHP, SLSN	Regulación transporte intraflagellar durante el ensamblaje ciliar	Axonema, cilio, uniones célula-célula, zona de transición
SCAPER **[367] (-)**	*611611	15q24.3	Desconocido	-	Desconocido	Cilio, núcleo
Síndrome de Joubert						
INPP5E **[117,118] (JBTS1)**	*613037	9q34.3	2%-4%	BBS, MORM	Fosfatasa. Control del crecimiento ciliar	Aparato de Golgi, axonema, cilio, citosol, membrana plasmática, núcleo

Gen (Locus)	Número MIM	Locus	Casos BBS, JBTS o SLSN atribuidos al gen [86,351,352]	Asociado a otras ciliopatías	Rol en el cilio	Localización
TMEM216 **[368] (JBTS2)**	*613277	11q12.2	2%-3%	MKS	Parte del complejo MKS. Ciliogénesis específica de tejido. Regula composición membrana ciliar	Cilio, citosol, cuerpo basal, zona de transición
AHI1 **[369] (JBTS3)**	*608894	6q23.3	7%-10%	RP	Parte del complejo MKS. Tráfico de vesículas, ciliogénesis, recluta RAB8A en el cuerpo basal	Centrosoma, cilio, citosol, cuerpo basal
NPHP1 **[370] (JBTS4)**	*607100	2q13	1%-2%	BBS, NPHP, SLSN	Regulación transporte intraflagelar durante el ensamblaje ciliar	Axonema, cilio, uniones célula-célula, zona de transición
CEP290 **[371] (JBTS5)**	*610142	12q21.32	7%-10%	BBS, LCA, MKS, SLSN	Parte del complejo MKS. Organización de la zona de transición. Ensamblaje del BBSoma	Centrosoma, citosol, cuerpo basal, núcleo, zona de transición
TMEM67 **[372] (JBTS6)**	*609884	8q22.1	6%-20%	BBS, COACH, MKS, NPHP, RHYNS	Parte del complejo MKS. Ciliogénesis temprana y específica de tejido. Regula composición membrana ciliar.	Centrosoma, cuerpo basal, membrana ciliar, retículo endoplasmático, zona de transición
RPGRIP1L **[373,374] (JBTS7)**	*610937	16q12.2	1%-4%	COACH, MKS	Regula la actividad proteasómica en el cilio primario	Axonema, centrosoma, cilio, cuerpo basal, zona de transición
ARL13B **[375] (JBTS8)**	*612291	3q11.1-q11.2	<1%	-	Controla la estructura del axonema ciliar. Mantiene la asociación entre los complejos IFTA e IFTB	Axonema, cilio, citosol, membrana ciliar
CC2D2A **[376] (JBTS9)**	*612013	4p15.32	8%-11%	COACH, MKS	Parte del complejo MKS. Ciliogénesis y señalización Shh	Citosol, cuerpo basal, zona de transición
OFD1 **[377] (JBTS10)**	*300170	Xp22.2	<1%	OFD, RP, SGBS	Biogénesis del cilio. Regula señalización Wnt. Regulación ciliogénesis	Centrosoma, citosol, cilio, cuerpo basal, núcleo
TTC21B **[378] (JBTS11)**	*612014	2q24.3	Desconocido	BBS, NPHP, SRTD	Parte del complejo IFTA. Modula	Axonema, cilio, citoesqueleto,

Gen (Locus)	Número MIM	Locus	Casos BBS, JBTS o SLSN atribuidos al gen [86,351,352]	Asociado a otras ciliopatías	Rol en el cilio	Localización
					negativamente la transducción de la señal Shh	citoplasma, punta ciliar
KIF7 **[379] (JBTS12)**	*611254	15q26.1	<1%	ACLS, BBS	Complejo kinesina. Regula la señalización Shh. Regula la dinámica microtubular	Cilio, citoplasma, cuerpo basal, punta ciliar
TCTN1 **[380] (JBTS13)**	*609863	12q24.11	<1%	-	Parte del complejo MKS. Regulador de la señal Shh	Citosol, cuerpo basal, zona de transición
TMEM237 **[381] (JBTS14)**	*614423	2q33.1	<1%	-	Ciliogénesis	Cilio, zona de transición
CEP41 **[382] (JBTS15)**	*610523	7q32.2	<1%	BBS	Ciliogénesis	Centriolo, centrosoma, cilio, citosol, cuerpo basal, membrana
TMEM138 **[382] (JBTS16)**	*614459	11q12.2	<1%	-	Ciliogénesis	Cilio, membrana
CPLANE1 **[383] (JBTS17)**	*614571	5p13.2	8%-14%	OFD	Ciliogénesis. Reclutamiento proteínas IFTA periféricas a los cuerpos basales	Cilio, membrana, zona de transición
TCTN3 **[384] (JBTS18)**	*614815	10q24.1	<1%	OFD	Parte del complejo MKS. Ciliogénesis específica de tejido. Regula composición membrana ciliar.	Cilio, membrana ciliar, núcleo
ZNF423 **[385] (JBTS19)**	*604557	16q12.1	<1%	NPHP	Factor de transcripción, actúa como activador o represor, según contexto	Núcleo
TMEM231 **[383] (JBTS20)**	*614949	16q23.1	<1%	-	Parte del complejo MKS. Ciliogénesis y señalización Shh	Membrana ciliar, zona de transición
CSPP1 **[386] (JBTS21)**	*611654	8q13.1-q13.2	2%-4%	-	Papel en la organización de microtúbulos dependiente del ciclo celular	Centrosoma, citoplasma, cuerpo basal
PDE6D **[387] (JBTS22)**	*602676	2q37.1	<1%	-	Modula direccionamiento ciliar de proteínas diana. Aumenta la afinidad de ARL3 por GTP	Cilio, citosol, cuerpo basal

