Cytogenetic and Neurobiological Advances in Down syndrome

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Abstract: Down syndrome is an autosomal trisomy that traditionally has been studied independently from fields such as medicine, biology or psychology. In this article, we intend to go further and incorporate a multidisciplinary approach that includes, on the one hand, the main findings of these disciplines and, the theories that attempt to explain the complex relationships that occur between such findings. With this aim, we review the progress that has been made in the field of genetics, neuroanatomy and neurochemistry in relation to this syndrome, as well as the explanations that have been developed to try to understand the neuropsychological profile associated with this condition. We believe that the incorporation of this perspective will help achieve an overview of the psychobiological correlates of Down syndrome.

Key words: Down syndrome; genetics; neuroanatomy; neurochemistry; neuropsychology; psychology.

Introduction

Down syndrome (DS) is the most common genetic cause of intellectual disability (Nadel, 2003; Patterson, 2007). It was first described by Langdon Down in 1866, in an article published in the London Hospital Reports (Parajuy-Pozo & Casis-Argueta, 2000; Sherman, Freeman, Allen & Lamb, 2005). In this publication was already highlighted the presence of intellectual disability and a range of distinctive facial features, which to the author were similar to those of some eastern populations. The presence of these characteristic facial features allowed Langdon Down to define DS as a specific clinical entity (Carvajal, Iglesias & Loeches, 1994; Mégabane et al., 2009).

Initially many hypotheses about the origin of DS were raised that alluded that DS was a throwback to more primitive races, or it was due, either diseases or addictions of parents, problems during pregnancy, endocrine disorders, age of the mother or even gynecological irregularities (Mar-domingo, 1995).

Afterwards, Waardenburg raised its genetic origin in 1932 (Capone, 2001). But it was not until 1959 when Lejeune and his colleagues confirmed the presence of an extra chromosome in pair 21 in nine children with DS (Lejeune, Gautier & Turpin, 1959). Moreover, the advancement of cytogenetic techniques also revealed the existence of three underlying chromosomal abnormalities on the onset of the syndrome: regular trisomy, translocation trisomy and mosaicism (Patterson, 2007; Serés, Cuatrecasas & Catalá, 2005). In the first and second case, the error occurs in the formation of gametes or the first mitotic division so it affects all somatic cells of the organism, the main difference being that in the case of regular trisomy has 47 whole chromosomes whereas in the translocation case has 46 chromosomes and an extra portion of chromosome 21 (HSA21). In contrast, in mosaicism the error occurs from the second mitotic division, which gives rise to two different cell lines, one with 47 chromosomes and one with a normal genetic endowment.

Currently, it is widely known that between 90-95% of cases are due to regular trisomy of HSA21, while the rest are due to HSA21trisomy by translocation or mosaicism (Bornstein et al., 2010; Patterson, 2007).

When it was compared the phenotype of people with regular trisomy against the phenotype of people with DS due to translocation no significant differences were found. In any case, the varying extension of certain phenotypic traits seems to be related to the amount of additional genetic material, but this does not seem to exert influence on the intellectual level (Bornstein et al., 2010; Devlin & Morrison, 2004a; Loeches, Iglesias & Carvajal, 1991). However, when this comparison has been carried out with cases caused by mosaicism it was observed that the latter present less accentuated phenotypic characteristics in relation to the number of cells affected (Devlin & Morrison, 2004b; Dreux et al., 2008; Serés et al., 2005). However, as in the previous case, the existence of this relationship has not been demonstrated in the case of intellectual level (Carvajal et al., 1994).

In addition to its relationship with intellectual disability, regular trisomy appears to lead to a specific neuropsychological profile (Kittler, Krinsky-McHale & Devenny, 2006; Menghini, Costanzo & Vicari, 2011; Ruggieri & Arberas, 2003), mainly characterized by : a) deteriorated language skills, affecting this impairment more to the production than to the comprehension, as well as to the phonological and morphological domain than the semantic and pragmatic (Fildler, Philofsky & Hepburn, 2007; Galeote, Soto, Sebastián, Rey & Checa, 2012; Vicari, Caselli, Gagliardi, Tonucci...
& Volterra, 2002); b) a visuospatial skills characterized by the presence of a dissociation between processing of perceptual aspects such as color and shape, that would be very deteriorated, and spatial processing, that would be best preserved (Jarrold, Nadel & Vicari 2008; Silverman, 2007; Vicari, 2006); c) a executive dysfunction especially in subprocesses, such as working memory for verbal material (Edgin, Pennington & Mervis, 2010; Lanfranchi, Jerman, Dal, Alberti & Vianello, 2010; Rowe, Lavender & Turk, 2006) and d) a deterioration in episodic memory linked to advancing age (Krinsky-McHale, Kittler, Brown, Jenkins & Devenny, 2005; Vicari, 2004).

