Neuropsychological profile of Williams-Beuren syndrome and genetic modulators

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DOCTORAL THESIS UPF / 2015

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A mis padres,
a Héctor, Axel y Elan
a Saúl,
aEkaitz e Inara

AKNOWLEDGEMENTS

A todas las familias que han participado en este estudio. Gracias por vuestra generosidad y enseñarme el significado de la palabra fortaleza.

A todos los niños y adultos con síndrome de Williams por su felicidad, sus abrazos y sus sonrisas.

A Luis, por darme la oportunidad de alcanzar este objetivo. Por enseñarme el valor de la constancia y por su dedicación y enseñanza constante.

A Carme, por tantas cosas; por todo el aprendizaje, por tu refuerzo positivo constante, por tu confianza, por enseñarme a ver las cosas desde otra perspectiva.

A Raquel, por ser una gran compañera de mesa. Por las risas, por tu paciencia. Por sonreír siempre. Gracias por tu apoyo estos años.

A Ivon, por ser estar siempre dispuesta a ayudar.

A Clara, por el tiempo compartido en la consulta multidisciplinar. Ha sido un placer.

Al grupo de genética de la UPF y qGenomics (Judith, Armand, Cristina, Aida, Mariví, Roser, Marta, Andreu, Benja, Olaya, Manel, Sonia y Cristina H.) por el tiempo compartido.

A la Asociación española de síndrome de Williams, Asociación síndrome de Williams Cantabria, Asociación catalana síndrome de Williams, ElisabetGabau, BlancaGener y a Angie por ayudarme a contactar con nuevasfamilias.

A Estrella, Irma, Zahra e Monica. Porque empezamos juntas este gran desafío y aunque hemos seguido caminos separados dentro de él, lo que se creó de esa experiencia siempre estará ahí. Sois unas grandes compañeras de viajes y desafíos.

To Carolyn, for given me the opportunity to work in her lab. It has been a great experience.

To Danielle, you are the best office partner I could ever have! Thank you for the laughs, for all the sharing, for the help, for the time together! You made things easier.

To Holley, Celenia and Myra, for their help and for all the time spend together. It has been a great experience. To Jenny, Adam and all the undergrads students, it was really nice to work with all of you.Thankyou!!

A mis padres y mis hermanos, por estar siempre ahí. Por confiar en mí y apoyarme en cada momento. Gracias por dejarme perseguir mis sueños y enseñarme el valor de dedicarnos a algo que nos apasione.

A Saúl, por ir de mi mano en todas las aventuras de esta vida y esta tesis, sin duda, ha sido una de ellas. Gracias por levantarme cuando tropiezo, por disfrutar conmigo de las alegrías y de saltar conmigo al vacío en cada proyecto.

A Ekaitz e Inara, por ser las personitas que más me enseñan cada día. Porque todo es más fácil cuando vosotros sonreís.

GRACIAS

ABSTRACT

Williams-Beuren syndrome is a neurodevelopmental disorder caused by a heterozygous deletion of 25-27 coding genes in 7q11.23. Despite the great advances on the knowledge of the clinical phenotype and the molecular causes of the syndrome during the last two decades, multiple aspects still remain unknown. The aim of the present work is to deep into the different aspects of the neuropsychological profile of WBS, distinguish syndrome-specific from unspecific symptoms and detect clinical associations and possible causes and modifiers, such as cultural or genetic. The results have provided a description of the main behavioral problems of WBS, how cognitive function, personality or cultural environment can influence behavior, the correlations among lateral dominance patterns and cognition, and finally some genetic modifiers of the profile. A better definition of the profile, the genes involved and the possible modifiers might in the long term lead to better diagnostic tools and targeted treatments and therapies for the specific features of WBS.

RESUMEN

El síndrome de Williams-Beuren es un trastorno del desarrollo causado por una deleción heterozigota de 25-27 genes codificantes en 7q11.23. A pesar de los grandes avances en el conocimiento sobre el fenotipo clínico y las causas moleculares del trastorno, múltiples aspectos todavía se desconocen. El objetivo del trabajo que se presenta es profundizar en los diferentes aspectos del perfil neuropsicológico, distinguir los síntomas clínicos no específicos así como detectar asociaciones y posibles causas y modificadores, como culturales o genéticas. Los resultados proporcionados muestran la descripción de los principales problemas de conducta del SWB, como la función cognitiva, la personalidad o el entorno cultural puede influir en la conducta, las correlaciones entre el patrón de dominancia lateral y la cognición, y finalmente modificadores genéticos del perfil. Una mejor definición del perfil, los genes implicados y los posibles modificadores podría conllevar, a largo plazo, a mejor herramientas diagnósticas y terapias y tratamientos más específicos para el SWB.

PROLOGUE

The neurospychological profile of Williams-Beuren syndrome is associated with high sociability, attention deficits, high levels of anxiety and poor visuospatial skills. Different neuropsychological instruments help to define and assess the profile and additional complications and symptoms. The molecular basis of the syndrome is a deletion that includes 25 to 27 genes being almost identical in the great majority of individuals. Some of the deleted genes are known to be relevant for the neurocognitive profile but little is known about the causes of the significant clinical variability among individuals. Genetic advances and novel molecular techniques, along with detail neuopsychological phenotyping on multiple individuals may be used to define possible genetic modulators of the profile.

This thesis presents the results of trying to contribute to a better description of the neuropsychological profile of Williams-Beuren syndrome and genetic modifiers of the profile.

The present work is divided in several chapters following the classical structure.

In the **introduction** a general overview of the clinical profile, the molecular mechanism and the main genotype-phenotype associations of Williams-Beuren syndrome are described. A brief description of the neuropsychological instruments and molecular techniques used are explained.

Methods section explains the neuropsychological instruments used to assess the neuropsychological profile and molecular analyses performed.

In the **main** body, different **articles** describe and present the different studies performed.

A general **discussion** providing all the obtained results and the possible interpretations is provided. In the final chapter the main **conclusions** of the thesis are summarized.

CONTENTS

LIST OF FIGURES

Introduction

METHODS

CHAPTER 4

LIST OF TABLES

INTRODUCTION

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

INTRODUCTION

1. WILLIAMS-BEUREN SYNDROME

1.1. History

Dr. Williams in 1961, a cardiologist at the Greelane Hospital in Auckland (New Zealand), and his colleagues described four children (figure 1) with the same cardiologic problem (supravalvular aortic stenosis), unusual facial features and Intellectual Disability (ID) (1).

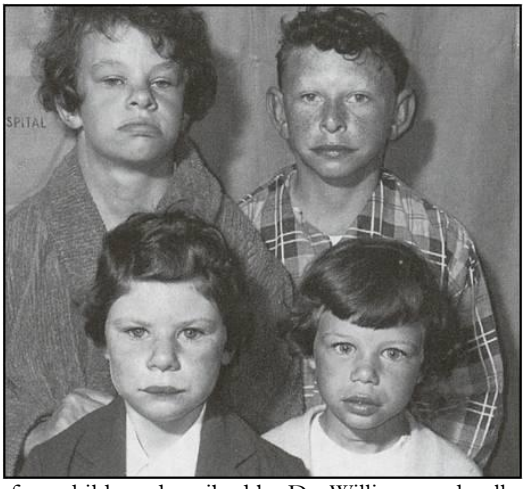

FIGURE 1: First four children described by Dr. Williams and colleagues (1).

The following year, Dr. Beuren, a German pediatrician described four children with the same clinical phenotype: supravalvular aortic stenosis, certain facial resemblance, intellectual disability and high sociability (2). The findings of both groups with the first description of the same neurodevelopmental condition led to the current name of the disorder: Williams-Beuren syndrome (WBS).

1.2. Cause and diagnostic tools

During more than three decades after the first clinical description, the diagnosis was based on clinical features. In 1993, the molecular basis were found after the discovery of a family with supravalvular aortic stenosis but no other features segregating a translocation that disrupted the elastin gene on chromosome 7 (3). Immediately after, hemizygosity at the *ELN* locus due to a larger deletion was demonstrated by fluorescent in situ hybridization (FISH) in patients

with WBS (4), and led to the first laboratory test of the syndrome. Further research in the past years has led to a rather deep knowledge of the molecular cause and mechanisms: WBS is a neurodevelopmental disorder caused by heterozygous deletion of 1.55 million to 1.83 million base pairs (Mb) on chromosome band 7q11.23 (figure 2) (5).

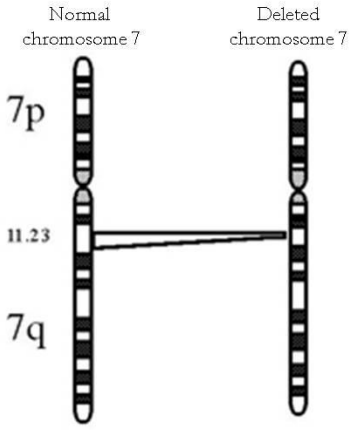

FIGURE 2: Illustration of normal chromosome 7 and deleted chromosome 7 on band 7q11.23 (WBS).

Nowadays, there are different molecular tools available for a confirmatory molecular diagnosis of WBS (figure 3). The increased knowledge and awareness, both of the general populations and the medical community, along with the genetic tools available, have also allowed an earlier recognition of this entity with an average age at diagnosis currently around 3 to 5 years old.

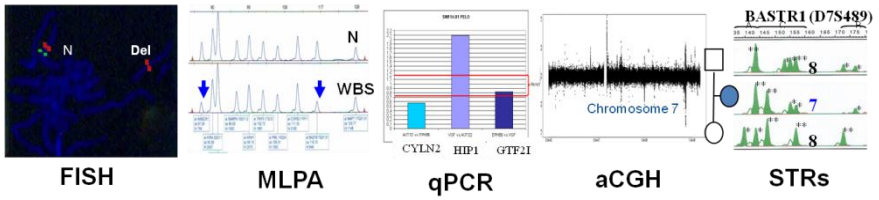

FIGURE 3: Different diagnosis tools used to detect WBS; FISH: Fluorescence in situ Hybridization, MLPA: Multiplex ligation-dependent probe amplification, qPCR: Quantitative polymerase chain reaction, aCGH: array comparative genomic hybridization, STRs: Short tandem repeats

1.3. Prevalence

Data of the occurrence of WBS based on population studies (epidemiology studies) are very limited. There is only a study with follow-up of in Norway estimating a prevalence of WBS of 1 in 7500-10000 newborns (6). Because of its low prevalence it is considered a rare disease (a disease that affects less than 1/2000 of the population).

1.4. Clinical phenotype

A clinical phenotype with specific medical problems and a defined neuropsychological profile, albeit with significant variability, has been well defined since the first four children with WBS were described.

1.4.1. Facial Features

There are some dysmorphic features that complete a characteristic cranio-facial gestalt including flat nasal bridge, short upturned nose, periorbital puffiness, long philtrum, starry iris pattern (generally blue), wide mouth, small chin, small and widely spaced teeth (7).

1.4.2. Cardiovascular problems

Cardiovascular abnormalities, mainly stenosis (narrow) of medium and large arteries, are very common in WBS due to thickening of the vascular media from smoothmuscle overgrowth.

70% of individuals have supravalvular aortic stenosis (SVAS), with a narrowing just above the valve that connects the aorta with the heart (figure 4) (8, 9). The SVAS can range from trivial to severe, requiring corrective surgery in around the 30% of the cases (usually before age 5).

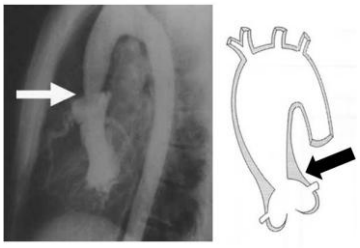

FIGURE 4: Image and drawing of the most common cardiovascular abnormality in WBS: : Supravalvular aortic stenosis (SVAS).

The second most common cardiovascular abnormality is pulmonary artery stenosis (PAS) (10). PAS can be noticed at birth and usually improves spontaneously as children grow.

The arteriopathy is generalized and other stenosis can occur, including aortic arch, coronary, renal, mesenteric or intracranial arteries. Hypertension is also a common problem. Around 50% develop hypertension and need pharmacological treatment (11).

Cardiovascular problems and complications are the major cause of death in the syndrome. Individuals with WBS are at much higher risk of sudden death than the general population (12). They should be placed on regular control of the cardiovascular system during their entire life span.

1.4.3. Endocrinological problems

Growth is usually delayed by a combination of factors. There may be some prenatal growth retardation with low birth weight and length. Infants may also show some delay related to feeding difficulties and occasional gastrointestinal problems. Later, puberty usually starts a couple of years earlier and the growth spur is shorter. Then, the final height of WBS individuals is 10-15 cm shorter than expected for the family (13).

Transient hypercalcemia, which was one of the features in the initial description of the syndrome, may occur generally during the first 18 months of life. A mild abnormality of the calcium metabolism with delayed clearance of calcium overload can persist into adulthood (14, 15). A possible complication of persistent hypercalcemia is nephrocalcinosis.

Around 15 to 30% of individuals with WBS present subclinical hypothyroidism, with normal thyroid hormone and mildly elevated thyroid-stimulating hormone (TSH), which usually remains stable during the years and does not require clinical intervention (16).

Another usual endocrine problem in adults with WBS is impaired glucose tolerance, either a pre-diabetes status or frank diabetes mellitus (17).

1.4.4. Other common medical problems

In early childhood, babies suffer hypotonia, feeding difficulties, infantile colic and constipation (that can be present during all the life span). In a high percentage hernias are presented, essentially inguinal hernia, that need surgery during the first year of life (18).

Other medical problems that occur during infancy are chronic otitis media, dental problems (85% have malocclusion) and ophthalmologic problems (e.g. esotropia, hyperopia) (19, 20). Sleep disturbance is also a common problem and concern during childhood and continues to be a complaint during adolescents and young adults (21, 22). Young children and adults can present musculoskeletal problems, usually lordosis and scoliosis (23).

Some urinary tract abnormalities occur more often in the syndrome than in the general population such as renal structural defects, bladder diverticulae, and nephrocalcinosis. 50% of the children present enuresis while 30% of the adults have recurrent urinary tract infection (24).