Gen (Locus)	Número MIM	Locus	Casos BBS, JBTS o SLSN atribuidos al gen [86,351,352]	Asociado a otras ciliopatías	Rol en el cilio	Localización
KIAA0586 **[127] (JBTS23)**	*610178	14q23.1	2%-7%	SRTD	Ciliogénesis y señalización Shh. Posible papel en ciliogénesis temprana	Centrosoma, cuerpo basal
TCTN2 **[388] (JBTS24)**	*613846	12q24.31	1%	MKS	Parte del complejo MKS. Ciliogénesis y señalización Shh	Citoplasma, cuerpo basal, membrana ciliar
CEP104 **[389] (JBTS25)**	*616690	1p36.32	<1%	-	Ciliogénesis e integridad estructural en la punta ciliar	Centriolo, cilio, citoplasma, huso mitótico
KATNIP **[390] (JBTS26)**	*616650	16p12.1	<1%	-	Estabilidad de los microtúbulos	Axonema, citoplasma, cuerpo basal
B9D1 **[391] (JBTS27)**	*614144	17p11.2	<1%	MKS	Parte del complejo MKS. Ciliogénesis y señalización Shh	Axonema, centrosoma, cuerpo basal, citosol, zona de transición
MKS1 **[391] (JBTS28)**	*609883	17q22	2%-6%	BBS, MKS	Parte del complejo MKS. Ciliogénesis temprana. Papel en la regulación de longitud/número ciliar	Centriolo, centrosoma, citosol, cuerpo basal
TMEM107 **[392] (JBTS29)**	*616183	17p13.1	<1%	MKS, OFD	Ciliogénesis, localización de las proteínas del complejo MKS en la zona de transición del cilio. Señalización Shh	Cilio, membrana, zona de transición
ARMC9 **[393] (JBTS30)**	*617612	2q37.1	<1%	-	Ciliogénesis. Regulador positivo señalización Shh	Centriolo, citoplasma, cuerpo basal, punta ciliar
CEP120 **[394] (JBTS31)**	*613446	5q23.2	<1%	SRTD	Duplicación y maduración del centriolo en la mitosis y ciliogénesis posterior	Centriolo, centrosoma, citoplasma
SUFU **[395] (JBTS32)**	*607035	10q24.32	<1%	-	Regulador de la vía de señalización Shh	Cilio, citoplasma, núcleo
PIBF1 **[396] (JBTS33)**	*607532	13q21.33-q22.1	<1%	-	Ciliogénesis	Centrosoma, citoplasma, núcleo
B9D2 **[127] (JBTS34)**	*611951	19q13.2	<1%	MKS	Parte del complejo MKS	Axonema, cuerpo basal, núcleo
ARL3 **[397] (JBTS35)**	*604695	10q24.32	<1%	RP	Proteína de unión a GTP.	Centrosoma, cilio, citoplasma,

Gen (Locus)	Número MIM	Locus	Casos BBS, JBTS o SLSN atribuidos al gen [86,351,352]	Asociado a otras ciliopatías	Rol en el cilio	Localización
					Citocinesis y señalización ciliar. Dirige proteínas al cilio	microtúbulos, núcleo
***FAM149B1* [398] (JBTS36)**	*618413	10q22.2	<1%	-	Localización de proteínas en el cilio y ensamblaje del cilio. Regulación indirecta de funciones ciliares	Cilio
***TOGARAM1* [399] (JBTS37)**	*617618	14q21.2	<1%	-	Organización haces de microtúbulos del axonema	Cilio, cuerpo basal, microtúbulos
***KIAA0753* [400] (JBTS38)**	*617112	17p13.1	<1%	OFD, SRTD	Duplicación del centriolo	Centriolo, centrosoma, citosol
***TMEM218* [401] (JBTS39)**	*619285	11q24.2	<1%	-	Biogénesis y función ciliar	Cilio, membrana
***IFT74* [402] (JBTS40)**	*608040	9p21.2	<1%	BBS	Parte del complejo IFTB. Ciliogénesis. Media transporte de tubulina en el cilio	Centrosoma, cilio, núcleo
Síndrome de Senior-Løken						
***NPHP1* [403] (SLSN1)**	*607100	2q13	Desconocido	BBS, JBTS, NPHP	Regulación transporte intraflagellar durante el ensamblaje ciliar	Axonema, cilio, uniones célula-célula, zona de transición
***INVS* [404] (SLSN2)**	*243305	9q31.1	Desconocido	NPHP	Regulador en la vía de señalización Wnt	Cilio, citoplasma, membrana, microtúbulo, núcleo
***NPHP3* [403] (SLSN3)**	*608002	3q22.1	Desconocido	MKS, NPHP	Regulador en la vía de señalización Wnt	Cilio, citosol
***NPHP4* [405] (SLSN4)**	*607215	1p36.31	Desconocido	BBS, NPHP	Construcción de cilios funcionales	Centrosoma, citosol, cuerpo basal, núcleo, zona de transición
***IQCB1* [129] (SLSN5)**	*609237	3q13.33	17%-18%	-	Ciliogénesis, al asociarse con CEP290. Regula integridad del BBSoma	Centriolo, centrosoma, cilio, microtúbulos, núcleo
***CEP290* [406] (SLSN6)**	*610189	12q21.32	Desconocido	BBS, JBTS, LCA, MKS	Parte del complejo MKS. Organización de la zona de transición. Ensamblaje del BBSoma	Centrosoma, citosol, cuerpo basal, núcleo, zona de transición