All these neuropsychological characteristics are closely related to neurochemical and neuroanatomical alterations presented by people with DS. And therefore with the role of specific regions of HSA21 in the processes of brain development. For this reason, today, addressing its study is carried out from a multidisciplinary perspective that attempts to clarify the links between the underlying genetic and molecular mechanisms, and the cognitive and behavioral profile they present (Lott, Patterson & Malick, 2007; Nadel, 2003).

Moreover, the sequencing of the human genome, the possibility of generating animal models of DS, the use of neuroimaging techniques, tissue analysis at the cellular level, as well as advances in neuropsychological assessment and functional knowledge of the brain have helped improve our understanding of the complex relationships between genes and neuroanatomical, neurochemical and neuropsychological characteristics. The following are the latest findings in the field of genetics, neuroanatomy and neurochemistry in relation to the study of DS.

**Genetic advances**

Since Lejeune and his collaborators confirmed the presence of an extra chromosome in the chromosome pair 21 (Lejeune et al, 1959), the genetic study of DS has experienced a breakthrough. The discovery of the human genome, especially the complete sequencing of HSA21, published in Nature (The chromosome 21 mapping and sequencing consortium, 2004) and the study of trisomic and transgenic animal models have led to considerable improvement in the knowledge of the syndrome and the consequences of this trisomy (Patterson, 2007; Scorza & Cavalheiro, 2011). For example, thanks to the sequencing of this chromosome, to date, have been identified more than 530 genes (Park, Song & Chung, 2009; Sturgeon & Gardiner, 2011). Although, in spite of its identification, 45% of these genes function is still uncertain (Kahlem, 2006).

Within this extraordinary progress, one of the findings with greater impact was the discovery, in the 1980s, that complete triplication of HSA21 was not necessary for the distinctive DS features to appear (Petersen et al., 1990). In fact, these features appear even when only the band 21q22 is tripled (Wilkie, Amberger & McKusiek, 1994). Specifically, observations of people with partial trisomy of the 21q22.3 sub-band allowed to suggest that most signs of the syndrome, including intellectual disability, depend on the expression of this region. For this reason, is considered a critical region for DS, receiving the name of Down syndrome critical region (DSCR) (Chabert et al., 2004; Rachidi & Lopes, 2008). However, it must be taken into consideration that the triplication of this region is regarded necessary but not sufficient to explain the phenotypic features of DS (Olson et al., 2007). Probably genes from other regions are also involved in the expression of certain features and may need to interact with them and between each other, to give rise to the characteristic phenotype of DS (Galdzicki & Siarey, 2003).

Currently, most studies are based on animal models, mainly by the multiple advantages they present (Liu et al., 2011; Vacano, Duvak & Patterson, 2012). These models date back to 1973-74, when the first two genes located on HSA21 were identified and for the first time mice with trisomy of chromosome 16 (MMU16), chromosome that is homologous to HSA21, were produced (Salehi, Faizi, Belchenko & Mobley, 2007).

In animal models we can find two strategies; trisomic mice based models and transgenic mice based models. Mice trisomic based models are focused on producing mice with triplication of a set of genes located on HSA21 homologous regions. On the other hand, models based on transgenic mice use mice in which a specific gene is inserted to assess the effect of its overexpression on the phenotype. Both strategies are useful, because the first allow us to assess the effects produced by the interaction between genes and are models closer to reality; and the latter allow to identify the phenotypic features dependent on the triplication of a particular gene.