Finally, a quite specific manifestation of WBS, most likely of neurologic origin, is hyperacusis or odinoacusis. Children and adults with WBS show oversensitivity to sounds (25).

1.4.5. Neuropsychological phenotype

Profile is characterized by some relative strengths and weaknesses: strength in auditory rote memory, and in select aspects of language, and remarkable weakness in visuospatial and visuomotor skills (26).

1.4.5.1. Cognitive profile

Standardized testing demonstrates a full-scale intelligence quotient (IQ) averaging 50–60, indicative of mild to moderate ID in most cases, though IQs range from 40 to 100 (although only very few individuals score higher than 70) (27). Usually, verbal IQ scores are higher than performance IQ, in some cases with significant among scores.

Although few longitudinal studies have been conducted, results show almost identical mean IQ with less progress relative to their general population peers and little evidence of regression (28,29,30).

1.4.5.2. Visuospatial problems

Visuospatial construction, generally assessed by drawing or pattern construction tasks is an extreme weakness in the profile (figure 5) (31). Specific deficits on visuospatial processing with on poor mental imagery and poor orientation, discrimination and spatial representational processes have been described (32, 33, 34). With aging, skills show improvement but remain generally poor and became a special factor to considerer during learning and everyday activities.

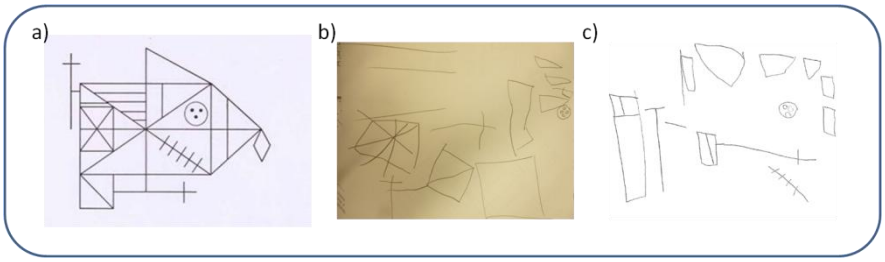

FIGURE 5: Rey-Osterrieth Complex Figure Test (a, from (35)) copy condition in two adults with WBS (b & c).

1.4.5.3. Personality and social behavior profile

A distinctive personality profile with high sociability, empathy and excessive anxiety has been described. Children are anxious to establish interactions with other individuals (children or adults), are frequently noticed by others and show a high emotional empathy and/or sensitivity to other people's emotions (36). This distinctive personality pattern changes with aging. Adults generally present lower extraversion and lower emotional stability (37).

High sociability behavior is often inappropriate because of excessive approach to others and difficulties in social adjustment or understanding. This behavior leads, especially during adulthood, to social vulnerability (38, 39).

1.4.5.4. Language profile

Language development is delayed, in part because of motor delays (40). Development trajectory of the language has shown typical pattern for verbal comprehension, phrase repetition, mean length utterance and for object categorization and atypical trajectory for phonological processing and morphology appear (27).

As mention before, language is considered a relative strength in the syndrome. Children are unusually loquacious and highly expressive compared with mental-age peers (41). On narrative tasks, children and adolescents include remarkable language of affective and motivation of the characters and try to engage the audience's attention (42). Overall language expression level is in almost all the cases higher than overall comprehension level. Although loquacious abilities, children present often stereotypical conversation and inappropriate initiate conversations than individuals (43).

As the overall neuropsychological profile, language profile presents some strengths and weaknesses; concrete vocabulary and phonological skills are relative strengths while grammatical abilities, relational language and pragmatics (taking into account the overall intellectual abilities) are described weaknesses (44).

For the overall cognitive profile, language abilities present a clear dissociation with visuospatial abilities (figure 6).

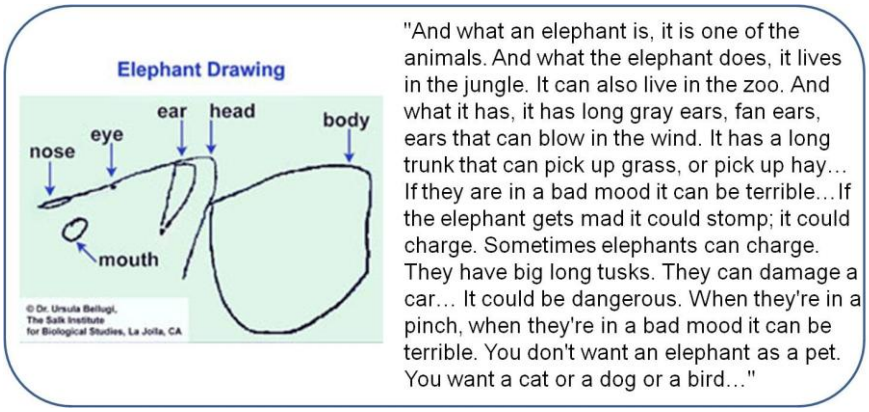

FIGURE 6:Dissociation between language and spatial cognition: drawing and description of an elephant made by a 15 year old with WBS (IQ of 49). From (45).

1.4.5.5. Behavior and emotional profile

Research on behavior and emotional problems in the syndrome have reported to show overall more problems than other children with ID (46). Attention Deficit Hyperactivity Disorder (ADHD), generally Inattentive, is the most common diagnosis by clinical interviews during childhood. 65% of the children meet Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for clinical diagnosis (47).

Anxiety disorders are really common in WBS, with Specific Phobia and Generalized Anxiety Disorder (GAD) being the most prevalent diagnosis. 53 % of children meet criteria diagnosis for Specific Phobia and longitudinal studies reveal that 82% of the children met at some point of their childhood diagnostic criteria for an anxiety disorder (48). Low prevalence of externalizing behavior problems has been reported.

Emotional and behavior problems persist in adulthood (49). During adolescent and in adulthood depression symptoms can appear, in part related with individual's social adjustment (social isolation) (50).

Restricted and repetitive behaviors (RRB) and restricted interests have also been described in children (51, 52).

As in other neurodevelopmental disorders, prevalence of Autism Spectrum Disorder (ASD) is higher than in general population with an estimation of 12% (53).

1.4.5.6. Executive function profile

Impairments in different measures of executive function have been reported recently in individuals with WBS. Inhibition deficits, poor planning and global impairment of working memory components are some of the most described impairments (54, 55, 56). These deficits have been associated with the personality and behavioral profile. One of the most common relations described is high social behavior probably related with an inhibitory deficit for social response (57, 58).

1.4.5.7. Lateral preference

Atypical lateral preference with higher prevalence of left handedness compared to general population has been described on WBS. A casual link between lateral preference and cognition has been suggested (59).

1.4.6. Special musical abilities

Music abilities have been considered as a special trait in the syndrome. Children and adults with WBS generally display high musical interest with higher music skills than overall cognitive abilities (60). They show high enjoyment to music and display higher emotional response to it (61). This fact makes music as a great therapy tool. Music therapy is commonly used to reduced anxiety and in educational settings as a tool for learning different concepts.

Although results are not conclusive, higher prevalence of absolute pitch in the syndrome than in general population has been suggested (62).

1.4.7. Neurological phenotype

Neurological phenotype, based on studies done by magnetic resonance imaging (MRI), has revealed that cerebral volume is reduced around 10 to 15% (63). Image studies have also shown disproportionately reduced areas such as right and left superior parietal lobes, right occipital lobe and brainstem (64, 65). On the other hand, frontal and temporal limbic structures and superior temporal gyrus seem to be relatively preserved areas (63).

The most common structural abnormality associated to the syndrome with estimated prevalence of 10 % is Arnold Chiari type I (66).

Amygdala dysfunction has been brain area suggested to be related to high sociability (67). On tasks showing different stimuli of pictures displaying happy, fearful and neutral expressions individuals with WBS present heightened amygdala response to happy faces and diminished amygdala response to negative facial expressions (68, 69).

Although the neurological profile is not associated with severe neurological problems, mild neurological signs involving cerebellar functions and the extrapyramidal system have been described (70).

2. MOLECULAR BASIS OF WILLIAMS-BEUREN SYNDROME

WBS is one of the so called "genomic disorders" or "contiguous gene syndromes", which are due to recurrent rearrangements that occur in several chromosomal regions facilitated by the local structure of the human genome. There have been tremendous advances in the understanding of the mechanisms underlying the occurrence of these genomic disorders (71).

Segmental duplications are large blocks of DNA that share high level of sequence identity (>95%). The segmental duplications encompass around 5% of the human genome and have been generated during the hominoid evolution, with many of them being human-specific. In addition to been driving forces that contribute to speciation and intra-species variation, the regions enriched in segmental duplications predispose the local genome to additional rearrangements (72).

2.1. The Williams-Beuren syndrome deletion

WBS is a model genomic disorder caused by a recurrent heterozygous deletion on chromosome band 7q11.23 that encompasses 1.55 to 1.83 Mb and contains 26-28 single-copy genes (5). The Williams-Beuren syndrome critical region (WBSCR) were deletions occur has a complex genomic architecture, including a 1.2 Mb single copy interval flanked by a complex set of segmental duplications specific of the chromosome 7. These spontaneous chromosomal rearrangements are almost always sporadic and arise from unequal crossover during the meiosis.

2.1.1 Mechanisms of deletions

WBS deletions occur by the wrong alignment between segmental duplications that facilitate unequal recombination during meiosis between non-allelic blocks of paralogous sequences (figure 7)(73). This mechanism of non-allelic homologous recombination (NAHR) is common to other genomic disorders. In WBS, NAHR may occur inter-chromosomally in the 66% of the cases, while it is intra-chromosomal (inter-chromatid) in the 34% of the cases)

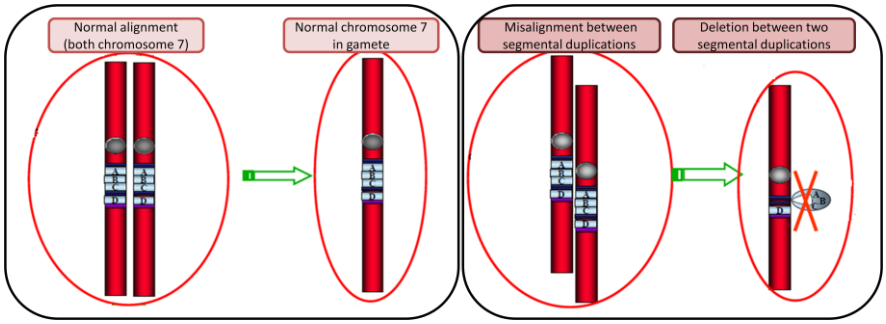

FIGURE 7:A. Schematic representation of the normal pairing of the two chromosome 7 homologs during meiosis, with the resulting segregation of an entire chromosome 7 to the gamete. B. Representation of abnormal pairing of the two chromosome 7 homologs mediated by the 7q11.23 segmental duplications (dark blue and the resulting gamete carrying a chromosome 7 with the WBS deletion (A-C light blue squares) after NAHR.

Chromosomal breakpoints have been determined with reasonable accuracy in individuals with WBS and are located within the segmental duplications. These segmental duplications are about 300-400 kilobases (kb) of length, and each of them is made of three blocks called A, B, C (74). Most of the deletions (1,55 Mb) happen between concrete blocks (Bc/Bm), which are in tandem, share >99.5% sequence identity, and contain three genes (*GTF2I*, *NCF1* and *GTF2IRD2*) in the medial position (figure 8). In around 10% of the cases, de recombination happens between blocks Ac/Am (75), with deletions 1,83 Mb in size. The majority of cases of WBS are sporadic, indicating a high rate of formation of the novo deletions ~ 0.5x10-4 per gamete and per generation.

A few exceptional patients have been described with deletions affecting the WBSCR either smaller o larger. These deletions are non-recurrent and all these patients display atypical phenotypes partially overlapping with WBS features, less severe in smaller deletions and more severe in larger deletions, as expected.

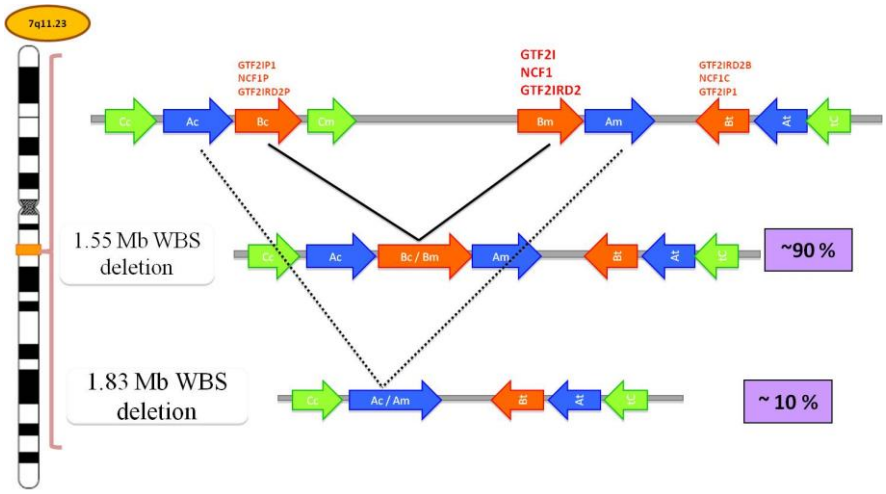

FIGURE 8:On the left, there is and ideogram of the chromosome 7 showing the 7q11.23 band that is expanded and represented on the right. The illustration on the right depicts the blocks of segmental duplications as colored arrows that indicate their relative orientation. Deletions mediated by blocks B (red), 1.55 Mb in size, and deletions mediated by blocks A (blue, 1.83 Mb, are shown along with their frequency.

2.1.2. Genetic variants that predispose to the deletion

The most common variant found in the transmitter parents is an inversion of the all the interval (around 2Mb) between external segmental duplications (figure 9). Inversion-mediated deletions have been reported to account for approximately 25% of patients (76, 5), while the estimated population frequency of this inversion polymorphism is 4-5%. Inversion carriers have no obvious phenotype, although heterozygosis for the inversion represents an additional risk factor for occurrence of the WBS deletion in the sperm or egg of the person. The estimated risk for individuals with an inversion of having a child with WBS is around 1 in 1500, a 5-10 fold increase over the population risk.