Gen (Locus)	Número MIM	Locus	Casos BBS, JBTS o SLSN atribuidos al gen [86,351,352]	Asociado a otras ciliopatías	Rol en el cilio	Localización
SDCCAG8 **[363] (SLSN7)**	*613524	1q43-q44	3%-4%	BBS	Ciliogénesis, localización centrosoma	Centrosoma, citosol, cuerpo basal, zona de transición
WDR19 **[407] (SLSN8)**	*616307	4p14	Desconocido	NPHP, SRTD	Parte del complejo IFTA. Se asocia al BBSoma para mediar transporte ciliar	Cilio, citoplasma, cuerpo basal
TRAF3IP1 **[408] (SLSN9)**	*616629	2q37.3	Desconocido	-	Parte del complejo IFTB. Regula organización del citoesqueleto de microtúbulos y su estabilidad. Ciliogénesis	Axonema, centrosoma, cilio, cuerpo basal, zona de transición

ACLS, síndrome acrocalloso; BBS, síndrome de Bardet-Biedl; COACH, síndrome de COACH (en inglés, *Cerebellar vermis hypo/aplasia, Oligophrenia, Congenital ataxia, ocular Coloboma, and Hepatic fibrosis*); DCB, distrofia de conos-bastones; GTP, guanosín trifosfato; GTPasa, guanosina trifosfatasa; IFTA, complejo A del transporte intraflagelar; IFTB, complejo B del transporte intraflagelar; JBTS, síndrome de Joubert; LCA, amaurosis congénita de Leber; MKKS, síndrome de McKusick-Kaufman; MKS, síndrome de Meckel-Gruber; MORM, síndrome MORM (*Mental retardation, truncal Obesity, Retinal dystrophy, and Micropenis*); NPHP, nefronoptisis; OFD, síndrome oro-facio-digital; RP, retinosis pigmentaria; RHYNS, síndrome de RHYNS (*Retinitis pigmentosa, Hypopituitarism, Nephronopthisis, and mild Skeletal dysplasia*); SLSN, síndrome de Senior-Løken; SRTD, displasia torácica de costillas cortas con o sin polidactilia.

Anexo II. Genes candidatos causales y/o modificadores para los síndromes de Bardet-Biedl (BBS), Joubert (JBTS) y Senior-Løken (SLSN).

La información relacionada con el rol de cada proteína en el cilio o su localización ha sido tomada de Uniprot (https://www.uniprot.org/; último acceso marzo de 2022).

Gen	Número MIM	Locus	Ciliopatía candidata	Rol en el cilio	Localización
ADIPOR1	*607945	1q32.1	BBS [409]	Desconocido	Membrana plasmática
C2CD3	*615944	11q13.4	JBTS [410]	Regula elongación del centriolo. Formación del cilio primario. Señalización Shh	Centriolo, centrosoma, citosol, cuerpo basal
CCDC28B	*610162	1p35.2	BBS [411]	Ciliogénesis	Centrosoma, citoplasma
CELSR2	*604265	1p13.3	JBTS [412]	Ciliogénesis	Membrana plasmática
CEP164	*614848	11q23.3	BBS [413]	Organización/mantenimiento microtúbulos para la formación del cilio primario	Centriolo, centrosoma, citosol, fibra de transición ciliar, núcleo
CEP19	*615586	3q29	BBS [414]	Ciliación. Participa en la formación del cilio reclutando vesículas ciliares. Anclaje de microtúbulos a los centrosomas	Centriolo, centrosoma, cilio, citoplasma, cuerpo basal
CEP41	*610523	7q32.2	BBS [220]	Ciliogénesis	Centriolo, centrosoma, cilio, citosol, cuerpo basal, membrana
CORO2B	*605002	15q23	BBS [219]	Desconocido	Citoesqueleto de actina, citoplasma
EXOC8	*615283	1q42.2	JBTS [415]	Desconocido	Citosol, membrana plasmática
HYDIN	*610812	16q22.2	JBTS [416]	Motilidad ciliar	Axonema, cilio
HYLS1	*610693	11q24.2	JBTS [417]	Ciliogénesis	Centriolo, centrosoma, cilio, citosol, membrana plasmática, núcleo
IFT172	*607386	2p23.3	JBTS [418]	Parte del complejo IFTB. Mantenimiento y formación del cilio	Axonema, cilio, cuerpo basal, punta ciliar
IFT80	*611177	3q25.33	JBTS [419]	Parte del complejo IFTB. Desarrollo y mantenimiento del cilio	Axonema, centrosoma, cilio, citoplasma, cuerpo basal, punta ciliar
INPP5E	*613037	9q34.3	BBS [420]	Fosfatasa. Control del crecimiento ciliar	Aparato de Golgi, axonema, cilio, citosol, membrana plasmática, núcleo
KIF7	*611254	15q26.1	BBS [421]	Complejo kinesina. Regula la señalización Shh. Regula la dinámica microtubular	Cilio, citoplasma, cuerpo basal, punta ciliar
LRRC34	*619037	3q26.2	JBTS [422]	Desconocido	Citoplasma, núcleo

