



Adult height and long-term outcomes after rhIGF-1 therapy in two patients with PAPP-A2 deficiency

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ABSTRACT

PAPP-A2 deficiency is a novel syndrome characterized by short stature due to low IGF bioactivity, skeletal abnormalities and decreased bone mineral density (BMD). Treatment with recombinant human IGF-1 (rhIGF-1) for 1 year demonstrated to increase growth velocity and BMD, without reported adverse effects, but data regarding the long-term efficacy and safety of rhIGF-1 administration in this entity has not yet been reported.

Two Spanish siblings with short stature due to a homozygous loss-of-function mutation in the PAPP-A2 gene (p.D643fs25*) were treated with rhIGF-1 twice daily for six years. Growth velocity continued to increase and both patients achieved their target height. Free IGF-1 concentrations increased notably after rhIGF-1 administration, with serum IGFBP-3, IGFBP-5 and ALS levels also being higher during treatment. BMD was progressively normalized and an increase in lean mass was also noted during treatment. No episodes of hypoglycemia or any other adverse effects were documented. An increase in the growth of kidney and spleen length was observed in one of the patients.

1. Introduction

In 2016, we described a novel syndrome due to loss of function in the pregnancy-associated plasma protein-A2 (PAPP-A2) gene [1] in two unrelated families. PAPP-A2 is a highly specific metalloproteinase that increases insulin-like growth factor (IGF)-1 bioactivity by proteolytic cleavage of IGFBP-3 and -5 in the ternary complexes, thus, regulating the dissociation of IGF-1 from secondary and ternary complexes [2]. PAPP-A2 deficiency is characterized by postnatal growth retardation, high peripheral levels of total IGF-1, IGFBP-3, IGFBP-5 and ALS levels but with low free IGF-1 concentrations and IGF bioactivity and very low or undetectable PAPP-A2 levels, skeletal abnormalities and decreased bone mineral density [3]. The two Spanish siblings were treated with recombinant human IGF-1 (rhIGF-1) and we demonstrated that this treatment was effective, improving short-term growth [4] as well as bone mass and body composition after 1 year [5].

The objectives of this study were to assess the auxological,

biochemical and metabolic outcomes after six years of rhIGF-1 administration, as well as the long-term safety of this treatment.

2. Subjects and methods

2.1. Clinical characteristics

Our patients were two Spanish siblings, a 10.5-year-old girl (patient 1) and a 6-year old boy (patient 2), who carried a homozygous frame-shift mutation in the PAPP-A2 gene (p.D643fs25*), resulting in undetectable PAPP-A2 levels. Their growth patterns revealed progressive growth deceleration with short stature relative to target height (1.7 and 1.3 SDS below target height, respectively).

Recombinant hIGF-1 (Mecasermin, Increlex; Ipsen) was administered subcutaneously twice daily. The initial dose was 40–80 µg/kg/dose and progressively increased to 120 µg/kg during the first year. During the following years, the dose was maintained between 90 and

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120 µg/kg. In patient 1, treatment was interrupted at 16.5 years of age, after six years of treatment, whereas patient 2 is still under treatment. Patient 1 also received GnRH analog treatment (Triptorelin, 3.75 mg/28 days), since she started puberty after initiation of rhIGF-1 [4] treatment.

Physical and anthropometric evaluation were carried out every 6 months. Continuous glucose monitoring was performed during treatment. Biochemical analysis, radiography of the left hand and wrist and abdominal ultrasound were evaluated every year. Kidney length standard deviation scores (SDS) for height (using the mean of the left and right renal lengths) were computed according to Han and Babcock [6] and splenic length SDS for height using the norms from Megremis et al. [7].

Bone mineral density (BMD) and body composition measurements were performed using dual-energy X-ray absorptiometry (DXA; DXA Discovery Wi, software version 13.3; Hologic, Inc., Waltham, MA, USA). Data for BMD are expressed adjusting for height-for-age Z scores [8]. Body composition data obtained by DXA were compared from baseline to the end of the study [9].