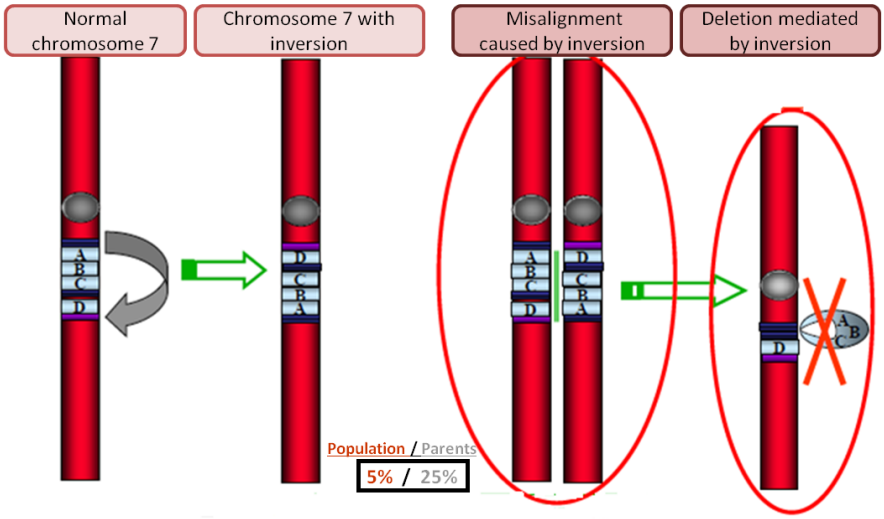

FIGURE 9:: Illustration of the mechanism generating the inversion polymorphism by intra-chromosomal rearrangement between the external blocks of segmental duplications (left). In a heterozygous carrier of the inversion polymorphism, the likelihood of meiotic misalignment of chromosome 7 homologs increases, leading to a deletion of the 7q11.23 region when NAHR occurs at specific locations (right).

Other described variants present in around 5% of the transmitting parents, also found in 1% of the general population, are large copy number variants (CNVs), either deletions or duplications at the flanking segmental duplications (77).

2.2. Genes within the WBSCR and function

Depending on the breakpoints, a total of 25 to 27protein-coding genes are included in the recurrent WBS deletions. There are also two microRNAs (*MIR4284* and *MIR590*) and two anti-sense non-coding transcripts (*ABDH11-AS1* and *LOC101926943*). During the past years the function of many of these genes has been discovered.

TABLE 1:Protein-coding genes deleted in the Williams Beuren syndrome and function (From NCBI/Genecards)

Official Symbol	Official Full Name	Function (defined or predicted)
NSUN5	NOP2/Sun domain family, member 5	This gene encodes a member of an evolutionarily conserved family of proteins that may function as methyltransferases. There are two pseudogenes for this gene located in the same region of chromosome 7.
TRIM50	Tripartite motif containing 50	The protein encoded by this gene, E3 ubiquitin-protein ligase, is a member of the tripartite motif (TRIM) family. The TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region.
FKBP6	FK506 binding protein 6, 36kDa	The protein encoded by this gene is a cis-trans peptidyl-prolyl isomerase that may function in immunoregulation and basic cellular processes involving protein folding and trafficking.
FZD9	Frizzled class receptor 9	Members of the 'frizzled' gene family encode 7-transmembrane domain proteins that are receptors for Wnt signaling proteins. FZD9 is expressed predominantly in brain, testis, eye, skeletal muscle, and kidney.
BAZ1B	Bromodomain adjacent to zinc finger domain, 1B	This gene encodes a member of the bromodomain protein family. The bromodomain is a structural motif characteristic of proteins involved in chromatin-dependent regulation of transcription.

BCL7B	B-cell CLL/lymphoma 7B	This gene encodes a member of the BCL7 family including BCL7A, BCL7B and BCL7C proteins. This member is BCL7B, which contains a region that is highly similar to the N-terminal segment of BCL7A or BCL7C proteins. The BCL7A protein is encoded by the gene known to be directly involved in a three-way gene translocation in a Burkitt lymphoma cell line.
TBL2	Transducin (beta)-like 2	This gene encodes a member of the beta-transducin protein family. Most proteins of the beta-transducin family are involved in regulatory functions. This protein is possibly involved in some intracellular signaling pathway.
MLXIPL	MLX interacting protein-like	This gene encodes a basic helix-loop-helix leucine zipper transcription factor of the Myc/Max/Mad superfamily. This protein forms a heterodimeric complex and binds and activates, in a glucose-dependent manner, carbohydrate response element (ChoRE) motifs in the promoters of triglyceride synthesis genes.
VPS37D	Vacuolar protein sorting 37 homolog D (S. cerevisiae)	VPS37D is a component of the ESCRT-I complex, a regulator of vesicular trafficking process. Required for the sorting of endocytic ubiquitinated cargos into multivesicular bodies. May be involved in cell growth and differentiation.
DNAJC30	DnaJ (Hsp40) homolog, subfamily C, member 30	This intronless gene encodes a member of the DNAJ molecular chaperone homology domain-containing protein family.
WBSCR22	Williams Beuren syndrome chromosome region 22	This gene encodes a protein containing a nuclear localization signal and an S-adenosyl-L-methionine binding motif typical of methyltransferases, suggesting that the encoded protein may act on DNA methylation.
STX1A	Syntaxin 1A (brain)	This gene encodes a member of the syntaxin superfamily. Syntaxins are nervous system-specific proteins implicated in the docking of synaptic vesicles with the presynaptic plasma membrane. Syntaxins possess a single C-terminal transmembrane domain, a SNARE [Soluble NSF (N-ethylmaleimide-sensitive fusion protein)-Attachment protein Receptor] domain (known as H3), and an N-

		terminal regulatory domain (Habc). Syntaxins bind synaptotagmin in a calcium-dependent fashion and interact with voltage dependent calcium and potassium channels via the C-terminal H3 domain. This gene product is a key molecule in ion channel regulation and synaptic exocytosis.
ABHD11	Abhydrolase domain containing 11	This gene encodes a protein containing an alpha/beta hydrolase fold domain.
CLDN3	Claudin-3	The protein encoded by this intronless gene, a member of the claudin family, is an integral membrane protein and a component of the epithelial cell tight junction strands, which regulate movement of solutes and ions through the paracellular space. It is also a low-affinity receptor for Clostridium perfringens enterotoxin, and shares sequence similarity with an apoptosis-related protein found in rat.
CLDN4	Claudin-4	Anotherintronless gene encoding aclaudin family member.
WBSCR27	Williams Beuren syndrome chromosome region 27	This gene encodes a protein belonging to ubiE/COQ5 methyltransferase family.
WBSCR28	Williams-Beuren syndrome chromosome region 28	This gene encodes a 265 amino acids protein of unknown function.
ELN	Elastin	Elastin is one of the two components of elastic fibers. It is a protein rich in hydrophobic amino acids such as glycine and proline, which form mobile hydrophobic regions bounded by crosslinks between lysine residues. Deletions and mutations in this gene are associated with supravalvular aortic stenosis (SVAS) and autosomal dominant cutis laxa.
LIMK1	LIM domain kinase 1	LIMK1 is a serine/threonine kinase that regulates actin polymerization via phosphorylation and inactivation of the actin binding factor cofilin. This protein is

		ubiquitously expressed during development and plays a role in many cellular processes associated with cytoskeletal structure. This protein also stimulates axon growth and may play a role in brain development.
EIF4H	Eukaryotic translation initiation factor 4H	This gene encodes one of the translation initiation factors, which functions to stimulate the initiation of protein synthesis at the level of Mrna utilization.
LAT2	Linker for activation of T cells family, member 2	This gene consists of at least 14 exons, and its alternative splicing generates 3 transcript variants.
RFC2	Replication factor C (activator 1) 2, 40kDa	The elongation of primed DNA templates by DNA polymerase delta and epsilon requires the action of the accessory proteins, proliferating cell nuclear antigen (PCNA) and replication factor C (RFC). Replication factor C, also called activator 1, is a protein complex consisting of five distinct subunits. RFC2 is the 40Kd subunit responsible for binding ATP and that may help promote cell survival.
CLIP2	CAP-GLY domain containing linker protein 2	The protein encoded by this gene belongs to the family of cytoplasmic linker proteins, which have been proposed to mediate the interaction between specific membranous organelles and microtubules. This protein was found to associate with both microtubules and an organelle called the dendritic lamellar body.
GTF2IRD1	GTF2I repeat domain containing 1	The protein encoded by this gene contains five GTF2I-like repeats and each repeat possesses a potential helix-loop-helix (HLH) motif. It may have the ability to interact with other HLH-proteins and function as a transcription factor or as a positive transcriptional regulator under the control of Retinoblastoma protein
GTF2I	general transcription factor Iii	This gene encodes TFII-I a phosphoprotein containing six characteristic repeat motifs. The encoded protein binds to the initiator element (Inr) and E-box element in promoters and functions as a regulator of transcription.
NCF1	neutrophil cytosolic factor 1	The protein encoded by this gene is a 47 kDa cytosolic subunit of neutrophil NADPH oxidase. This oxidase is a multicomponent enzyme that is activated to

		produce superoxide anion.
GTF2IRD2	GTF2I repeat domain containing 2	This gene is one of several closely related genes on chromosome 7 encoding proteins containing helix-loop-helix motifs. These proteins may function as regulators of transcription. The encoded protein is unique in that its C-terminus is derived from CHARLIE8 transposable element sequence.

2.3. Genotype-phenotype relationships in Williams-Beuren syndrome

During the last years research has tried to find clinical-molecular correlations with the aim of understanding which gene or genes are responsible for each aspect of the clinical phenotype of the syndrome. Although the deletions include multiple genes, only those that are sensitive to dosage (that show haploinsufficiency) will contribute to the phenotype. Comprehending molecular data will lead to more effective treatments for children and adults with WBS. A phenotypic map of the 7q11.23 deletion region is being defined based on studies in subjects with partial deletions of the WBSCR and partial phenotypes (78, 79), as well as with the analysis of the generated mouse models (80). However, other than for the cardiovascular and some of the neurobehavioral phenotypes, many of the associations are still weak and the main genes and pathways responsible for other aspects of the WBS phenotype have not been completely defined (figure 10). In addition, it is likely that most clinical manifestation cannot be attributed to specific genes in a simplistic manner. Additive effects of haploinsufficiency for deleted genes along with other genetic and environmental factors contribute to the final phenotype.

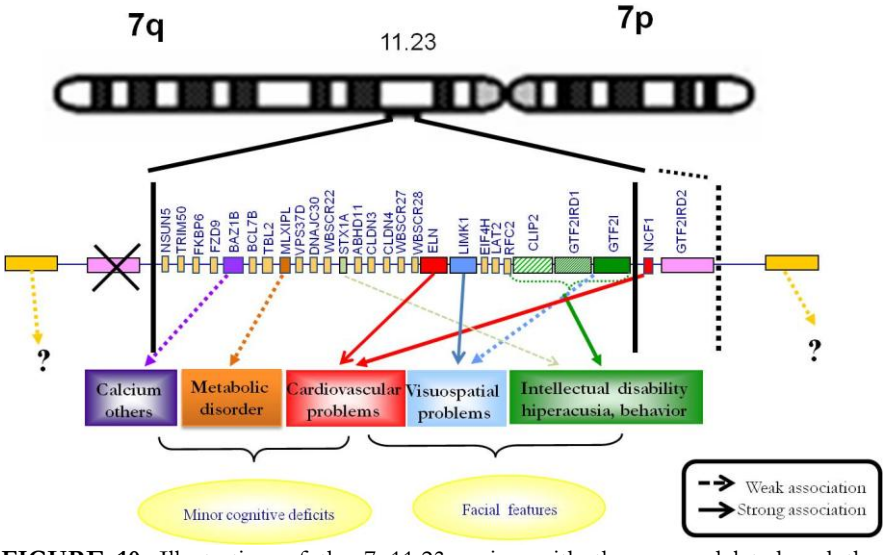

FIGURE 10: Illustration of the 7q11.23 region with the genes deleted and the morbid map representing the genotype/phenotype associations.

2.3.1. Cardiovascular phenotype: *ELN* and *NCF1*

The first gene linked to the specific phenotype was the gene coding elastin (*ELN*). Deletion, disruption or mutation of this gene causes the cardiovascular and connective tissue manifestations of the syndrome.

Elastin is the major component of elastic fibers, which are slender bundles of proteins that provide strength and flexibility to connective tissue (tissue that supports the body's joints and organs). In the clinical profile, *ELN* gene has been demonstrated to be the main responsible for the cardiovascular problems in individuals with WBS (4). Elastin deficiency due to the genomic deletion leads to deficient elastic fiber formation and increased smooth muscle in the arterial walls as compensatory mechanisms during development. Arterial wall stiffness causes narrowing at several sites and the risk of arterial hypertension.

Probably, the deletion of *ELN* is also responsible for other problems of the connective tissue that occur in the syndrome, including some facial features (tissue increase in the periorbital zone and lips), inguinal hernias and the possibility of diverticulosis in the wall of the bladder and/or colon.

More recently, deletion of a functional *NCF1* gene copy has been shown to protect a proportion of WBS patients against hypertension (81). *NCF1* encodes one subunit of the NADPH-oxidase; decreased *NCF1* function might exert the protective role by decreasing long-life oxidative stress (81).

2.3.2. Neuropsychological phenotype: *LIMK1, STX1A, GTF2I, GTF2IRD1, GTF2IRD2.*

Based on studies performed in subjects with partial deletions of the region as well as in mouse models, the genes at the distal part of the deletion (*GTF2I, GTF2IRD1*) have been proposed as the main contributors to the neuropsychological profile of the syndrome. Other two genes, *LIMK1* and *STX1A,* might also be responsible of some neurobehavioral features. *LIMK1* has been controversially associated with deficits in spatial cognition (82), and *STX1A* has been suggested

to be a component of the cellular pathway modifying human intelligence in WBS (83).