Gen	Número MIM	Locus	Ciliopatía candidata	Rol en el cilio	Localización
LRRCC1	*617791	8q21.2	JBTS [423]	Desconocido	Centriolo, centrosoma, citoplasma
NPHP4	*607215	1p36.31	BBS [243,244]	Construcción de cilios funcionales	Centrosoma, citosol, cuerpo basal, núcleo, zona de transición
POC1B	*614784	12q21.33	JBTS [424]	Ensamblaje y estabilidad del centriolo. Ciliogénesis. Longitud del cilio	Centrosoma, centriolo, citoplasma, cuerpo basal
SCLT1	*611399	4q28.2	BBS [425], SLSN [426]	Anclaje del cilio a la membrana plasmática	Centriolo, centrosoma, cilio, fibra de transición ciliar
TMEM17	*614950	2p15	JBTS [422]	Componente transmembrana del complejo MKS. Ciliogénesis y señalización Shh	Membrana ciliar, zona de transición
TMEM67	*609884	8q22.1	BBS [427], SLSN [428]	Parte del complejo MKS. Ciliogénesis temprana y específica de tejido. Regula composición membrana ciliar.	Centrosoma, cuerpo basal, membrana ciliar, retículo endoplasmático, zona de transición
TTC21B	*612014	2q24.3	BBS [429]	Parte del complejo IFTA. Modula negativamente la transducción de la señal Shh	Axonema, cilio, citoesqueleto, citoplasma, punta ciliar

BBS, síndrome de Bardet-Biedl; IFTA, complejo A del transporte intraflagelar; IFTB, complejo B del transporte intraflagelar; JBTS, síndrome de Joubert; SLSN, síndrome de Senior-Løken.

Anexo III. Casos ERS no Usher caracterizados molecularmente en nuestro laboratorio desde octubre de 2020.

Desde octubre de 2020 hasta enero de 2022, se caracterizaron 11 nuevos casos con ERS no Usher totalmente y 3 parcialmente.

ID	Clasificación fenotípica *a priori*	Clasificación fenotípica final	Status en el artículo 2	Gen	Alelo 1	Alelo 2	Modo de herencia (cigosidad)	Técnica molecular
Caracterizados totalmente								
RP-1722	*"ESPECÍFICO"* (COH-*like*)	*"DR+DI"*	Negativa	*MORC2*	NM_001303256.3: c.79G>A; p.(Glu27Lys)	wt	AD *de novo* (het)	WGS
RP-2172	*"NO-SIND"*	*"DR+DI"* (LCA+MC+DA+ linfedema)	No aparece (reclasif. clínica)	*KIF11*	NM_004523.3: c.2548-2A>G; p.(?)	wt	AD, herencia materna (het)	CES – reanálisis (nuevos datos clínicos)
RP-2394	*"CILIOPATÍA"* (JBTS-*like*)	*"DR+DI"* (DR+DI+MC+ Malf SNC)	VUS monoalélica	*RERE*	NM_001042681.2: c.3959G>A; p.(Arg1320Gln)	wt	AD, no segregada (het)	WES
RP-2499	*"DR+AE"* (DR+AE+AN)	*"DR+AE"* (DR+AE+AN)	Negativa	*RERE*	NM_001042681.2: c.4313_4318del; p.(Leu1438_His1439del)	wt	AD, no segregada (het)	CES – nueva versión
RP-3103	*"DR+PA"*	*"DR + PA"*	No aparece (nuevo caso)	*PRPS1*	NM_002764: c.46T>C; p.(Ser16Pro)	wt	XL (hemi)	CES
RP-3187	*"DR+OTROS"*	*"DR+OTROS"* (DR+ataxia)	No aparece (nuevo caso)	*SCA7*	Expansión de 39 repeticiones	wt	AD	Secuenciación directa
RP-3193	*"ESPECÍFICO"* (Síndrome de HARP)	*"ESPECÍFICO"* (Síndrome de HARP)	No aparece (nuevo caso)	*PANK2*	NM_153638.3: c.629T>C; p.(Leu210Pro)	NM_153638.3: c.629T>C; p.(Leu210Pro)	AR (homo)	CES
RP-3428	*"DR+PA"*	*"ESPECÍFICO"* (WFS)	No aparece (nuevo caso)	*WFS1*	NM_006005.3: c.2051C>T; p.(Ala684Val)	wt	AD (het)	CES
RP-3500	*"CILIOPATÍA"* (JBTS)	*"CILIOPATÍA"* (JBTS)	No aparece (nuevo caso)	*AHI1*	NM_017651.4: c.910dup; p.(Thr304Asnfs*6)	NM_017651.4: c.2492+1G>A; p.(?)	AR (het_comp)	CES