2.2. Biochemical analyses

Serum levels of total IGF-I and IGFBP-3 were determined by chemiluminescence immunoassay from Diasorin (Saluggia, Italy), free IGF-I, IGFBP-5 and ALS were measured by ELISA (Ansh Labs, Webster, Tx, USA) and insulin by an immunoradiometric assay (DIASource ImmunoAssays, Louvain-La-Neuve, Belgium). In the sixth year of treatment, an oral glucose tolerance test (OGTT, 75 g glucose) for determination of both glucose and insulin levels was performed. The sensitivity and intra- and inter-assay variability for these assays, as well as comparisons with other assays has been previously reported [1,4].

3. Results

3.1. Clinical and auxological outcomes

After the first year of treatment with rhIGF-1, that has been previously reported [4], both patients increased height SDS progressively (Fig. 1, Table 1). Currently, patient 1 has already reached her adult size. Patient 2 is over his target height (with a slight increase in bone maturation). In fact, in patient 2, an acceleration in skeletal maturation was observed during treatment, currently remaining consistent with chronological age (Δ chronological age - bone age = -0.5). The BMI in both patients increased progressively; being at present within the normal range.

Kidney and spleen size in patient 1 increased moderately during the treatment, particularly throughout the pubertal period (spleen: + 3.92 SDS; kidney: + 2.40 SDS after the sixth year of treatment; Table 1).

3.2. Biochemical and metabolic parameters

Levels of total IGF-1, IGFBP-3 and IGFBP-5 continued to increase throughout the treatment. Free IGF-1 values were also higher, mostly immediately (up to two hours) after administration of rhIGF-1 (Fig. 2). On the other hand, values of ALS did not vary during the first years of treatment, rising in both patients during mid-puberty. [9]. No episodes of hypoglycemia were documented in neither patient, during the Continuous glucose monitoring.

The OGTT performed in both patients during the sixth year of treatment showed glucose intolerance (Table 2). An insulin tolerance test was performed simultaneously revealing insulin resistance that was more severe in patient 2 (Table 2).

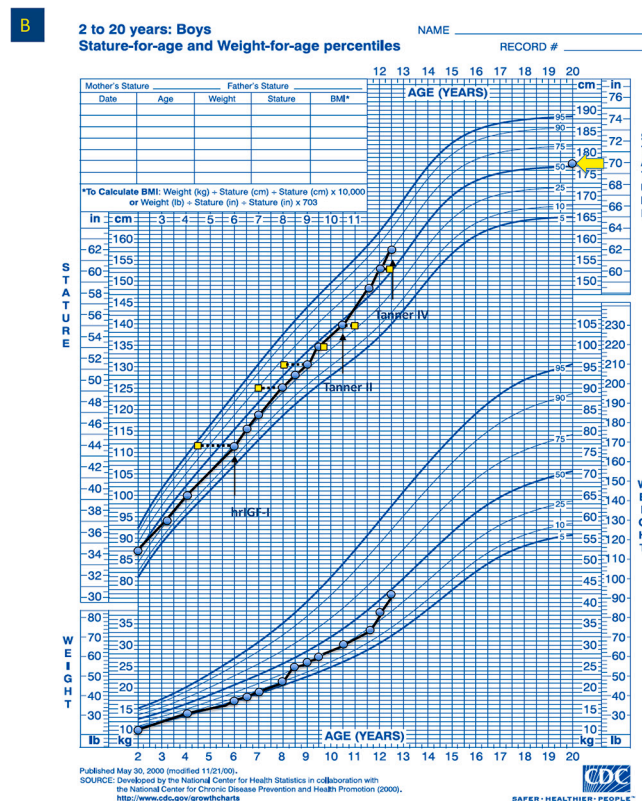
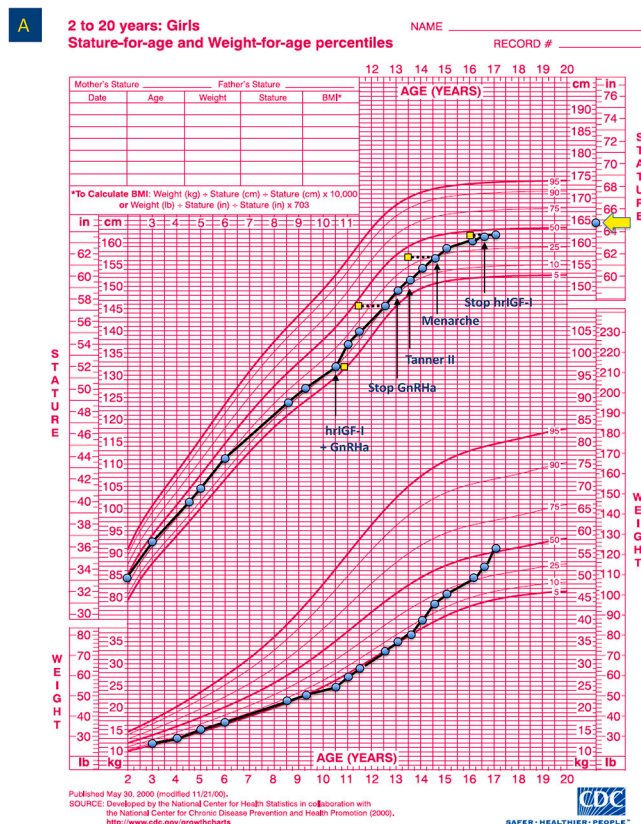