The strongest association for the neurocognitive profile has been found with *GTF2I* and *GTF2IRD1* (79; 84). Not only cognition but also other traits of the neuropsychological profile have been related to these two genes. *GFT2IRD1* has been associated with visual special construction and *GTF2I* has been suggested to contribute to WBS social behavior (85). In knock out mice, *Gtf2ird1* has been associated with motor coordination and anxiety (86) and to auditory threshold (87). Finally, the main contribution of *GTF2I* to the neurodevelopmental and cognitive abnormalities of WBS has been validated in studies in mice, showing that restoring Gtf2i expression levels in specific brain areas of mica with a complete deletion of the interval can rescue most aspects of the phenotype (88, 89).

Recent data have suggested that *GTF2IRD2*, a gene belonging to the same family of transcriptional regulators as *GTF2I* and *GTF2IRD1*, could modulate many of the key features of WBS (mainly neurobehavioral). *GTF2IRD2* (GTF2I repeat domain containing 2) may or not be affected by the deletion depending on deletion breakpoints and it is likely a modifier of the function of structurally related genes included in the common WBS critical region (*GTF2I* and *GTF2IRD1*) (90). A previous report has shown that this gene might be related with differences in executive function among WBS individuals (91).

2.3.3. Other associations

MLXIPL, and also *STX1A*, have been proposed to play a role in the metabolic abnormalities in the syndrome (92). *BAZ1* encodes a protein that acts on the chromatin remodeling required for promoter activation of the vitamin D receptor, which might participate in the regulation of calcium metabolism (93). Interestingly, a genome-wide epigenetic dysfunction secondary to the haploinsufficiency of some of the genes involved in chromatin remodeling has been implicated in the possible pathogenesis of WBS (94).

3. INFLUENCES AND MODIFIERS OF THE WBS NEUROPSYCHOLOGICAL PROFILE

As in other neurodevelopmental disorders, WBS shows clinical variability among children and adults. This variability is probably due to environmental and genetics influences and/or modifiers.

3.1. Possible environmental modifiers of the phenotype and interrelationships of features

Many biological and psychosocial risk factors that occur during prenatally and early childhood have been described to compromises children's development, having effects on brain structure and function (95). Prenatal infections or drug exposure, early life medical severe complications or life events are some examples of recognize factors modifiers of children's development.

One well established factor influencing children's cognitive development is family environment. Parents care and stimulation during childhood has direct influence on neurospychological profile. An example of a defined associated environmental factor is of the families socioeconomic status (SES)(96). As results found on typical developing children, maternal education has been found to influence on verbal intelligence in WBS (97). Children with mothers with higher education levels present higher verbal IQ.

Finally, other fact to take into account when trying to define and understand the neuropsychological profile in WBS is interrelationship between cognitive and psychological features. Distinctive personality, IQ and executive function problems are probably related to behavior in the syndrome.

3.2. Possible genetic modifiers

There are several possible genetic modifiers of the clinical phenotype caused by a genomic disorder such as WBS. Of course, the finding of different deletion sizes could be used to define the small region of overlap responsible for the relevant phenotypic features. This strategy has been used with the few patients reported having smaller deletions and atypical phenotypes (figure 11, C). However, since deletion size is

very similar among WBS individuals despite significant clinical variability, other approaches are required for genotype-phenotype relations. Other possible modulators could be genetic variants in the not deleted allele (figure 11, A) or in the same allele nearby (figure 11, B), as well as more subtle variation at the specific breakpoint. The parent origin could also have an affect on the profile (figure 11, D). Finally, genetic variants elsewhere in the genome, such as single nucleotide polymorphisms, (SNPs) or copy number variants, could also influence in the clinical phenotype (figure 11, D).

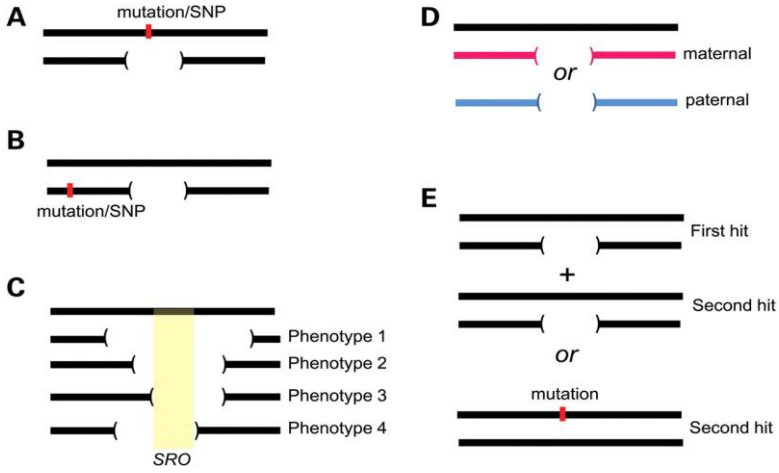

FIGURE 11: Illustration of possible causes for phenotypic variability; A: recessive mutations or functional polymorphism within the CNV region; B: Single-nucleic changes altering the expression pattern of the genes in the region, C: different deletion size, D: effects by parent of origin, E: second hits models (From (70)).

4.THE 7q11.23 MICRODUPLICATION SYNDROME

4.1. History

The first individual with 7q11.23 duplication syndrome (Dup7, the reciprocal duplication of the WBS region) was described 10 years ago. The child presented mild physical manifestations (mild dysmorphic features and growth retardation) along with a severe delay in expressive language (98). Since then, other cases reports have been published describing 45 children and 15 adults and a larger study with 64 individuals with Dup7. Some of the cases reported were foun screening for diagnosis in populations with ASD (99) and schizophrenia (100). All these studies have provided preliminary phenotype description.

4.2. Clinical phenotype

Facial features include brachycephaly, broad forehead, straight eyebrows and deep set eyes, broad nasal tip, micrognathia, short philtrum, thin upper lip, minor ear anomalies, and facial asymmetry (101, 102). Cardiovascular abnormalities, mainly an aortic dilation and patent ductus arteriosus,, have been reported in several cases, although the natural history and possible complications remain unknown (103, 104). Other medical problems described in several individuals are seizures, growth hormone deficiency, constipation, and structural renal abnormalities (105).

The neuropsychological profile is somehow the opposite of WBS. Cognitive abilities range from moderate intellectual disability to high average ability although generally individuals with Dup7 are in the low average range. A phenotypic characteristic is severe speech delay and autistic features (106, 107, 108).

The largest sample of individuals with Dup7 assessed (63 children, 16 toddler and 12 adults) to define the neuropsychological profile demonstrated that most of the children met criteria of at least one anxiety disorder other than Specific Phobia, with Social Phobia and Selective Mutism most common diagnosis (109). One-third met criteria for ADHD and one-fourth was diagnosed with Oppositional

Defiant Disorder (ODD) or Disruptive Behavior Disorder—Not Otherwise Specified (DBD-NOS).

Many individuals with Dup7 showed abnormalities in brain MRI studies, such as decreased cerebral white matter volume, cerebellar vermis hypoplasia, and ventriculomegaly (105, 110).

4.3. Molecular basis

Dup7 is caused by duplications of identical size of the WBS deletions. Duplications are generated by the exact reciprocal mechanism of the deletions, and are expected to occur with similar frequency, mostly through inter-chromosomal events. Even though the research has not yet advanced as much as in WBS, the genes and pathways implicated in Dup7 pathogenesis are thought to be the same as in WBS by reciprocal dosage changes. Reciprocal genome-wide epigenetic dysfunction was also detected in Dup7 individuals when compared to WBS(94).

METHODS

1. INSTRUMENTS USED FOR THE NEUROPSYCHOLOGICAL PHENOTYPE

Neuropsychological profile of the syndrome can be assessed by different instruments available. Instruments used in this work are exposed.

1.1. Intelligence quotient

Different instruments assess cognitive abilities. One of the most common instruments used are the Wechsler Intelligence Scales. Scales differ by individual's age. The Wechsler Preschool and Primary Scale of Intelligence III (WPPSI–III) (111) is designed for children from 2 years 6months to 7 years and 7 months. The Wechsler Intelligence Scale for Children-R (WISC-R) (112) is a battery of tests for children aged 6 to 16 years old. Finally, the Wechsler Adult Intelligence Scale III (WAIS-III) (113) is used for individuals older than 16 years old. Different subtests are assessed individually. Subtest scaled scores have a mean of 10 and a standard deviation of 3 while quotient and composite scores have a mean of 100 and a standard deviation of 15. All three scales provide Verbal and Performance IQ scores as well as a Full Scale IQ score.

Differential Ability Scales (DAS) (114) is another common instrument used to assess intellectual abilities in WBS. Two forms are available by age; the Early Years form is administered to children from 4 to 8 years old and the School Age form to children from 9 to 17 years old. Instrument is divided in six core subtests that are slip up into three clusters of two subtests each: Verbal, Nonverbal Reasoning, and Spatial. The General Conceptual Ability (GCA; similar to Full-Scale IQ) is derived from performance on the six core subtests, and the Special Nonverbal Composite (SNC; similar to Performance IQ) is based on performance on the four core subtests included in the Nonverbal Reasoning and Spatial clusters.

1.2. Behavior problems

Child Behavior Checklist 4-18 & 6-18 (CBCL) (115, 116) is a questionnaire that assesses children's behavioral and emotional problems. Parents are asked on 113 items to score on a 3 point Likert scale (with 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true) different possible behavior o emotional problems score.

Based on factor analyses CBCL includes empirically based syndrome scales: Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Rule-Breaking Behavior and Aggressive Behavior. Intrument in the 2001 version also provides six DSM-oriented scales: Depressive Problems, Anxiety Problems, Somatic Problems, Attention Deficit/Hyperactivity Problems, Oppositional Defiant Problems and Conduct Problems. Raw scores are converted into T scores taking into account age (6-11 and 12-18) and gender. Normal range is assigned to T scores of 64 or lower. Scores of 65 to 69 are considered in the borderline range and T scores of 70 or higher are in the clinical range.

Three higher-order factor scales: Internalizing Problems (Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints), Externalizing Problems (Rule-breaking Behavior, Aggressive Behavior) and Total Problems (with all items of the instrument).

From Child Behavior Checklist 4-18 to 6-18 six items changed.

1.3. Visuospatial abilities

The *Rey*-Osterrieth Complex *Figure* Test (ROCF) (35, 117) evaluates visuospatial constructional ability and visual memory. The ROCF consists in three test condition; copy, immediate recall and delayed recall. For the copy condition, subjects are given the ROCF stimulus card and are asked to draw the exactly the same figure.

1.4. Lateral dominance

Lateral dominance can be measured by different instruments. One of the most common ways is to asses by asking the subject to perform different tasks or activities in situ. Eye, hand, foot and ear dominance

is measured with different materials by defining tasks that require skills and that are not influence by other facts.

1.5. Sociability (approach to strangers)

The approachability task was a task created to assess social judgment (118). Modified version of the task was made for WBS (119) selecting 42 photographs of unfamiliar human faces from the original set of 100 stimuli. The selection was made based on those that had previously received the most negative, and the most positive, ratings from general population. The final instrument contains a global of forty-two stimuli (black and white photographs of unfamiliar adult faces) that are presented to be rated. Upon seeing each photograph, subjects are asked to rate how much they would like to go up to each person and begin a conversation with them. Response ratings are given on a five-point color-coded Likert scale (individuals need to point the response). Higher scores denoting a greater desire to approach and talk to the person. Each response is coded numerically on a scale from -2 to +2. Before beginning the task, subjects are familiarized with the rating scale using a two sample stimuli.

1.6. Language

1.6.1. Narrative production

"Frog, where are you?" is a wordless picture book that describes a boy and his dog looking for their lost frog (120). The book has been previously use as a narrative production instrument in WBS with criteria for its assessment (121, 122, 123).

1.6.2. Receptive vocabulary

The Peabody Picture Vocabulary Test, fourth edition (PPVT-4) (124) measures the receptive vocabulary in children and adults for ages 2 years 6 months to 90+. Instrument has 228 items distributed across 19 items sets. Examiner presents an item with 4 pictures and reads a word describing one of the pictures shown. The subject needs to point which picture describes the word that has said.

1.6.3. Expressive vocabulary

Expressive Vocabulary Test, second edition (EVT-2) (125) is a measure of expressive vocabulary and word retrieval for ages 2-90. Forms (parallel forms A and B) are administered individually and contain 190 test items in increasing difficulty. Examiner presents each item (picture) and reds a stimulus question. Subjects responds with one word that considers fits the picture.

1.7. Executive function

Several instruments have been created to assess executive Function. One possible way to evaluate executive function problems is the Behavior Rating Inventory of Executive Function Parent form (BRIEF-P)(126). The inventory is an 86 items questionnaire indicating how frequently a behavior occurs (never, sometimes, often) design to be completed by one of the caregivers (usually mother or father). Instrument provides eight clinical scales: Inhibit scale, Shift scale, Emotional control scale, Initiate scale, Working memory scale, plan/organize, Organize of materials and Monitor. The clinical scales provide two indexes; Behavioral Regulation Index (BRI) and Metacognition index (MI). The Global Executive Composite (GEC) is a summary score of the eight clinical scales. T scores higher than 65 are considered in the clinical range.

1.8. Personality profile

The Big Five Questionnaire (BFQ) is based on the personality theory approximation that defines personality as five superordinate factors (often referred as the Big five). Instrument has two forms based on the age of the subject. The Big Five Questionnaire- Children and Adolescents (BFQ- NA) (127) is the in children and adolescents. The questionnaire is asked by the principal caregiver. 65 items are answered in a 5 Likert scale. The five personality dimensions are: conscientiousness, openness, extraversion, agreeableness and emotional instability.

Adult form (116) is based on 132 items with five possible responses graded from 5 to 1 (5; almost always; to 1; hardly ever). Instrument is divided in several scales and subscales:

Energy scale (extraversion), subscales: dynamism and dominance. Friendliness scale, subscales: cooperativeness and politeness. Conscientiousness scale, subscales: scrupulousness and perseverance. Emotional stability scale, subscales: emotional control and impulse control.
Openness scale, subscales: openness to culture and openness to experience.
Instrument also provides a lie scale that tries to identify the false profiles.