ID	Clasificación fenotípica *a priori*	Clasificación fenotípica final	Status en el artículo 2	Gen	Alelo 1	Alelo 2	Modo de herencia (cigosidad)	Técnica molecular
RP-3612	*"DR+DI"*	*"ESPECÍFICO"* (COH)	No aparece (nuevo caso)	*VPS13B*	NM_017890.4: c.5998_5999del; p.(Leu2000Alafs*2)	Deleción exón 34-39	AR (het_comp)	CES
RP-3616	*"CILIOPATÍA"* (BBS)	*"CILIOPATÍA"* (BBS)	No aparece (nuevo caso)	*BBS12*	NM_152618.3: c.1616G>A; p.(Gly539Asp)	NM_152618.3: c.1616G>A; p.(Gly539Asp)	AR (homo)	CES
Caracterizados parcialmente								
RP-0304	*"CILIOPATÍA"* (BBS)	*"DR+DI"*	Negativa	*PRPF31*	NM_015629.4: c.757G>C; p.(Gly253Arg)	wt	AD *de novo* (het)	CES – reanálisis
RP-2882	*"CILIOPATÍA"* (ALMS-*like*)	*"DR+PA"* (DR+PA+TE)	VUS monoalélica	*ADAMTSL4*	NM_001288607.2: c.767_786del; p.(Gln256Profs*38)	NM_001288607.2: c.2477G>A; p.(Arg826His)	AR (het_comp)	CES – reanálisis
RP-3557	*"DR+PA+DI"*	*"DR+PA+DI"*	No aparece (nuevo caso)	*TULP1*	NM_003322.6: c.99+1G>A; p.(?)	NM_003322.6: c.1204G>T; p.(Glu402*)	AR (het_comp)	CES

AD, autosómico dominante; AE, alteración esquelética; ALMS, síndrome de Alström; AN, alteraciones neurodesarrollo; AR, autosómico recesivo; BBS, síndrome de Bardet-Biedl; CES, secuenciación del exoma clínico (*Clinical exome sequencing*); COH, síndrome de Cohen; DA, dificultad de aprendizaje; DI, discapacidad intelectual; DR, distrofia de retina; hemi, hemicigosis; het, heterocigosis; het_comp, heterocigosis compuesta; homo, homocigosis; JBTS, síndrome de Joubert; Malf SNC, malformaciones Sistema Nervioso Central; MC, microcefalia; NO-SIND, enfermedad hereditaria de la retina no sindrómica; PA, pérdida de audición; TE, trastornos endocrinos; VUS, variante de significado incierto; WES, secuenciación del exoma completo (*Whole exome sequencing*); WFS, síndrome de Wolfram; WGS, secuenciación del genoma completo (*Whole genome sequencing*); wt, alelo *wild-type*; XL, ligado al cromosoma X.

Anexo IV. Listado de las combinaciones trialélicas de variantes relacionadas con el síndrome de Bardet-Biedl.

Las combinaciones fueron obtenidas de la base de datos de DIDA (*Digenic Diseases Database*) y de nuestro propio estudio (artículo 3, Perea-Romero *et al*., sin publicar). Los 11 genes marcados en negrita son aquellos genes encontrados en nuestro estudio y que no aparecen en DIDA como parte de alguna combinación.