Fig. 1. Growth chart of the female patient with pregnancy-associated plasma protein-A2 (PAPP-A2) deficiency before and after 6 years of treatment with recombinant human insulin-like growth factor-1 (rhIGF-1).

A. Growth chart of the male patient with PAPP-A2 deficiency before and after 6 years of treatment with rhIGF-1.

Table 1

Anthropometric, clinical and biochemical data over six years of recombinant human insulin-like growth factor-1 (rhIGF-1) therapy.

Clinical characteristics		Patient 1				Patient 2			
		Baseline	2 years	4 years	6 years	Baseline	2 years	4 years	6 years
Clinical data	Chronological age, years	10.5	12.5	14.5	16.5	6.0	8.0	10.0	12.0
	Bone age, years	10.75	12.5	13.5	16.0	4.5	7.5	9.5	12.5
	Growth velocity, cm/year (SDS)	3.7 (−1.5)	5.9 (−0.9)	5.7 (+0.7)	0.5 (−0.4)	5.8 (−1.6)	6.0 (+0.5)	5.6 (+1.5)	9.6 (+5.2)
	Height, SDS	−1.25	−0.86	−0.04	+0.11	−0.74	−0.31	−0.15	+1.02
	BMI, Kg/m ² (SDS)	14.1 (−1.5)	15.6 (−1.3)	17.7 (−1.1)	20.2 (−0.4)	13.4 (−1.7)	14.1 (−1.3)	15.9 (−0.9)	16.3 (−0.9)
	Δ Height-target height, SDS	−1.94	−1.55	−0.73	−0.58	−1.17	−0.74	−0.58	+0.59
Biochemical data	Total IGF-1, ng/ml	957	1135	1190	1502	882	1060	1366	1709
	Free IGF-1, ng/ml	1.39	4.64	5.48	5.29	0.27	3.94	1.27	4.10
	IGFBP-3, ng/dl	5912	8460	8940	9660	4850	6235	8250	11,500
	IGFBP-5, ng/dl	997	1587	1671	2042	853	893	899	1861
	ALS, U/L	3745	3353	5971	3798	3625	3353	3690	3528
Densitometric data	Total BMD, g/cm ² (SDS)	0.79 (−2.0)	0.87 (−1.0)	1.01 (−0.2)	1.07 (−0.2)	0.76 (−2.3)	0.83 (−1.3)	0.87 (+0.7)	0.95 (+0.5)
	Lumbar spine BMD, g/cm ² (SDS)	0.58 (−1.7)	0.68 (−1.3)	0.82 (−1.1)	0.93 (−0.6)	0.49 (−1.4)	0.53 (−0.4)	0.57 (−0.4)	0.71 (0.0)
	Total body fat, %	31.7	29.9	27.4	31.7	27.6	24.2	27.5	18.9
Ultrasound data	Spleen lenght, cm (SDS)	10 (+0.8)	11 (+1.1)	12.6 (+3.3)	13 (+3.9)	10 (+1.9)	10.5 (+1.8)	11 (+2.0)	11.7 (+1.8)
	Mean renal lenght, cm (SDS)	10.2 (+1.1)	10.7 (+1.3)	11.3 (+2.0)	12.1 (+2.4)	8.5 (+0.7)	9.2 (+0.4)	9.5 (+0.3)	10.3 (+0.7)
Treatment dosage	rhIGF-1, µg/kg/twice daily	105	110	100	100	105	120	120	91

SDS: standard deviation score; rhIGF-1: recombinant human insulin growth factor-1; BMD: bone mineral density.