2. MOLECULAR CHARACTERIZATION OF WILLIAMS BEUREN SYNDROME DELETIONS AND POTENTIAL MODIFIERS

Nowadays, different methods are available for molecular analysis of WBS deletion and region. DNA samples from the proband and both parents are ideally required.

2.1. Characterization of WBS deletions

The molecular characterization of the deletion size and parental origin can be done with the analysis of single and multiple-copy microsatellites to determine the size and parental origin of the deletion (5) (figures 12 and 13). To further define the breakpoint of the deletions and detect those mediated by inversions, site-specific nucleotides (SSNs) or Paralogous sequence variants (PSVs) are genotyped. SSNs are nucleotides that are different between the blocks of segmental duplications. In order to determine the frequency of inversion-mediated deletions in patients with 1.55 Mb WBS deletions, a specific SSN within the *GTF2IRD2* gene is analyzed (5). At these positions close to the end of the ~105-kb alignment between blocks Bm and Bt, there should always be a gain of a Bt-type sequence and loss of a Bm-type sequence if the rearranged WBS chromosome was originated by an inter-chromosomal unequal exchange in an inversion carrier (figure 14). By PCR amplification followed by digestion with restriction enzymes and size fractioning in agarose gels, the relative intensities of the products are quantified and a dosage quotient is calculated to determine a gain or loss of specific blocks (5).

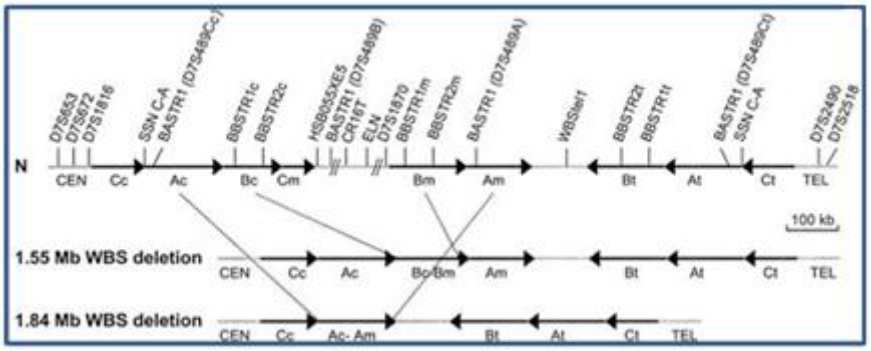

FIGURE 12:Schematic representation of the 7q11.23 genomic region in normal chromosomes (N) and chromosomes with the WBS deletions (1.55. and 1.83 Mb deletions) and the relevant polymorphic markers used in for the molecular analysis of the region are indicated. From (5).

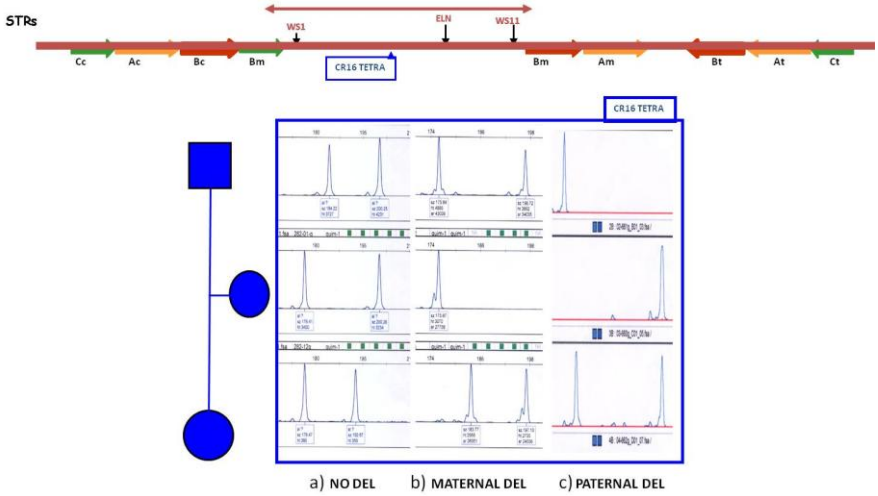

FIGURE 13:Results of analyzing several STRs to identify the parental origin of the deletion. Illustration shows results when a) No deletion, b) Maternal origin deletion and c) Paternal origin deletion.

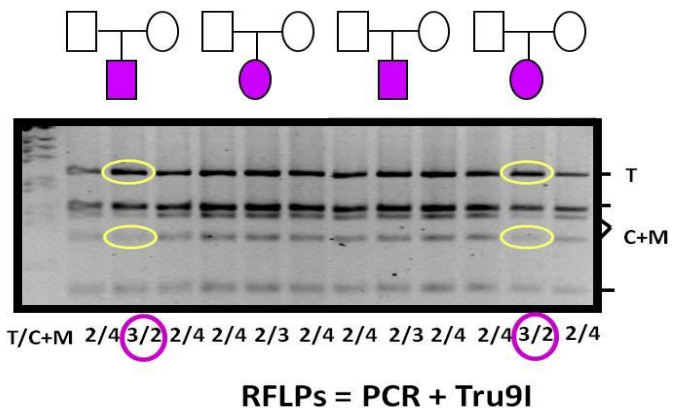

FIGURE 14:Quantification of SSNs or PSVs in WBS probands (purple symbols) and parents to identify whether deletions have been mediated by inversion polymorphisms in parents. After PCR and restriction with Tru9I, the labeled bands correspond to the amplification products of the telomeric block B (T) or de centromeric and medial blocks B (C+M). In inversions mediated deletions, a gain of telomeric type copy is detected in the WBS patients (purple circles).

The 1.55 Mb deletion can occur at different points along the block B. When the NAHR occurs at the *GTF2I* gene, the two functional copies of *NCF1* and the two medial and telomeric copies of *GTF2IRD2* (2T+2M) are kept (Breakpoint 1, B1). When the crossover takes place at *NCF1* gene, there is a loss of a functional copy of the gene but *GTF2IRD2* telomeric and medial copies are maintained (2T+2M). In the third case, the breakpoint occurs at *GTF2IRD2* and there is a loss of NCF1 gene and the creation of a chimeric copy of *GTF2IRD2*, with the final exons belonging to the centromeric copy and the initial exons belonging to the medial copy. Finally, the fourth scenario is the inversion-mediated deletion, were there is a loss of *NCF1* gene and a gain of the telomeric copy of *GTF2IRD2*. In the 1.83 Mb deletion, the crossover occur between the centromeric and medial A blocks, result in a loss of *NCF1* gene and the medial copy of *GTF2IRD2* (1M+2T) (figure 15).

Secondary to gene conversion events between *NCF1* gene and its pseudogenes, approximately 15% and 1% of individuals with WBS present three or four copies of *NCF1,* respectively (81).

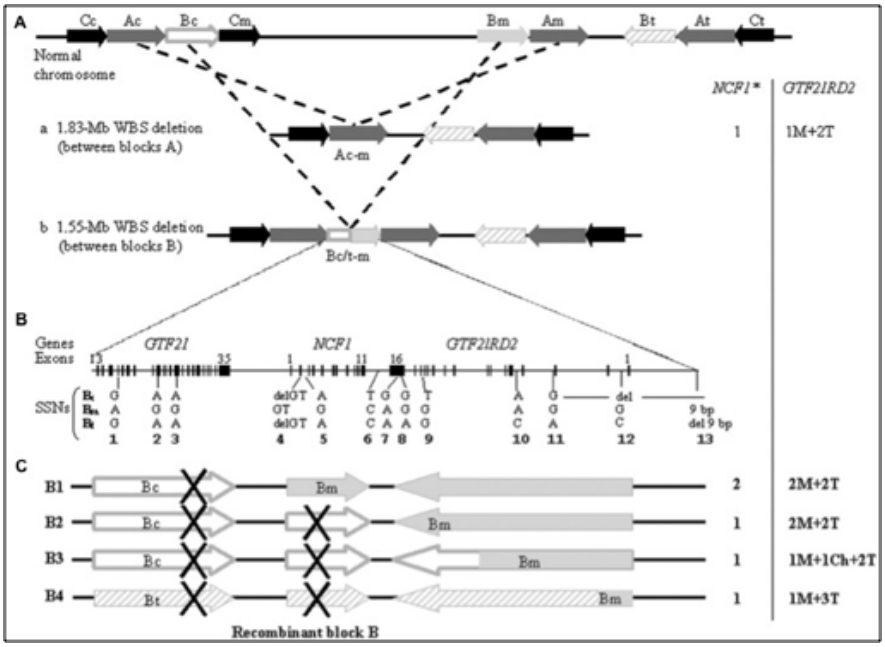

FIGURE 15:Schematic representation of the 1.55 Mb and 1.83 Mb deletions and breakpoint characterization. A. Representation of the 7q11.23 region, in black are represented the blocks C, in grey the A blocks and in white the B blocks. The top of the figure depicts the 1.83 Mb and 1.55 Mb deletions. B. Representation of the genomic content of the B blocks, as well as the location of the 13 genotyped site-specific nucleotides (SSNs) to refine the breakpoints of the deletion (75). C. Scheme of the different locations of the 1.55 Mb deletion. In breakpoint 1 (B1) the crossover occurs at *GTF2I*, therefore *NCF1* gene content and the functional copy of *GTF2IRD2* are not affected. In B2, the breakpoint is located at *NCF1*, therefore the patient presents with only one functional copy but *GTF2IRD2* remains the same. In B3, the breakpoint occurs at *GTF2IRD2* generating a chimeric copy with the final exons belonging to the centromeric block and the initial exons belonging to the medial block. Finally B4, which is the product of the inversion-mediated deletion, has a loss of *NCF1* gene and a gain of the telomeric *GTF2IRD2* copy.

2.2. Other potential genetic modifiers of the neurobehavioral phenotype

Considering the possible influence of genetic variation on the non-deleted allele as well as in candidate genes with known relevance for the neurocognitive function elsewhere in the genome, some variations were are considered as strong candidates. Single nucleotide polymorphisms (SNPs) in 7q11.23 can be identified in several blocks of strong linkage disequilibrium in the region (www.hapmap.org). With a total of five SNPs at different intervals within the regions

(rs799160, rs471803, rs4717820, rs6460068 and rs2528997), the almost entire region can be tagged for common variants.

Variation at several genes in the genome has been consistently reported to influence cognitive function and other aspects of the WBS phenotype, such as anxiety and attention. Although the list is quite large and growing with the genome wide association studies, a few candidate genes with known functional SNPs are worth special consideration.

BDNF encodes the brain-derived neurotrophic factor that acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses. In the brain, it is active in the hippocampus, cortex, and basal forebrain—areas vital to learning, memory, and higher thinking. BDNF itself is important for long-term memory. Although the vast majority of neurons in the mammalian brain are formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural stem cells during neurogenesis (129).

ADORA2A encodes a protein which is one of several receptor subtypes for adenosine. Its activity is mediated by G proteins which activate adenylyl cyclase to induce synthesis of intracellular cAMP. The A_{2A} receptor is expressed in the brain, where it has important roles in the regulation of glutamate and dopamine reléase (130).

HTR1A & HTR2A. Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter that occupies an important place in neurobiology because of its role in many physiologic processes such as sleep, appetite, thermoregulation, pain perception, hormone secretion, and sexual behavior. Abnormality of the serotonergic system has been implicated in a number of human diseases such as mental depression, migraine, epilepsy, obsessive-compulsive disorder, and affective disorder.. Like other neurotransmitters, 5-HT is released into the synaptic junction and exerts its effect on specific receptors on the postsynaptic membranes, such as HTR1A and HTR2A (131).

COMT: encodes Catechol-O-methyltransferase, one of the major mammalian enzymes involved in the metabolic degradation of catecholamines. COMT catalyzes the transfer of a methyl group from S-adenosyl-methionine to a hydroxyl group on a catechol nucleus (e.g., dopamine, norepinephrine, or catechol estrogen). Variation at this gene has been implicated in several psychiatric conditions (132).

All selected SNPs were genotyped using the Sequenom MassArray iPLEX system (Sequenom Inc.).

HYPOTHESIS

1) Some of the behavioral problems associated to WBS may not be specific to the syndrome but related to intellectual disability.

2) The behavioral profile of WBS could be influenced by other factors of the neuropsychological profile as well as by cultural differences of societies.

3) The lateral preference pattern of individuals with WBS can be affected and related to the neurosychological profile.

4) Genetic variation at the deletion breakpoint, at the non-deleted allele and/or at several candidate genes, can contribute to the clinical variability of the neuropsychological profile among WBS individuals.

OBJECTIVES

Main goal

1) To contribute to understanding the entire spectrum of the WBS neuropsychological phenotype, contributing to the knowledge of the pathogenic mechanisms of WBS by integrating clinical research with molecular genetics.

Secondary goals

2) Contribute to a better definition of the behavioral phenotype of WBS by comparison with Fragile X syndrome and non-specific intellectual disability as control groups.

3) Assess the possible influence of executive function, intelligence quotient and personality in the behavioral profile of WBS.

4) Examine similarities and differences in the behavioral profile in children with Williams-Beuren syndrome by countries/societies.

5) Analyze possible link between lateral preference and the neuropsychological profile of WBS.

6) Identify some of the possible genetic modulators on the neuropsychological profile by studying deletion breakpoints, parental origin of the deletion, genetic variants in the non-deleted allele and/or genetic variants in candidate genes elsewhere in the genome (SNPs).

CHAPTER 1

Behavioral features of Williams-Beuren syndrome compared to Fragile X syndrome and subjects with intellectual disability without defined etiology.

D. Pérez-García, R. Granero, F. Gallastegui, LA. Pérez Jurado C. & C. Brun-Gasca

Research in Developmental Disabilities, 2011, 32(2), 643-652

Knowledge on the behavioral features associated to WBS has significantly increased during the past years. Some of the emotional and behavioral problems that have been described are common on children and adults with WBS are also common on other neurodevelopmental disorders. `

The aim of the present study is to try to understand with behavioral problems could be syndrome-specific than those that could secondary to intellectual disability. Two controls groups are used; individuals with Fragile X and individuals with intellectual disability without defined etiology.

We also analyze possible influence of intelligence quotient is associated with anxiety in WBS.