Origen	ID	Gen primario	Alelo 1	Alelo 2	Gen modificador	Alelo 1	Alelo 2
DIDA	dd111	*ARL6*	c.92C>T (p.Thr31Met)	c.92C>T (p.Thr31Met)	*TTC8*	c.405G>A (p.Met135Ile)	wt
DIDA	dd095	*BBS1*	c.724-8_726del (p.?)	c.1169T>G (p.Met390Arg)	*BBS10*	c.886G>A (p.Ala296Thr)	wt
DIDA	dd096	*BBS1*	c.887del (p.Ile296Thrfs*7)	c.887del (p.Ile296Thrfs*7)	*BBS10*	c.2144A>G (p.His715Arg)	wt
DIDA	dd080	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	*BBS2*	c.1046T>G (p.Leu349Trp)	wt
DIDA	dd105	*BBS1*	c.1645G>T (p.Glu549*)	c.1645G>T (p.Glu549*)	*BBS2*	c.1659+3A>G (p.?)	wt
DIDA	dd081	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	*BBS4*	c.1414A>G (p.Met472Val)	wt
DIDA	dd0107	*BBS1*	c.1169T>G (p.Met390Arg)	c.1645G>T (p.Glu549*)	*BBS9*	c.396G>C (p.Gln132His)	wt
DIDA	dd084	*BBS1*	c.339T>G (p.Tyr113*)	c.1169T>G (p.Met390Arg)	*CCDC28B*	c.330C>T (p.Phe110=)	wt
DIDA	dd204	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	*CCDC28B*	c.330C>T (p.Phe110=)	wt
DIDA	dd085	*BBS1*	c.1169T>G (p.Met390Arg)	c.1645G>T (p.Glu549*)	*CCDC28B*	c.330C>T (p.Phe110=)	wt
DIDA	dd078	*BBS1*	c.871C>T (p.Gln291*)	c.1169T>G (p.Met390Arg)	*MKKS*	c.706T>C (p.Ser236Pro)	wt
DIDA	dd079	*BBS1*	c.1169T>G (p.Met390Arg)	c.1642del (p.Leu548Trpfs*31)	*MKKS*	c.973A>C (p.Thr325Pro)	wt
DIDA	dd0106	*BBS1*	c.1473+4A>G (p.?)	c.1473+4A>G (p.?)	*TTC8*	c.1011C>G (p.Phe337Leu)	wt
DIDA	dd091	*BBS10*	c.164T>C (p.Leu55Pro)	c.821_822del (p.Glu274Valfs*29)	*BBS12*	c.355G>A (p.Gly119Ser)	wt
DIDA	dd091	*BBS10*	c.164T>C (p.Leu55Pro)	c.821_822del (p.Glu274Valfs*29)	*BBS12*	c.787T>C (p.Tyr263His)	wt
DIDA	dd108	*BBS10*	c.1598_1601del (p.Thr534Ilefs*21)	c.1598_1601del (p.Thr534Ilefs*21)	*BBS12*	c.355G>A (p.Gly119Ser)	wt
DIDA	dd103	*BBS10*	c.271dup (p.Cys91Leufs*5)	c.1867C>T (p.Gln623*)	*BBS2*	c.1885G>A (p.Glu629Lys)	wt
DIDA	dd110	*BBS10*	c.1333C>A (p.Leu445Ile)	c.1333C>A (p.Leu445Ile)	*BBS2*	c.311A>C (p.Asp104Ala)	wt

Origen	ID	Gen primario	Alelo 1	Alelo 2	Gen modificador	Alelo 1	Alelo 2
DIDA	dd089	*BBS10*	c.273C>G (p.Cys91Trp)	c.2119_2120del (p.Val707*)	*BBS5*	c.551A>G (p.Asn184Ser)	wt
DIDA	dd104	*BBS10*	c.145C>T (p.Arg49Trp)	c.145C>T (p.Arg49Trp)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
DIDA	dd102	*BBS10*	c.271dup (p.Cys91Leufs*5)	c.271dup (p.Cys91Leufs*5)	*MKKS*	c.1015A>G (p.Ile339Val)	wt
DIDA	dd093	*BBS10*	c.271dup (p.Cys91Leufs*5)	c.1230T>G (p.His410Gln)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
DIDA	dd090	*BBS10*	c.271dup (p.Cys91Leufs*5)	c.1677C>A (p.Tyr559*)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
DIDA	dd109	*BBS10*	c.1090del (p.Asn364Thrfs*5)	c.1090del (p.Asn364Thrfs*5)	*MKKS*	c.926G>A (p.Arg309His)	wt
DIDA	dd113	*BBS12*	c.2023C>T (p.Arg675*)	c.476C>T (p.Pro159Leu)	*BBS1*	c.1535G>A (p.Arg512His)	wt
DIDA	dd094	*BBS12*	c.1438del (p.Asp480Metfs*3)	c.1438del (p.Asp480Metfs*3)	*BBS4*	c.1463C>A (p.Thr488Lys)	wt
DIDA	dd077	*BBS2*	c.944G>A (p.Arg315Gln)	c.117+1G>C (p.?)	*BBS1*	c.1169T>G (p.Met390Arg)	wt
DIDA	dd099	*BBS2*	c.661del (p.Leu221Phefs*25)	c.661del (p.Leu221Phefs*25)	*BBS10*	c.271dup (p.Cys91Leufs*5)	wt
DIDA	dd101	*BBS2*	c.256_278dup (p.Val94Serfs*8)	c.256_278dup (p.Val94Serfs*8)	*BBS12*	c.1571A>G (p.Tyr524Cys)	wt
DIDA	dd100	*BBS2*	c.661del (p.Leu221Phefs*25)	c.1705C>T (p.Gln569*)	*BBS12*	c.1223A>G (p.Lys408Arg)	wt
DIDA	dd082	*BBS2*	c.1237C>T (p.Arg413*)	wt	*BBS4*	c.1508C>T (p.Pro503Leu)	wt
DIDA	dd075	*BBS2*	c.1673C>T (p.Thr558Ile)	c.1673C>T (p.Thr558Ile)	*BBS4*	c.1091C>A (p.Ala364Glu)	c.1091C>A (p.Ala364Glu)
DIDA	dd083	*BBS2*	c.1928G>A (p.Arg643His)	wt	*BBS4*	c.137A>G (p.Lys46Arg)	wt
DIDA	dd087	*BBS2*	c.311A>C (p.Asp104Ala)	c.1895G>C (p.Arg632Pro)	*BBS7*	c.171G>A (p.Val57=)	wt
DIDA	dd098	*BBS2*	c.646C>T (p.Arg216*)	c.661del (p.Leu221Phefs*25)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
DIDA	dd097	*BBS2*	c.646C>T (p.Arg216*)	c.72C>G (p.Tyr24*)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
DIDA	dd073	*BBS2*	c.72C>G (p.Tyr24*)	c.72C>G (p.Tyr24*)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
DIDA	dd074	*BBS2*	c.72C>G (p.Tyr24*)	c.175C>T (p.Gln59*)	*MKKS*	c.442C>T (p.Gln148*)	wt