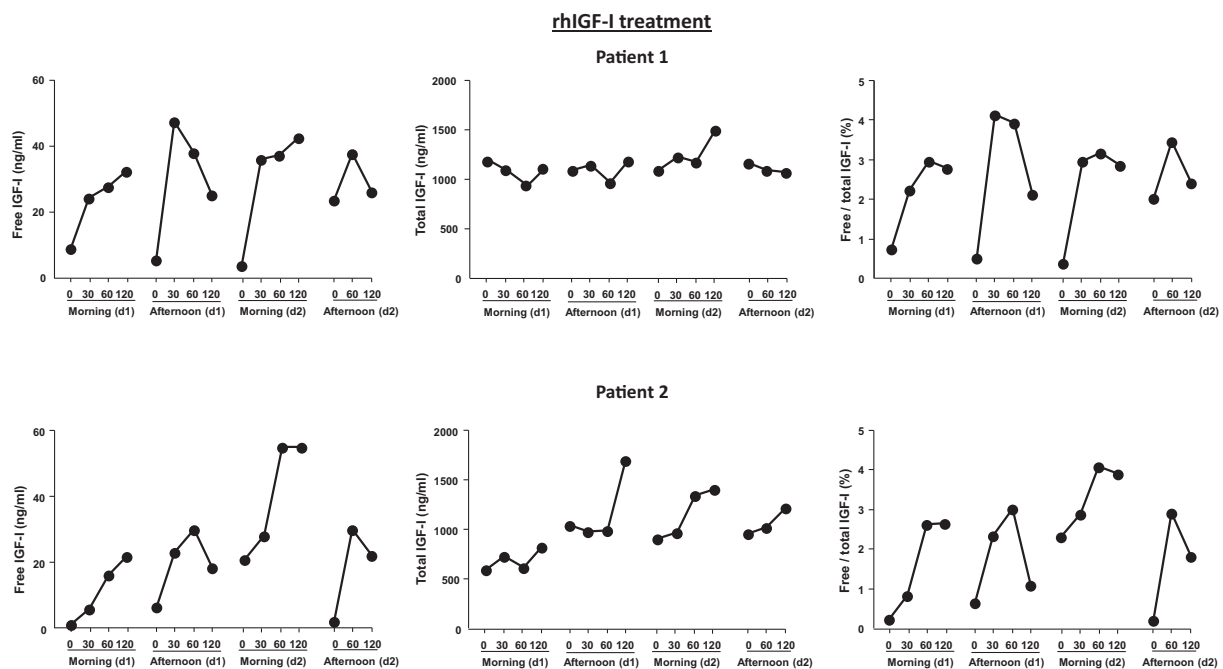


Fig. 2. Changes in circulating free and total insulin-like growth factor-1 (IGF-1) after the administration of recombinant human IGF-1. Measurements were performed at baseline and every 30 min up to 120 min after morning and afternoon injections of rhIGF-1 on two consecutive days (d1 & d2).

Table 2

Oral glucose tolerance test and insulin tolerance test in the two patients after 6 years of rhIGF-1 therapy.

Time (minutes)	Patient 1				Patient 2			
	0	30	60	120	0	30	60	120
Oral Glucose Tolerance Test (OGTT; mg/dl)	90	162	180	158	81	134	199	166
Insulin Tolerance Test (ITT; µU/ml)	10.3	83.6	94.2	109.0	34.5	63.5	192.0	253.0

3.3. Body composition and bone mineral density

BMD at diagnosis was below the normal range in both patients (Z-score −2 and −2.3 SDS). In both patients, BMD increased during the subsequent years of treatment. In patient 1 from 0.788 to 1.066 g/cm²; in patient 2 to from 0.763 to 0.950 g/cm². Additionally, the percentage of total body fat mass was reduced in both patients throughout the

treatment (up to −8.7% in patient 2; up to 4% in patient 1, but increasing again to basal values, 6 months after the discontinuation of the treatment (Table 1).