**Trisomic mice**

Most of these models are based in mice with triplication of HSA21 or DSCR homologous regions. Their main finding is having been shown that mere triplication of homologous regions to HSA21 and DSCR, like the partial trisomy of MMU16, result into neuroanatomical and behavioral features similar to those present in individuals with DS. Between these neuroanatomical characteristics worth mentioning their implication in the development of cerebral hypoplasia, abnormal dendritic arborizations, as well as the fewer number of granule cells and the smaller size of the cerebellum and hippocampus (Aldridge, Reeves, Olson & Richtsmeier, 2007; Belichenko et al., 2009; Bianchi et al., 2010; O’Doherty et al., 2005; Rueda et al., 2010; Siarey, Villar, Epstein & Galdzicki, 2005). Likewise, in relation to the behavioral features it has been shown the involvement of genes in these regions in the appearance of motor dysfunctions, hypoaactivity, impaired spatial learning and decrease in the frequency of exploratory behavior (Fernandez & Garner, 2008; Galante et al., 2009; Olson et al., 2004; Sago et al., 2000; Salehi et al., 2009; Villar et al., 2005).
Furthermore, there is also evidence of the involvement of the MMU16 in the emergence of cholinergic degeneration associated with advancing age, similar to that found in adults with DS and in people from the general population with Alzheimer's disease (Contestabile, Giani & Contestabile, 2008; Granholm, Sanders & Grnic, 2000; Hunter, Bachman & Granholm, 2004; Salehi et al., 2006).

Transgenic Mice

To date, the genes that have been overexpressed in mice to understand their contribution are APP, CBS, DSCR-1, Dyrkla, S100β, SIM2, C21orf5 and SOD1 (Gardiner, 2009; Rachidi & Lopes, 2008; Salehi et al., 2007).

The most remarkable finding of these studies is to have demonstrated the involvement of the APP gene in the development of Alzheimer's disease. Since, at a behavioral level, mice with triplication of this gene present increases in spontaneous locomotor activity and changes in spatial learning; at a neurobiological level these behavioral findings have been associated with age-related cholinergic degeneration and morphological alterations in the hippocampus, cortex and cerebellum found in these mice and which are similar to those found in people with Alzheimer's disease (Epstein, 2000; Lyle, Gehrig, Neergaard-Henrichsen, Deutsch & Antonarakis, 2004; Millan et al., 2012; Simón et al., 2009).

These models also demonstrated the importance of overexpression of other genes on certain phenotypic traits, although its influence has not been established so clearly (Rachidi & Lopes, 2008). In particular, it has been found that overexpression of SOD1, Dyrkla, SIM2, C21orf5 and S100β genes influence the onset of both neuroanatomical and behavioral features similar to those presented by people with DS (Gardiner, 2009; Salehi et al., 2006). Specifically, it has been found a relationship between the overexpression of these genes and the emergence of alterations in brain plasticity, in the neuronal branches extension and cerebral apoptosis processes. And as to the behavioral characteristics it has been found a link between these genes and alterations in spatial learning, acquisition of locomotion or the exploratory behavior (Altafaj et al., 2001; Chrast et al., 2000; Dierssen et al., 2011; Donato, 2003; Lopes, Chettouh, Delabar & Rachidi, 2003; Martinez et al., 2008; Park et al., 2009; Yahut, Domagauer & D’Arcangelo, 2010).

Although the findings of these animal models are still scarce and its practical application is still limited, it may be considered that, in addition to providing basic information about the underlying etiology to DS, it is possible that their study also provide information, which on its basis early pharmacological interventions could be developed that prevent or compensate the appearance of some of the neuro-psychological manifestations of DS (Gardiner, 2009; Rueda et al., 2012; Scorza and Cavalheiro, 2011, Liu et al., 2011; Vacano et al., 2012).

Neurobiological advances

Neuroanatomy

The first neuroanatomical data on the DS were obtained from measurements post-mortem and, subsequently, were confirmed by using neuroimaging techniques (Dierssen, 2012; Lott & Dierssen, 2010; Pinter, Eliez, Schmitt, Capone & Reiss, 2001; White, Alkire & Haier, 2003).