Origen	ID	Gen primario	Alelo 1	Alelo 2	Gen modificador	Alelo 1	Alelo 2
DIDA	dd072	*BBS2*	c.503dup (p.Leu168Phefs*3)	c.646C>T (p.Arg216*)	*MKKS*	c.1495T>G (p.Cys499Gly)	wt
DIDA	dd076	*BBS7*	c.632C>T (p.Thr211Ile)	c.632C>T (p.Thr211Ile)	*BBS1*	c.700G>A (p.Glu234Lys)	wt
DIDA	dd086	*BBS7*	c.187G>A (p.Gly63Arg)	c.601+2T>C (p.?)	*BBS4*	c.341del (p.Leu114Trpfs*28)	wt
DIDA	dd112	*BBS7*	c.632C>T (p.Thr211Ile)	c.632C>T (p.Thr211Ile)	*TTC8*	c.284A>G (p.Lys95Arg)	wt
DIDA	dd071	*MKKS*	c.110A>G (p.Tyr37Cys)	c.110A>G (p.Tyr37Cys)	*BBS2*	c.209A>G (p.Asp70Gly)	wt
Esta Tesis (cap. 3)	RP-1219 (07/1158)	*BBS2*	c.565C>T (p.Arg189*)	c.565C>T (p.Arg189*)	***MKS1***	c.1136C>G (p.Ala379Gly)	wt
Esta Tesis (cap. 3)	RP-1325 (08/1281)	*BBS4*	arr([GRCh37] 15q24.1(72,685,818-72,686,251)x0)	arr([GRCh37] 15q24.1(72,685,818-72,686,251)x0)	***NPHP4***	c.1571C>A (p.Ser524*)	wt
Esta Tesis (cap. 3)	GBB23 (2128)	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	***ALMS1***	c.11638C>T (p.His3880Tyr)	wt
Esta Tesis (cap. 3)	RP-2634 (16/2256)	*BBS1*	c.1169T>G (p.Met390Arg)	c.951+58C>T (p.?)	*BBS5*	c.620G>A (p.Arg207His)	wt
Esta Tesis (cap. 3)	RP-1322 (08/1168)	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	*BBS7*	c.1235A>G (p.Asp412Gly)	wt
Esta Tesis (cap. 3)	RP-0129 (1518)	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	***IFT172***	c.3073C>G (p.Pro1025Ala)	wt
Esta Tesis (cap. 3)	RP-2996 (18/2222)	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	***IFT172***	c.4363C>T (p.Asp412Gly)	wt
Esta Tesis (cap. 3)	RP-2228 (13/2606)	*BBS1*	c.118del (p.Cys40Alafs*2)	c.1645G>T (p.Glu549*)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
Esta Tesis (cap. 3)	RP-1377 (09/0025)	*BBS1*	c.1097T>A (p.Va366Asp)	c.1097T>A (p.Va366Asp)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
Esta Tesis (cap. 3)	RP-0063 (97/1078)	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	*MKKS*	c.724G>T (p.Ala242Ser)	wt
Esta Tesis (cap. 3)	RP-0289 (95/0307)	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	*MKS1*	c.1318G>A (p.Gly440Ser)	wt
Esta Tesis (cap. 3)	RP-1731 (10/2087)	*BBS1*	c.1169T>G (p.Met390Arg)	c.1169T>G (p.Met390Arg)	***NPHP4***	c.2962G>A (p.Ala988Thr)	wt