4. Discussion

We have demonstrated how treatment with rhIGF-1 is effective in

patients with PAPP-A2 deficiency for the promotion of long-term growth by increasing their growth, allowing our two patients to reach or exceed their target height. Patient 1 has reached her target height and has discontinued treatment, whereas patient 2 is currently over his expected height and continues under treatment. During the first year of treatment, the patients had height velocities of 7.6 and 7 cm, respectively (+1.6 and + 1.06 SDS, respectively) and a mean of 6 cm/year throughout the prepubertal period. Patient 1 grew 12.6 cm during the pubertal period (since aGnRH treatment was interrupted to adult height) and patient 2 had grown 14.1 cm, since he started puberty.

Levels of total IGF-1, free IGF-1, IGFBP-3, IGFBP-5 continued to increase during treatment, rising even more with pubertal maturation in both patients. Basal free IGF-1 values and free IGF-1/total IGF-1 ratio increased notably following rhIGF-1 administration, which is in agreement with the continued increase in growth.

In these patients, treatment with rhIGF-1 has also been found to be safe. They did not experience any side effects (visual disturbances or signs of intracranial hypertension, among others). Furthermore, they did not experience any episode of hypoglycemia, although this is the most common adverse event documented in patients under treatment with rhIGF-1, having been reported in up to 49% of treated subjects [10].

However, mild to moderate splenomegaly and nephromegaly was observed in one of our patients. Disproportionate growth of these two organs (spleen and kidneys) has already been previously reported in response to treatment with rh-IGF-1. In a study that evaluated the long-term efficacy and safety of rh-IGF-1 for children with severe short stature, increased kidney length SD scores for height were reported in 5/23 patients, whereas spleen length for height was over 2 SDS in 2/23 subjects [10].

Both patients also exhibited mild basal hyperinsulinemia, that became more severe during puberty, as well as insulin resistance and glucose intolerance. In other GH-IGF axis disturbances, for example IGF-ALS deficiency, mild hyperinsulinemia has also been reported [11]. This is most likely due to the fact that IGF-1 has insulin-like activities. In a recent study performed in a mouse model with a mutation in PAPP-A2, fasting glucose levels were not altered, but GTT and ITT analyses revealed glucose intolerance and insulin resistance. Furthermore, insulin resistance correlated with the decrease in serum free IGF-1 levels [12]. Nevertheless, further research is needed to elucidate the consequences of PAPP-A2 deficiency on glucose and insulin metabolism.

Treatment with rhIGF-1 also increased bone mineral density and induced changes in body composition, decreasing the percentage of total body fat, throughout 6 years of treatment in our two patients. This is similar to that observed in PAPP-A2-deficient mice, where changes in trabecular and cortical mineral density have been described and associated with increased serum levels of IGFBP-5 and reduced peripheral levels of factors related to bone turnover [13]. Furthermore, treatment with recombinant IGF-1 modulated bone composition and remodeling in Pappa2-deficient mice, improving bone strength and density [14].

Recently, we have described a new homozygous nonsense mutation (p.Glu886* in exon 7) in the PAPP-A2 gene in a family from Saudi Arabia, with two affected males. The younger male is currently under treatment with rhIGF1 and an increase in growth and height velocity has been reported [15].

Therefore, rhIGF-1 should be considered as a safe and effective therapeutic option to increase the peak of bone mass and bone density achieved during childhood and puberty, mostly taking into account that human recombinant PAPP-A2 is currently unavailable for treatment. Follow-up of these and other PAPP-A2 deficient patients during adulthood will provide more knowledge about the natural history of this disease and the physiological functions of this protease [16].

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Author's contributions

J.A had the original idea and contributed to conception and planning the design, interpretation of the findings and preparation of manuscript; Á. M-R contributed to planning the design and acquiring the data; V-B performed and discussed biochemical analyses. All authors have been involved in drafting the article, have given final approval and agree to be accountable for all aspects of the work.

Declaration of Competing Interest

All authors declare that they have no conflict of interest.

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