Pérez-García D, Granero R, Gallastegui F, Pérez-Jurado LA, Brun-Gasca C. Behavioral features of Williams Beuren syndrome compared to Fragile X syndrome and subjects with intellectual disability without defined etiology. Res Dev Disabil. 2011 Mar;32(2):643–52. DOI: 10.1016/j.ridd.2010.12.005

CHAPTER 2

Influence of personality traits, intelligence quotient and executive function in Williams-Beuren syndrome behavioral profile.

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Submitted to Journal of Intellectual Disability Research

Individuals with WBS present a quite specific neurobehavioral profile, being attention problems, phobias and anxiety the most common emotional and behavioral problems of the profile. Other neuropsychological characteristics are a distinctive personality profile with high empathy and sociability and poor executive function skills.

Research in the last years has focused in the description of the phenotype buy only few studies have tried to analyze interrelationships between the main features. Understanding how other features influence in behavior would help to better create better therapeutic tools.

The aim of this study was to define how different neurocognitive traits, such as intelligence, executive function and personality, influence on WBS children and adults emotional and behavioral profile.

Influence of personality traits, intelligence quotient and executive function in the Williams-Beuren syndrome emotional and behavioral profile.

D. Pérez-García, A. Fornieles, C. Brun-Gasca, & L.A. Pérez-Jurado

Abstract

Williams-Beuren syndrome (WBS) is a rare genetic neurodevelopmental disorder with a well-defined and rather specific behavioral profile. Despite common traits in most patients, there is significant variability and the interrelationships among the different features are not well understood. The aim of our study was to define the possible influences of executive function, intelligence quotient (IQ) and personality in the behavioral profile of the syndrome. Twenty-four subjects (12 males, 12 females, age range 6-47 years) with a confirmed diagnosis of WBS were assessed by several tools: the Child Behavior Checklist, the Behavior Rating Inventory of Executive Function Parent form, the Big Five Questionnaires and the Weschler intelligence scales. We found significant correlations of the executive function with some behavioral problems, especially with externalizing problems. However, IQ showed no significant correlation with any trait of the behavioral profile. The role of executive function is discussed because and its influence in interventions.

Keywords: Williams-Beuren syndrome, executive function, personality, IQ, behavioral profile

Introduction

Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder caused by heterozygous deletion of 26-28 genes on chromosome band 7q11.23 (Bayes, Magano, Rivera, Flores, & Perez Jurado, 2003). The disorder is characterized by dysmorphic facial features, vascular stenoses, abnormalities of calcium and glucose metabolism, hyperacusis, visuospatial deficits and intellectual disability. Research in

the psychopathological profile has described WBS individuals as anxious, distractible, and hyperactive. Compared with chronological age-matched or mental age-matched controls, WBS individuals are more likely to experience difficulties with peer relationships (Leyfer, et al., 2006) and to manifest specific phobias (Dykens, 2003), as well as some sleep disturbances (Einfeld, Tonge, & Rees, 2001) and communication problems (Einfeld, Tonge & Florio, 1997). Longitudinal studies have demonstrated that the behavioral features persist into adulthood (Udwin, Howlin, Davies, & Mannion, 1998; Einfeld, et al., 2001).

Executive function is an umbrella term used to describe a constellation of cognitive processes, including sustained attention, response inhibition, working memory and error processing, which allow humans to guide behavior in a goal-directed and adaptive fashion (Miyake et al., 2000). Executive function is divided in two different aspects: 1) 'cool', which is elicited by abstract decontextualized problems and associated with the dorsolateral prefrontal cortex; and 2) 'hot', elicited by problems associated with socio-emotional decision making, affecting regulation and motivation, and associated with the ventral medial prefrontal cortex (Zelazo & Müller, 2002). To complete a task, the combination of both aspects is needed. Executive functioning is typically impaired in patients with frontal lobe damage (Baron-Cohen & Moriarty, 1995) and could be a core trait for the study of intellectual disability (ID) and intelligence in general (Henry, Cornoldi & Mähler, 2010). It has been suggested that children with ID have a specific profile of executive functioning (Danielsson, Henry, Messer & Rönnberg, 2012).

Working memory is one of the executive function processes that play a relevant role in daily activities, such as problem-solving, reading or reasoning. Working memory is responsible of the active maintenance and manipulation of information over brief time periods (Miyake & Shah, 1999). Children with low working memory show a distinctive developmental profile with behavioral problems and inattentive symptoms (Alloway, Gathercole, Kirkwood & Elliot, 2009).

A relationship between executive function and behavior has been described in WBS, with some aspects of executive function, such as working memory, planning and inhibition, positively influencing social and adaptive behavior (Menghini, Addona, Costanzo & Vicari 2010). Hypersocial behavior has been specifically linked to inhibition deficits (Porter, Coltheart & Langdon, 2007) and it has been suggested that part of the cognitive and behavioral phenotype of the syndrome could be attributable to deficits in planning, working memory and attention set-shifting (Rhodes et al., 2010).

Personality can also significantly influence the WBS behavioral profile. A theoretical approximation to personality defines its basic structure as five superordinate factors (often referred as the Big five): openness, extraversion, agreeableness, conscientiousness and neuroticism (Fiske, 1949; Norman, 1963). Openness is reflected in strong intellectual curiosity and a preference for novelty and variety. *Extraversion* refers to aspects such as activity, enthusiasm, assertiveness, and self-confidence. *Agreeableness* reflects concern and sensitivity towards others and their needs. *Conscientiousness* has to do with dependability, orderliness, precision, and the fulfilling of commitments. *Neuroticism (emotional stability)* pertains to a proneness to experience feelings of anxiety, depression, discontent, and anger; and *intellect/openness* is concerned with intellectual functioning, creativity, imagination, and social and cultural interest.

Personality traits are associated with psychopathology: low agreeableness, low conscientiousness, and high extraversion have been linked with externalizing problems, whereas high neuroticism has been related to internalizing problems (John, Caspi, Robins & Moffitt, 1994). Particularly, children with low scores in agreeableness and conscientiousness exhibit social and conduct problems, attention deficits, and hyperactivity, while children with low scores on openness to experience exhibit problems in social behavior, conduct, and attention. Neuroticism trait has been associated with anxiety and depression (Ehrler, Evans & McGhee, 1999).

WBS individuals show a distinctive personality profile: more approaching to others, more empathic, less shy, and more worrisome

and anxious than children with other developmental disabilities (Klein-Tasman & Mervis, 2003). Although psychopathological problems are somehow different in WBS, some of these complications seem to secondary to intellectual disability and not specific to the syndrome (Pérez-García et al., 2011).

In order to better define their causes, we investigate here the possible influence of executive function, intelligence quotient (IQ) and personality in the behavioral problems of WBS.

Methods

Sample

Participants were 24 individuals (12 males and 12 females) with a diagnosis of WBS confirmed by molecular genetics (1.55-1.83 Mb heterozygous deletion at chromosomal band 7q11.23), aged 6–47 years (mean = 16.71, SD = 10.38). Mean age and IQ for each gender were 18.8+/-12.1 years old and 56.4+/-10.1 for males and 14.7+/-8.4 and 56.4+/-6.5 for girls, respectively, with no significant differences (p=.590 & p=.799). For some analyses, the sample was divided in two groups (children / adults) on the basis on one of the instruments used (Big Five Questionnaires, older than 15 years old were considered adults).

Instruments

Child Behavior Checklist

We used the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001) to measure psychopathology, with the Spanish version for 6–18 year-old children as previously reported for individuals with ID (Graham, Rosner, Dykens & Visootsak, 2005). Parents were asked to rate their children's behavioral problems on an ordered scale from 0 to 2. The instrument measures eight constructs: anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, and aggressive behavior. The questionnaire also gives a profile of scales comprising problem items identified by experts as very consistent with Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV)

categories. In our study, Cronbach's alpha for Total Problems scales was excellent (.955).

Behavior Rating Inventory of Executive Function

Behavior Rating Inventory of Executive Function Parent form (BRIEF-P, Gioia, Isquith, Guy & Kenworthy, 2000) was used to assess executive function. The inventory is an 86 items questionnaire (8 clinical scales) to be completed by one caregiver indicating how frequently a behavior occurs (never, sometimes, often).

The inhibit scale assesses inhibitory control (the ability to inhibit, resist, or not act on an impulse) and the ability to stop ones' own behavior at the appropriate time. The shift scale assesses the ability to move freely from one situation, activity, or aspect of a problem to another as the circumstances demand. The emotional control scale addresses the ability to modulate emotional responses. The initiate scale contains items relating to beginning a task or activity, as well as independently generating ideas, responses, or problem solving strategies. The working memory scale measures the capacity to hold information in mind for the purpose of completing a task. The plan/organize scale assesses the ability to anticipate future events, set goals, develop appropriate steps ahead of time, carry out tasks in a systematic manner, and to understand and communicate main idea. The monitor scale relates to capacity to check work, assess performance, and keep track of own and others' efforts.

The clinical scales combine to form two indexes, behavioral regulation (BRI) that represents the ability to shift cognitive set and modulate emotions and behavior via appropriate inhibitory control and metacognition (MI) that represents the ability to initiate, plan, organize and sustain future-oriented problem solving in working memory. The Global Executive Composite (GEC) is a summary score of the eight clinical scales. In our study, Cronbach's alpha for the global scale was excellent (.949).

Big Five Questionnaires

The Big Five Questionnaire (BFQ, Caprara, Barbaranelli, Borgogni & Perugini, 1993) is based on the personality theory approximation that defines personality as five superordinate factors. It has 132 items with five possible responses graded from 5 to 1 (5; almost always; to 1; hardly ever). The energy scale (extraversion) has two subscales: dynamism (dynamics behaviors, fluency and enthusiasm) and dominance (ability to prevail, to excel, to assert their own influence on others). The friendliness scale with the subscales: cooperativeness (ability to understand and reflect the problems and needs of others and cooperate effectively with them) and politeness (friendliness, trust and openness to others). The conscientiousness scale with the subscales: scrupulousness (reliability, thoroughness and love of order) and perseverance (persistence and tenacity with which the tasks are performed). The emotional stability scale has two subscales: emotional control (control of tension states associated with emotional experience) and impulse control (keep control of their own behavior even in situations of discomfort, conflict and danger). The openness scale with the subscales: openness to culture (interest to stay informed, to lecture and to acquire knowledge) and openness to experience (favorable disposition toward the new, the ability to considerer everything from different perspectives and favorable aperture to values, styles, and different cultures or ways of life). The lie scale tries to identify the false profiles. Spanish version was used (Bermúdez, 1998).

The Big Five Questionnaire- Children and Adolescents (BFQ- NA, Barbaranelli, Caprara, Rabasca & Pastorelli, 2003) is the in children and adolescents form of the BFQ. The questionnaire has 65 items and can be answered by the child or the parents. The five personality dimensions are: conscientiousness (assess autonomy, order, precision and compliance with standards and commitments), openness (with items with intellectual aspects, creativity and cultural interest), extraversion (activity, enthusiasm, assertiveness and self-confidence), agreeableness (concern and sensitivity to others and their needs) and

emotional instability (anxiety, depression, unhappiness or madness). Spanish version was used (Del Barrio, Carrasco & Holgado, 2006).

Wechsler Intelligence Scales

The Wechsler Intelligence Scale for Children-R (WISC-R; Wechsler, 1974) is a battery of tests for children aged 6 to 16 years old, which evaluates intellectual abilities. The test has 10 core subtests and five supplemental ones. These subtests generate a Full Scale score (FSIQ), Verbal IQ and Performance IQ. Spanish version of the WISC- R was used (Wechsler, 2001a).

The Wechsler Adult Intelligence Scale III (WAIS-III; Wechsler, 1997) is used for individuals older than 16 years old. Provides scores for Verbal IQ, Performance IQ, and Full Scale IQ. Spanish version was used (Wechsler, 2001b).

Socioeconomic status

The socioeconomic status (SES; Hollingshead, 1975) is a four-factor index based on a composite of maternal education, paternal education, maternal occupational status, and paternal occupational status.

Procedure

Parents answered all the three questionnaires while the probands were evaluated by the clinician. In some cases the questionnaires were sent by post. The use of parents as informants for emotional experiences in WBS has proven to be reliable with high concordance between informants and respondents (Stinson, Tomlinson & Estes, 2012).

IQ was measured by an expert clinician in all cases. Two individuals had IQ recently measured by the McCarthy Scales of Children's Abilities (MSCA) and we considered the results comparable to those of WPPSI-R (Karr et al., 1993).

Statistical analyses

We used the Man-Whitney test to calculate possible differences by gender or age in behavioral problems, executive function and personality traits, multiple lineal regressions adjusted by IQ were done (enter mode) for correlations between behavioral problems and

executive function, and Spearman bi-variant correlations for correlations between behavioral problems and personality traits (categorize punctuation) in adults and children. Statistical analyses were carried out with SPSS 19.0.

Results

Phenotype description

The average IQ was 56.42 (SD=8.32), ranging from 40 to 73. The socioeconomic status ranged from low to high, with most families in the medium and medium low range (25.0% and 33.3%, respectively). As described before, anxious/depressed, somatic complaints, thought problems and attention problems were the most common behavioral problems in our patients. Internalizing problems were more common than externalizing problems. No significant sex or age differences were found. Half of the patients were in the subclinical-clinical range for total problems scale.

Using the oriented DSM-IV scales a 58.3% of cases met criteria for affective problems, 70.8% for anxiety problems, 25.0% for somatic complaints, 45.8% for ADHD, 16.7% for oppositional defiant problems and 12.5% for conduct problems.

In WBS, initiate, working memory, plan/organize and monitor were the executive function variables most affected. In the case of working memory and plan/organize, more than the 75.0% of the sample were in the clinical range. Inhibit was the executive function less affected showing significant differences by sex (U=27.0; p=.008) with boys presenting more problems than girls. There were more problems in metacognition than in behavioral regulation, with 75.0% and 45.8% of the individuals in the clinical range of the metacognition and behavioral regulation scales, respectively. For the total scale, Global Executive Composite, the 62.5% of the sample was in the clinical range. No significant differences by age were found in any of the scales.