These early studies showed that there were a number of structural abnormalities that were present in the majority of people with DS, which consisted of the presence of brachycephaly, smaller brain weight and size, especially significant in the cerebellum, frontal and temporal lobes, and in the brainstem. It was also reported a significant increase in the size of the cerebral ventricles, a smaller hippocampus and amygdala, a narrower superior temporal gyrus, and a decrease in the number and depth of the cortical sulci and convolutions. Likewise, since the first neuroanatomical studies was evident the presence of characteristic neuropathological features of Alzheimer's disease in the 4th decade of life (De la Monte & Hedley-Whyte, 1990; Wisniewski, Winsniewski & Wen, 1985).

Subsequent neuroimaging studies have allowed detecting these alterations and other previously not found by post-mortem studies. Among them, is noteworthy the presence of a smaller volume in the corpus callosum and the temporal plane as well as a greater bilateral volume in the parahippocampal gyrus and in the parietal lobe (Beacher et al., 2010; Frangou et al., 1997; Kesslak, Nagata, Lott & Nalcioglu, 1994; Pearlson et al., 1998; Pinter, Eliez et al., 2001; Teipel et al., 2003; White et al., 2003).

However, at subcortical level the structures tend to have a normal and even greater volume. Specifically, after adjusting it to the total intracranial volume, there is a greater volume in the basal ganglia, thalamus and hypothalamus. Whereas the hippocampus and amygdala show a bilateral reduction, particularly accentuated in the left hemisphere (Aylward et al., 1997; Beacher et al., 2010; Jernigan, Bellugi, Sowell, Doherty & Hesselink, 1993; Pinter, Brown et al., 2001; White et al., 2003).

Both the neuroimaging studies and those conducted post-mortem, those based on brain biopsies and the studies based on animals models also have provided data on cerebral morphological characteristics at the cellular level of people with DS. In particular, reports a fewer number of granule cells in the cerebellum, defects in cortical lamination, reductions in the amount of cortical neurons, presence of malformations in arborizations and dendritic spines and synaptic alterations (Becker, Mito, Takashima & Onodera, 1991; Belichenko et al., 2009; Golden & Hyman, 1994; Larsen et al., 2008; Vuksic, Petanjek, Rasin & Kostovic, 2002). Nevertheless, this reduction in the number and neuronal density does not affect equally all cortical layers, being especially pronounced in interneurons of cortical layers II and IV, and...
the pyramidal neurons of the cortical layer III (Golden & Hyman, 1994; Lott & Dierssen, 2010).

As just reviewed, the brain of people with DS, at the anatomical level, is characterized by alterations in very specific regions of both hemispheres. Among these alterations the most notable is the smaller volume presented by the hippocampus, amygdala, the temporal and frontal lobes, the brainstem, cerebellum and corpus callosum. Interestingly, some recent studies have linked these alterations in specific regions with certain characteristics of the neuropsychological profile of individuals with DS. Specifically, lower volume in the frontal and cerebellar regions have been linked with executive deficits and problems of fluency and verbal production showed by people with DS compared with people with intellectual disabilities due to other etiologies or of unknown origin (Lott & Dierssen, 2010; Menghini et al., 2011; Pearson et al., 1998; Pinter, Eliez et al., 2001). The lower volume on temporal cortical and subcortical regions (amygdala, hippocampus and temporal lobe) with verbal comprehension difficulties, memory and larger deficit presented in purely perceptual processing aspects such as color and shape (Krasuski, Alexander, Horwitz, Rapoport & Schapiro, 2002; White et al., 2003). The reduction in these regions and in the corpus callosum, coupled with the onset of the characteristic lesions of Alzheimer's disease, has also been related with the progressive deterioration of episodic memory and the subsequent onset of clinical signs of this dementia observed with advancing age (Prasher et al., 2003; Teipel et al., 2003). Similarly, the fact that most of the subcortical structures and, above all, the posterior cortical areas (pial and occipital lobe) are not morphologically altered has been linked with better performance on tasks of visuospatial processing and visual motor coordination (Krasuski et al., 2002; Pinter, Eliez et al., 2001).

**Neurochemistry**

Neurochemical studies are very scarce and most deal with the changes that people with DS experience when advancing age.

Since its inception these studies found abnormalities in both the brain and the cerebrospinal fluid and blood (Boullin & O’Brien, 1971; Mann, Yates, Marcyniuk & Ravindra, 1985; Scott, Becker & Pettit, 1983). Currently, these early findings have been refined. Following we proceed to describe in more detail the findings that have been obtained in relation to each of the neurotransmitters that appear to be altered in people with DS.