Origen	ID	Gen primario	Alelo 1	Alelo 2	Gen modificador	Alelo 1	Alelo 2
Esta Tesis (cap. 3)	RP-1378 (09/0858)	*BBS1*	c.1169T>G (p.Met390Arg)	c.837del (p.Lys280Serfs*9)	***PDE6B***	c.1798G>A (p.Asp600Asn)	wt
Esta Tesis (cap. 3)	B64 (95/366)	*BBS12*	c.323C>T (p.Pro108Leu)	c.323C>T (p.Pro108Leu)	***PDE6B***	c.1055G>T (p.Gly352Val)	wt
Esta Tesis (cap. 3)	RP-1011 (05/1184)	*BBS12*	c.1619G>A (p.Gly540Asp)	c.1619G>A (p.Gly540Asp)	***SDCCAG8***	c.245G>T (p.Arg82Leu)	wt
Esta Tesis (cap. 3)	RP-0333 (97/0826)	*BBS12*	c.1115_1116del (p.Phe372*)	c.1115_1116del (p.Phe372*)	***TRIM32***	c.1198C>T (p.Arg400Cys)	wt
Esta Tesis (cap. 3)	GBB18 (559831)	*BBS2*	c.565C>T (p.Arg189*)	c.565C>T (p.Arg189*)	***CORO2B***	c.386C>T (p.Ala129Val)	wt
Esta Tesis (cap. 3)	RP-0928 (04/1611)	*BBS7*	c.1372-1G>C (p.?)	c.896_897del (p.Lys299Argfs*4)	*BBS9*	c.376A>T (p.Met126Leu)	wt
Esta Tesis (cap. 3)	RP-3079 (18/3967)	*BBS9*	c.1789c>T (p.Gln597*)	c.2349C>A (p.Cys783*)	***CEP290***	c.7311_7313del (p.Gly352Val)	wt
Esta Tesis (cap. 3)	RP-2966 (18/1755)	***C8orf37***	c.156-1G>T (p.?)	c.156-1G>T (p.?)	***WDPCP***	c.1032C>A (p.Cys344*)	wt
Esta Tesis (cap. 3)	RP-1573 (10/0618)	*MKKS*	c.1232G>C (p.Gly411Ala)	c.1232G>C (p.Gly411Ala)	*BBS5*	c.551A>G (p.Asn184Ser)	wt
Esta Tesis (cap. 3)	RP-1372 (08/1888)	*MKKS*	c.432_435del (p.Phe144Leufs*14)	c.432_435del (p.Phe144Leufs*14)	***IFT172***	c.2015G>A (p.Arg672Gln)	wt
Esta Tesis (cap. 3)	RP-0496 (98/0825)	*MKKS*	c.748G>A (p.Gly250Arg)	c.748G>A (p.Gly250Arg)	***PDE6B***	c.292C>T (p.Arg98Cys)	wt

ALMS1 (NM_015120.4), *ARL6* (NM_177976.3), *BBS1* (NM_024649.5), *BBS10* (NM_024685.4), *BBS12* (NM_152618.3), *BBS2* (NM_031885.5), *BBS4* (NM_033028.5), *BBS5* (NM_152384.3), *BBS7* (NM_176824.3), *BBS9* (NM_198428.3), *C8orf37* (NM_177965.4), *CCDC28B* (NM_024296.5), *CEP290* (NM_025114.4), *CORO2B* (NM_006091.5), *IFT172* (NM_015662.3), *MKKS* (NM_170784.3), *MKS1* (NM_017777.4), *NPHP4* (NM_015102.5), *PDE6B* (NM_000283.4), *SDCCAG8* (NM_006642.5), *TRIM32* (NM_001099679.2), *TTC8* (NM_144596.4), *WDPDP* (NM_015910.7). Wt, *wild type*

Anexo V. Listado de publicaciones científicas derivadas de esta Tesis Doctoral.

Perea-Romero I*, Gordo G*, Iancu IF*, *et al*. (1/116). Genetic landscape of 6089 inherited retinal dystrophies affected cases in Spain and their therapeutic and extended epidemiological implications. *Sci Rep*. 2021

Bea-Mascato B, Solarat C, Perea-Romero I, *et al*. (3/8). Prevalent *ALMS1* Pathogenic Variants in Spanish Alström Patients. *Genes (Basel)*. 2021

Iancu IF, Avila-Fernandez A, Arteche A, *et al*. (9/12). Prioritizing variants of uncertain significance for reclassification using a rule-based algorithm in inherited retinal dystrophies. *NPJ Genom Med*. 2021

Beigi F*, Del Pozo-Valero M*, Martin-Merida I, *et al*. (4/7). Apparent but unconfirmed digenism in an Iranian consanguineous family with syndromic Retinal Disease. *Exp Eye Res*. 2021

Lopez-Rodriguez R*, Lantero E*, Blanco-Kelly F, *et al*. (7/16). *RPE65*-related retinal dystrophy: Mutational and phenotypic spectrum in 45 affected patients. *Exp Eye Res*. 2021

Galbis-Martinez L*, Blanco-Kelly F*, García-García G*, *et al*. (7/14). Genotype-phenotype correlation in patients with Usher syndrome and pathogenic variants in *MYO7A*: implications for future clinical trials. *Acta Ophthalmol*. 2021

Perea-Romero I, Blanco-Kelly F, Sanchez-Navarro I, *et al*. (1/19). NGS and phenotypic ontology-based approaches increase the diagnostic yield in syndromic retinal diseases. *Hum Genet*. 2021

Romero R, de la Fuente L, Del Pozo-Valero M, *et al*. (9/16). An evaluation of pipelines for DNA variant detection can guide a reanalysis protocol to increase the diagnostic ratio of genetic diseases. *NPJ Genom Med*. 2022