Personality aspects in children showed more variability than in adults. WBS children were mostly in the medium range in agreeableness and extraversion, in the medium and high ranges in emotional instability,

and between the low and very low range in openness and conscientiousness. WBS adults were in the low or very low range in almost all analyzed dimensions of personality: conscientiousness, emotional stability, energy and friendliness. Cooperativeness and politeness were the only subdimensions with half of the sample in the medium range.

TABLE 1:Mann-Whitney comparison of CBCL T-scores by sex

	Descriptives (T-Scores)				Mann-Whitney	Distribution of T-scores (%) (N=24)		
	Boys (N = 12)		Girls (N = 12)		p	T< 65	65≤ T <70	T ≥70
	Mean	SD	Mean	SD				
Anxious/depressed (I)	63.0	8.8	61.6	8.1	.932	45.8	25.0	29.2
Withdrawn/depressed (II)	58.5	6.6	58.2	7.0	.932	79.2	12.5	8.3
Somatic complaints (III)	61.6	8.9	65.8	11.2	.410	50.0	12.5	37.5
Social problems (IV)	65.2	5.9	62.3	8.2	.160	54.2	29.2	16.7
Thought problems (V)	65.2	10.4	64.9	7.0	.977	50.0	20.8	29.2
Attention problems (VI)	67.3	12.7	68.8	10.3	.551	41.7	33.3	25.0
Rule-breaking behavior (VII)	57.0	6.2	58.9	6.4	.977	87.5	0.0	12.5
Aggressive behavior (VIII)	59.6	11.2	55.9	7.9	.347	83.3	8.3	8.3
Internalizing problems	62.8	9.5	62.3	12.6	.590	37.5	37.5	25.0
Externalizing problems	57.6	9.5	55.9	8.1	.514	83.3	8.3	8.3
Total problems	64.3	8.8	63.8	7.6	.551	50.0	25.0	25.0

TABLE 2:Mann-Whitney comparison of BRIEF T-scores by sex

	Descriptives (T-score)				Mann-Whitney	Distribution of T-scores (%) (N=24)	
	Boys (N = 12)		Girls (N = 12)		P		
	Mean	SD	Mean	SD		T < 65	T ≥65
Inhibit (I)	63.3	11.5	52.5	9.7	.008*	70.8	29.2
Shift (II)	66.8	14.1	60.4	9.0	.410	58.3	41.7
Emotional Control (III)	62.7	9.2	57.5	9.5	.630	58.3	41.7
Initiate (IV)	66.1	11.3	68.3	9.6	.713	45.8	54.2
Working Memory (V)	71.4	12.8	74.8	8.2	.932	25.0	75.0
Plan/Organize (VI)	66.3	8.4	70.4	8.1	.347	25.0	75.0
Org. of Materials (VII)	61.6	10.7	58.2	10.6	.843	58.3	41.7
Monitor (VIII)	64.4	7.4	65.2	7.7	.068	45.8	54.4
BRI	66.3	9.9	58.4	8.9	.114	54.2	45.8
MI	68.2	9.5	71.4	7.3	.590	25.0	75.0
GEC	69.4	9.6	67.1	7.3	.319	47.5	62.5

All individuals scored between the medium and very high range in the lie dimensions. Significant differences by sex were found in emotional stability and the subdimension impulse control with girls measuring higher scores.

No significant correlations were found between the socioeconomic status and the executive function scales or the behavior scales.

Results are described by the dependent variables:

Correlations among variables

No correlation was found between any of the scales measuring behavioral problems and IQ.

Anxious/depressed scale presented a positive association with emotional control (r^2=.408; p=.004). Emotional control explained a 35.1% of the variability of anxious/depressed.

Executive function scales showed no significant correlations with somatic complaints or withdrawn/depressed. Withdrawn/depressed showed a high negative relation with extraversion in children (rho=-.539, p=.046) and with cooperativeness (rho=-.791, p=.006) and conscientiousness (rho=-.698, p=.025) in adults. Somatic complaints showed positive relations with dominance (rho=.669, p=.049) and openness to culture (rho=.802, p=.005) in adults.

Social problems showed no significant correlations with any personality dimension. When associated with executive function, a positive association with emotional control (r^2=.335; p=.014) and behavioral regulation index (r^2=.333; p=.014) were detected. A 26.9 % of the variability of social problems was explained by behavioral regulation index.

Thought problems presented a positive relation with perseverance in adults (rho=.683, p=.030) and a positive association with working memory (r^2=.280; p=.032), plan/organize, metacognition (r^2=.304; p=.022) and global executive composite (r^2=.365; p=.008). The global executive composite explained a 30.5% of the variability of thought problems.

Attention problems was the scale with more positive associations with executive function scales: emotional control (r^2=.440; p=.002), working memory (r^2=.379; p = .007), BRI (r^2=.422; p=.003), MI (r^2=.291; p=.027) and GEC (r^2=.451; p=.002). The 39.9 % of the variability of attention problems was explained by the GEC. When compared with personality traits a positive relation was found with emotional instability in children (rho=.573, p=.032) and in adults, dynamism presented a positive relation (rho=.815, p=.004) while emotional stability presented a negative relation (rho=-.718, p=.019).

Rule breaking behavior scale presented a positive relation with an emotional instability in children (rho=.554, p=.040) and with energy in adults (rho=.676, p=.032). Emotional control (r^2=.322; p=.017), BRI (r^2=.330; p=.015) and GEC (r^2=.280; p=.032) presented a positive association with rule-breaking behavior. The 21.1% of the variability of rule-breaking behavior was explained by GEC.

Aggressive behavior presented a positive association with inhibit (r^2=.478; p=.004), emotional control (r^2=.626; p=.000), BRI (r^2=.691; p=.000) and GEC (r^2=.460; p=.002). The 66.1 % of the variability of aggressive behavior was explained by BRI. Emotional instability (rho=.775, p = .001) in children and energy (rho=.716, p=.020), dominance (rho=.692, p=.039) and perseverance (rho=.776, p=.008) in adults presented a positive relation with aggressive behavior.

Internalizing problems only presented a positive relation with the personality trait energy (rho=.689, p=.027) in adults.

Externalizing problems presented a positive association with EF: inhibit (r^2=.408; p=.013), emotional control (r^2=.599; p=.000), BRI (r^2=.650; p=.000) and GEC (r^2=.456; p=.002). Personality traits as emotional instability in children (rho=.774, p=.001) and energy (rho=.740, p=.014) and dominance (rho=.698, p=.037) in adults presented a positive relation. The 61.7% of the variability of externalizing problems was explained by BRI.

Finally, for total problems a positive association was found with emotional control (r^2=.479; p=.001), BRI (r^2=.441; p=.002) and GEC (r^2=.374; p=.007). The 31.4 % of the variability of total problems was explained by GEC. For personality traits, emotional instability showed a positive relation with total problems (rho=.626, p=.017).

TABLE 3:Distribution of T-scores of the Big Five Questionnaires (%)

	Boys		Girls		Man-Whitney P	Very low	Low	Medium	High	Very high
BFQ- NA (N=14)										
	Mean	SD	Mean	SD						
Emotional instability	57.7	5.0	50.9	6.3	.081	0.0	14.3	35.7	42.9	7.1
Agreeableness	44.8	11.9	52.1	12.1	.282	21.4	7.1	42.9	21.4	7.1
Extraversion	47.5	6.9	45.5	9.6	.755	7.1	28.6	57.1	7.1	0.0
Openness	37.0	5.5	38.5	4.5	.573	35.7	64.3	0.0	0.0	0.0
Conscientiousness	38.8	5.9	38.9	9.2	.950	28.6	50.0	21.4	0.0	0.0
BFQ Adults (N=10)										
Energy	39.5	7.7	39.8	11.8	.762	40.0	40.0	10.0	10.0	0.0
Dynamism	42.8	9.5	39.5	12.7	.610	30.0	30.0	30.0	10.0	0.0
Dominance	38.8	12.3	36.3	11.5	.762	30.0	40.0	30.0	0.0	0.0
Friendliness	47.3	9.3	41.8	8.4	.352	10.0	50.0	20.0	20.0	0.0
Cooperativeness	42.0	8.5	38.8	12.2	.762	10.0	10.0	50.0	20.0	10.0
Politeness	52.5	13.8	48.8	8.7	.610	10.0	10.0	50.0	20.0	10.0
Conscientiousness	34.2	12.3	34.8	9.9	.610	70.0	10.0	10.0	10.0	0.0
Scrupulousness	36.3	10.9	43.0	21.2	.762	50.0	30.0	0.0	10.0	10.0
Perseverance	34.7	12.7	30.8	4.8	1.00	70.0	20.0	0.0	10.0	0.0
Emotional stability	30.3	3.2	36.8	3.7	.038*	80.0	20.0	0.0	0.0	0.0
Emotion control	31.0	2.4	34.5	3.8	.171	40.0	60.0	0.0	0.0	0.0
Impulse control	32.0	4.1	41.0	3.2	.019*	28.6	71.4	0.0	0.0	0.0
Openness	37.0	12.3	41.5	13.1	.610	50.0	10.0	40.0	0.0	0.0
Openness to culture	38.8	14.9	47.0	10.5	.352	30.0	30.0	20.0	20.0	0.0
Openness to experience	37.0	9.1	39.5	13.2	.914	50.0	20.0	20.0	10.0	0.0

TABLE 4:Significant regressions (R^2 corrected) between behavior problems and executive function and IQ

	A/D	W/D	SC	SP	TP	AP	RBB	AB	I	E	T
IQ	-	-	-	-	-	-	-	-	-	-	-
Inhibit	-	-	-	-	-	-	-	.399*	-	.320*	-
Shift	-	-	-	-	.116	.179	.114	.131	-	.150	.162
Emotional control	.351*	-	-	.272*	-	.386*	257*	.591**	.128	.560**	.429**
Initiate	-	-	-	-	.112	.115	-	-	-	-	-
Working memory	-	-	-	-	.262*	.319*	-	.134	-	.138	.134
Plan/Organize	-	-	-	-	.211*	-	-	-	-	-	-
Org. Materials	-	-	-	-	-	-	-	-	-	-	-
Monitor	-	-	-	-	-	-	-	-	-	-	-
BRI	.140	-	-	.269*	.167	.367*	.266*	.661**	-	.617**	.387*
MI	-	-	-	-	.238*	.223*	-	-	-	-	.112
GEC	.142	-	-	.128	.305*	.399*	.211*	.408*	-	.404*	.314*

*Significant mean difference (.05 level),** (.01). A/D: Anxious/depressed, W/D: Withdrawn/depressed, SC: Somatic Complaints, SP: Social Problems, TP: Thought problems, AP: Attention problems, RBB: Rule-breaking behavior, AB: Aggressive behavior, I: Internalizing, E: Externalizing, Total problems

TABLE 5:Spearman bivariate correlations between behavioral problems and personality in WBS children and adults

	A/D	W/D	SC	SP	TP	AP	RBB	AB	I	E	T
Emotional instability	.441	-.376	.238	.471	.331	.573*	.554*	.775**	.243	.774**	.626*
Agreeableness	-.010	.424	.221	.097	-.142	-.201	-.211	-.339	.178	-.315	.001
Extraversion	-.111	-.539*	.000	-.090	-.385	-.016	-.103	-.074	-.236	-.045	-.052
Openness	-.354	-.169	-.038	-.131	-.429	-.465	-.226	-.278	-.241	-.259	-.315
Conscientiousness	-.212	-.217	-.060	.010	-.501	-.474	-.256	-.342	-.257	-.307	-.273
Energy	.720*	.137	.423	.610	.360	.580	.676*	.716*	.689*	.740*	.603
Dynamism	.486	.209	.133	.329	.223	.812*	.430	.422	.479	.363	.436
Dominance	.385	-.443	.669*	.563	.408	-.005	.570	.692*	.410	.698*	.505
Friendliness	.039	-.131	-.206	-.053	.494	.141	-.030	-.096	-.129	-.020	-.003
Cooperativeness	-.383	-.791*	.045	-.138	.364	-.116	-.465	-.068	-.548	-.364	-.428
Politeness	.436	.385	.052	.153	.250	.402	.510	.244	.455	.460	.358
Conscientiousness	-.135	-.698*	.315	.023	.294	.108	.-015	-.174	-.159	-.023	-.007
Scrupulousness	-.039	-.284	.224	-.128	-.046	-.041	.122	-.020	.033	.020	.085
Perseverance	.135	-.610	.497	.622	.683*	.464	.326	.776*	.137	.501	.361
Emotional stability	-.175	-.044	.219	-.357	-.264	-.718*	-.135	-.354	-.088	-.176	-.349
Emotion control	-.407	-.350	.058	-.416	.059	-.539	-.480	-.530	-.412	-.529	-.524
Impulse control	-.071	.429	.072	-.510	-.288	-.367	.221	-.505	.180	-.072	-.036
Openness	.259	-.485	.543	.357	.536	.518	.420	.442	.320	.353	.467
Openness to culture	.367	-.547	.802*	.359	.589	.381	.540	.464	.464	.456	.486
Openness to experience	.160	-.472	.422	.154	.389	.400	.286	.228	.205	.171	.336

*Significant mean difference (.05 level),** (.01). A/D: Anxious/depressed, W/D: Withdrawn/depressed, SC: Somatic Complaints, SP: Social Problems, TP: Thought problems, AP: Attention problems, RBB: Rule-breaking behavior, AB: Aggressive behavior, I: Internalizing, E: Externalizing, Total problems

Discussion

As predicted and in agreement with previous research, executive function and personality traits are crucial for the behavioral problems in WBS. However, IQ does not seem to be related to the psychopathological profile (Dykens & Rosner, 1999; Leyfer et al., 2006).

The dimensions of personality in WBS change from childhood to adulthood with a notorious decrease in extraversion and in emotional stability (Van Lieshout et al., 1998). There is also more variability between individuals during childhood, which could explain the poor influence of personality traits on behavior in childhood.

WBS people have been described as friendly, although a remarkable low range in friendliness was found in WBS adults. This fact is due to a poor impulse control and the increase of social problems. It has been proposed that the behavior towards people could be explained by abnormal perceptual processing of their faces rather than by an overall bias at the level of behavior (Järvinen-Pasley, 2010). This distinctive social behavior also seems to be related with problems in executive function. Planning, inhibition and working memory are some of the executive function dimensions that have been associated with social and adaptive behavior (Menghini, Addona, Costanzo & Vicari, 2010). In our data, behavior regulation also played a big role, while personality traits were not related.