**Serotonergic function**

Early studies revealed a loss of serotonin in the brain, cerebrospinal fluid and blood (Whitaker-Azmitia, 2001). Consistent with these studies, more recent ones noted an earlier peak in the embryonic development of serotonergic receptors within the DS in comparison with the general population followed by a decline below normal levels at birth (Bar-Peled et al., 1991). In addition to reporting a decrease of these receptors in the frontal cortex and in the granular layer of the dentate nucleus in fetuses with DS who are among the 16 and 20 weeks of gestation (Whittle, Simone, Dierssen, Lubec and Singewald, 2007).

These results are particularly relevant, if we take into account that a decrease in serotonin levels in embryos produces a delay in the onset of neurogenesis, reductions in non-serotonergic synapse density and a decrease in brain plasticity (Berger-Sweeney & Hohmann, 1997; Brezun & Daszuta, 1999). Therefore it is expected that the serotonergic reduction experienced by individuals with DS during embryonic development plays an important role in the onset of neuroanatomical alterations presented later. The issue that still is not clear is to what extent this reduction can account for all abnormalities of brain development observed in DS (Whittle et al., 2007).

In adults also appear to exist alterations in serotonin levels. Specifically, higher levels have been found in frontal and occipital regions, as well as lower levels in the thalamus, caudate nucleus, cerebellum and temporal cortex (Gulessiann, Engidawork, Cairns & Lubec, 2000; Mann et al., 1985; Seidl et al., 1999).

Likewise, studies in animals have also provided data supporting the existence of a serotonergic loss in people with DS. For example, studies of mice with overexpression of the S100β gene found an association between overexpression of this gene and serotonin neuron loss of serotonergic neurons in temporal lobe (Salehi et al., 2007; Whitaker-Azmitia, 2001).

Based on these results, pharmacological treatments targeted at people with DS include for some time, serotonergic agents (Whitaker-Azmitia, 2001). Moreover, several studies suggest the usefulness of these in the treatment of self-injurious and aggressive behavior, as well as to produce improvements in cognitive functioning and depressive states (Gedye, 1991; Geldmacher et al., 1997; Hirayama, Kobayashi, Fujita & Fujino, 2004).

**Amino acids group neurotransmitters**

In relation to the levels of the Amino acids group neurotransmitters that present people with DS, only alterations in the levels of GABA and taurine have been found. Specifically, reductions of these levels are found in the frontal cortex during the embryonic period but not in adult life (Whittle et al., 2007).

When considering these reductions, it is important to note that in vitro studies have shown that activation of GABAA receptors promote the neuronal proliferation and differentiation (Represa & Ben-Ari, 2005) and that their antagonists are associated with reductions in neural ramifications (Barbin, Pollard, Gatursa & Ben-Ari, 1993). Similarly, studies in humans and animals have shown that maternal diets deprived of taurine are associated with decreased den-
dritic arborization and atypical cortical development (Whittle et al., 2007). It is therefore likely that these reductions also affect the brain development of people with DS.

**Dopamine**

In the case of dopamine, unlike what happens with levels of GABA and taurine, its reduction has been found both during the embryonic period as well as in adults with DS which show characteristic neuropathological signs of Alzheimer's disease (Whittle et al., 2007). This reduction mainly affects dopaminergic neurons of the ventral tegmental area (Mann & Esiri, 1989).

Dopamine is involved in the establishment of synaptic contacts. For example, has been noted that dopaminergic reductions, achieved by damaging the ventral tegmental area produced reductions in the cortical thickness (Kalmsbeek, Buijs, Hofman, Matthijssen & Pool, 1987). Therefore, it can be assumed that, together with the rest of neurochemical alterations present during the embryonic period, dopamine reduction contributes to the development of the morphological characteristics which exhibit later. In addition, reduced levels of dopamine in adult life, points to the possibility that this reduction is also involved in the onset of neuroanatomical and functional changes associated with Alzheimer's disease in DS.

**Norepinephrine**

Norepinephrine shows a pattern characterized by the presence of normal levels in childhood which are altered with advancing age (Whittle et al., 2007). In fact, it has been found that with advancing age, adults with DS present damage in ascending noradrenergic system, attributed to the loss of neurons in the locus coeruleus. This loss produces reductions of norepinephrine in the hypothalamus, which appears in parallel to the onset of Alzheimer's disease (Yates, Simpson & Gordon, 1986). This could imply that reductions of norepinephrine would also contribute to the appearance of the characteristic neuropathological changes of Alzheimer's disease and its clinical manifestations.

**Acetylcholine**

Acetylcholine does not present alterations neither during the prenatal period nor during childhood. Conversely, in adults with DS there is a loss of cholinergic neurons of the nucleus basalis of Meynert, similar to that observed in people from the general population with Alzheimer's disease, who exhibit a progressive degeneration of neurons that project to the hippocampus (Casanova, Walker, Whitehouse & Price, 1985; Head et al., 2001; Mann et al., 1985; Whitehouse et al., 1982). In animal models, like mice Ts65Dn or mice with overexpression of the APP gene, a similar pattern has been found, i.e.: a preserved cholinergic system in young mice that deteriorates with advancing age (Chang & Gold, 2004; Contestabile et al., 2008; Hunter et al., 2004; Millan et al., 2012; Salehi et al., 2006).

In addition, it has been found that the acetylcholinesterase inhibitors produce improvements in memory during aging (Boada-Rovir, Hernández-Ruiz, Badenas-Homiat, Buendia-Torras & Tárraga-Mestre, 2005; Dong et al., 2005), highlighting the relationship between cholinergic function and memory (Chang & Gold, 2004; Granholm et al., 2000). This relationship could be of great importance when evaluating the cholinergic loss in adults with DS and in adults with Alzheimer's disease (Parajú-Pozo & Casis-Arguea, 2000; Prasher, 2004).

In summary, during the embryonic period we can highlight the presence of alterations in neurotransmitters that affect the brain development. We should note the morphological alterations are not significantly revealed until the first year of life (Capone, 2001; Pinter, Eliez et al., 2001) and that its appearance has been attributed to alterations in the processes of brain development (Becker et al., 1991; Rachidi & Lopes, 2008; Vukie et al., 2002). From these data, we can assume that the observed alterations in the distribution of neurotransmitters are a cause and not a consequence of morphological abnormalities (Carvajal et al., 1994; Whittle et al., 2007).

Specifically, the neurotransmitters altered from the embryonic period are serotonin, GABA, dopamine and taurine. And such alterations would lead to lower total brain volume of the hippocampus, amygdala, cerebellum and brainstem, as well as the alterations in dendritic spines, in the synapses and in the cortical lamination, as the lower count of cortical neurons that characterize individuals with DS.

On the other hand, in light of the consequences that biochemical alterations during embryonic development involve, it is interesting considering the possibility that biochemical changes that occur with advancing age are responsible for the morphological and functional changes experienced by aging persons with DS and with the onset of Alzheimer's disease. Specifically, for now has been demonstrated the involvement of the reductions in the levels of dopamine and norepinephrine, and especially, the reductions in the levels of acetylcholine in the onset of morphological and functional changes that characterize Alzheimer's disease (Millan et al., 2012; Parajú-Pozo & Casis-Arguea, 2000; Whittle et al., 2007).

**Conclusions**

DS is the autosomal chromosomal condition showing a higher prevalence (1 in 800 to 1000 live births) (Brajenovic-Milik et al., 2008; Cocchi et al., 2010; Morris & Alberman, 2009). So its study has a major impact on the field of developmental disorders and particularly within the field of intellectual disability. In fact, this chromosomal condition constitutes approximately 25% of cases of intellectual disability (Serés et al., 2005) and in Spain affects approxi-
that linked the presence of specific neurochemical and neuroanatomical alterations with the strong and weak points that characterize the neuropsychological profile of persons with DS, as well as the changes experienced in their cognitive functioning with advancing age.

Although these conclusions should be regarded with caution since there are few studies that have focused on exploring the relationship between neurobiological data and cognitive performance, we want to finish this article pointing out the enormous progress achieved in recent years in the study of the DS psychobiological correlates. We also want to highlight the importance of incorporating a multidisciplinary perspective in the study of the DS which in the near future allows consider interventions that influence all the nervous system as a whole.

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