A worth mentioning aspect found with the personality instrument was the high score in the lie scale. As described by the manual, a high score in this dimension may be caused by a false profile, a try to give a good image. Although we cannot rule-out that parents might have tried to provide a better image of their children with WBS, it is possible that the high score in the lie scale could be related to the distinctive personality profile of the syndrome.

The relationship with executive function was more intense for externalizing than for internalizing problems. Internalizing problems such as anxiety have been previously related with executive function. The diagnosis of anxiety has been associated with increased scores on

behavioral regulation (Woodruff-Borden, 2010). Anxiety is related to energy, while depression and withdrawn symptoms are related to extraversion in children and cooperativeness and conscientiousness in adults. We did not find any influence of agreeableness and openness dimensions in the WBS behavior profile (Karsten et al. 2012).

Thought problems were described by parents as common and were highly influenced by problems in executive function, especially in metacognition. Emotional instability and emotional control in children and adults were related with externalizing problems.

One of the behavior difficulties most influenced by executive function was attention problems. Difficulties in executive function have already been implicated in the complex neuropsychology of Attention Deficit Hyperactivity Disorder (ADHD; Willcutt et al., 2005). Working memory plays an essential role in inattention with big influence in everyday life. Therefore, poor executive working memory can be a primary cause of the general difficulties the child is experiencing (Rhodes et al., 2010). Both, metacognition and behavioral regulation, play a relevant role in attention problems.

Executive function also influenced the rule-breaking and aggressive behaviors. Even though these behaviors are not common in WBS, they are a great concern for parents when present. Behavioral regulation and inhibition skills influence the presence of the behavior, especially in aggressive behavior. The personality trait energy in adults and emotional instability in children also has to be considered. Impulse control, mood dysregulation and perceived threat appear to underlie most of the aggressive behaviors reported in intellectual disability (Tsiouris, Kim, Brown & Cohen, 2011).

Inhibition problems have been reported as key characteristics of the WBS behavior (Menghini, et al., 2010). Our study reflects that all dimensions of executive function have to be considered, since different dimensions interfere with each behavior. Children with intellectual disability have a specific profile of executive functioning (Danielsson et al. 2012) although executive functioning is unrelated to general intelligence (Friedman et al., 2006).

In WBS, executive function should be considered to define the specific behavior problems. This relationship could help to explain why WBS individuals have limited adaptive skills with respect to Down syndrome (Edgin, Penningon & Mervis, 2010) or less success in managing household chores and acquiring job skills than Prader-Willi syndrome or Down syndrome peers (Rosner et al, 2004).

The principal limitation of our study is the sample size and the use of some instruments, especially personality test, that have not been validated in ID population.

Interestingly, twin studies have shown that prevalent forms of psychopathology may share a common genetic control with executive functions (Coolidge, Thede, & Jang, 2004), opening new ways to understand behavior problems, personality and executive function in ID. Future research should lead to specify the special role of executive function in WBS and its implication in behavioral problems. If the big influence of executive function in some behavior problems is confirmed, treatment programs should be probably revised. This will lead to more effective treatments not only for WBS but also for other individuals with ID.

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CHAPTER 3

Behavioral Profiles of 6 – 14 Year-Old Children with Williams-Beuren Syndrome from Spain and the United States: Cross-Cultural Similarities and Differences

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Submitted to American Journal on Intellectual and Developmental Disabilities

Research on cultural differences on behavior problems between societies (on typical developing children) reflects differences on emotional and behavior problems. On WBS, only two cross-cultural studies have been performed measuring differences on social behavior.

The aim of the present study is to find similarities and differences on the behavioral profile between two different countries, Spain and United States.

Pérez-García D, Brun-Gasca C, Pérez-Jurado LA, Mervis CB. Behavioral Profiles of Children With Williams Syndrome From Spain and the United States: Cross-Cultural Similarities and Differences. Am J Intellect Dev Disabil. 2017;122(2):156–72. DOI: 10.1352/1944-7558-122.2.156

CHAPTER 4

Part 1

Lateral preference in Williams-Beuren syndrome is associated with cognition and language

D. Pérez-García, R. Flores, C. Brun-Gasca & L.A. Pérez Jurado

Eur Child Adolesc Psychiatry, 2015, 24 (9), 1025-1033

Part 2

Lateral preference of children who have Williams Beuren syndrome and its association with cognition and language: A replication

D.Pérez-García, L.A. Pérez-Jurado and C.B. Mervis

In preparation

Atypical lateral preference has been described in WBS suggesting possible link with cognition.

The aim of the present study (and replication) is to define the atypical lateral preference in the syndrome and evaluate its possible association with features of the neuropsychological profile and some molecular variants in the syndrome. A second sample was assessed with the aim to replicate first study results.

Pérez-García D, Flores R, Brun-Gasca C, Pérez-Jurado LA. Lateral preference in Williams–Beuren syndrome is associated with cognition and language. Eur Child Adolesc Psychiatry. 2015 Sep 28;24(9):1025–33. DOI: 10.1007/s00787-014-0652-6

Lateral preference of children who have Williams Beuren syndrome and its association with cognition and language: A replication

Débora Pérez-García, Luis A. Pérez-Jurado and Carolyn B. Mervis

In preparation

Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder caused by a microdeletion of chromosome 7q11.23 and associated with mild to moderate intellectual disability and a specific pattern of intellectual strengths and weaknesses (Mervis & John, 2010). WBS also is associated with a higher frequency of mixed handedness (Carlier et al., 2011; Pérez-García et al., 2015) and a lower frequency of homogenous lateral preference than in the general population. Individuals with WBS who had mixed handedness had significantly lower full-scale IQ and significantly lower Verbal IQ than did individuals with defined handedness (Pérez-García et al., 2015). The aim of this study was to replicate Pérez-García et al. (2015) to assess the same types of abilities.

Methods

Sample

Participants were 27 children (16 males, 11 females) aged 5.02 – 9.84 years (mean: 7.34, SD: 1.62, Mdn: 7.21). All had genetically-confirmed WBS (1.55-1.83 Mb deletion at 7q11.23).

Instruments

Lateral preference was assessed in the same manner as in Pérez-García et al. (2015).

Intellectual abilities were assessed using the Differential Ability Scales II (DAS-II; Elliott, 2007). The DAS-II includes six core subtests distributed in three clusters (Verbal, Nonverbal Reasoning, Spatial). The General Conceptual Ability standard score (GCA; similar to IQ) is based on performance on all six core clusters. The Special Nonverbal Composite (SNC; similar to Performance IQ) is based on the subtests included in the Nonverbal Reasoning and Spatial clusters. Receptive vocabulary was

assessed using the Peabody Picture Vocabulary Test- Fourth Edition (PPVT-4; Dunn & Dunn, 2007), and expressive vocabulary was assessed using the Expressive Vocabulary Test, Second Edition (EVT-2; Williams, 2007). For all measures, the mean for the general population is 100 with a SD of 15.

Procedure

All children were assessed as part as an ongoing longitudinal study at the University of Louisville. All testing was completed either in one day or on two consecutive days.

Results

Of the 27 participants, 15 showed right hand preference, 3 left hand preference and 9 mixed handedness. Regarding foot preference, 19 exhibit right preference, 1 left, and 7 mixed. For eyedness, 13 children showed right preference, 9 left, and 5 mixed. Ear lateral preferences were 17 right, 3 left, and 7 mixed (Figure 16).

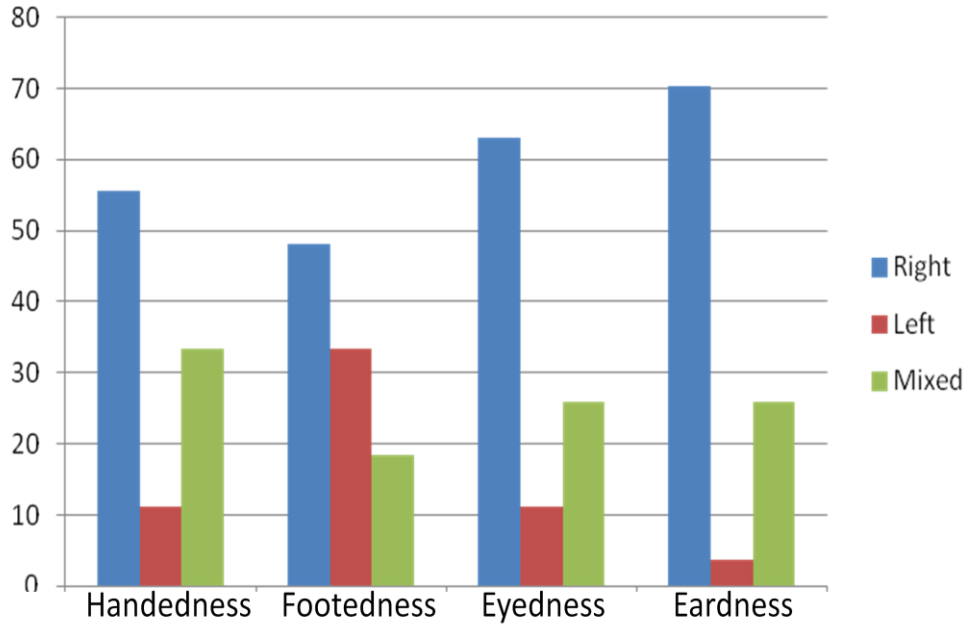

FIGURE 16:Percentage of children with each lateral preference

To compare the performance of the children with defined handedness to that of the children with mixed handedness, a series of Mann-Whitney U tests was performed. The two groups did not differ significantly in age distribution (Defined: median = 7.13 years, Mixed: median = 7.26 years, p = 1.00). Descriptive statistics for performance on the intellectual and vocabulary assessments are reported in Table 1. As indicated in the table, the distribution of standard scores was significantly higher for the Defined group than for the Mixed group for every comparison, with p-values ranging from .002 to <.0001.

TABLE 1:Descriptive statistics for intellectual and vocabulary assessments as a function of type of handedness (well-defined vs. mixed)

Instrument		Well-defined (N=18)			Mixed (N=9)			p
		Mean (SD)	Median	Range	Mean (SD)	Median	Range	
DAS-II	Verbal	83.72 (8.96)	83.00	66-100	52.78 (16.40)	49.00	31-81	.000*
	Nonverbal	81.72 (9.72)	83.00	62-96	62.00 (17.11)	61.00	43-101	.001*
	Spatial	61.06 (12.18)	59.50	34-78	39.22 (8.60)	34.00	32-54	.000*
	GCA	70.22 (8.70)	70.00	50-88	46.56 (11.10)	42.00	35-69	.000*
	SNC	67.11 (10.16)	65.50	45-84	44.11 (12.84)	40.00	30-68	.001*
PPVT-4		88.22 (10.76)	89.00	71-109	67.33 (16.19)	64.00	50-100	.002*
EVT-2		85.61 (10.96)	84.00	69-103	60.44 (20.53)	61.00	20-88	.001*

Abbreviations: DAS-II = Differential Ability Scales 2nd edition, SS = standard score, GCA = General Conceptual Ability (similar to IQ), SNC = Special Nonverbal Composite (similar to Performance IQ), PPVT-4 = Peabody Picture Vocabulary Test 4th edition, EVT-2 = Expressive Vocabulary Test 2nd edition.

Discussion

The present findings replicate those of Pérez-García et al. (2015) and Carlier et al. (2011) with regard to the higher prevalence of mixed handedness among individuals with WBS than in the general population. Furthermore, the present results not also replicate Pérez-García et al.'s (2015) finding that the distributions for Verbal IQ and Full-scale IQ are significantly higher for the defined handedness group than the mixed handedness group but also

document significant differences in favor of the defined handedness group for nonverbal reasoning SS, spatial SS, and both receptive vocabulary and expressive vocabulary SSs. There are several likely reasons for the stronger results in the present study, including a narrower age range, administration of the same assessment of intellectual ability to all participants, and use of assessments that were normed low enough (4 SDs below the general population mean) to prevent floor effects from obscuring differences in spatial abilities, the area of greatest weakness for individuals with WBS.

The increased prevalence of mixed handedness relative to the general population suggests that haploinsufficiency of one or more of the genes in the WBS region affects the establishment of lateral preference during development. An increased prevalence of mixed handedness also has been reported for other syndromes associated with intellectual disability (e.g., Carlier et al., 2011). Future research should focus on defining the possible morphological and functional brain differences, as well as the molecular mechanisms, that lead to a poorer definition of laterality during brain development in many WBS children.

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CHAPTER 5

GTF2IRD2 and *BNDF* modulate the Williams-Beuren syndrome neuropsychological profile

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In preparation

Neuropsychological profile is significantly variable among patients. We have searched for potential genetic modifiers of the neurobehavioral profile associated to the syndrome by characterizing deletion breakpoints and genotyping additional single nucleotide polymorphisms (SNPs).

GTF2IRD2 and *BNDF* modulate the Williams-Beuren syndrome neuropsychological profile

D. Pérez-García, R. Flores, C. Brun-Gasca & L.A. Pérez Jurado

ABSTRACT

The neuropsychological profile of Williams-Beuren syndrome (WBS), including hypersociability, asymmetric intellectual disability, anxiety, common phobias and behavioral problems, is significantly variable among patients. WBS is caused by a recurrent 1.55-18.3 Mb heterozygous deletion at 7q11.23, with hemizygosity at *GTF2I* as the main responsible for the neurobehavioral profile, but the causes of the clinical variability remain unknown. Depending on breakpoints, deletions affect differently *GTF2IRD2*, a gene with two functional copies only in humans whose product is thought to inhibit GTF2I action. We have searched for potential genetic modifiers of the WBS neurobehavioral profile by characterizing deletion breakpoints and genotyping additional single nucleotide polymorphisms (SNPs) at the undeleted allele and other candidate genes in 114 well-phenotyped WBS patients. Patients with a chimeric form of *GTF2IRD2* with potential dominant-negative effect showed higher verbal and global IQ than other groups (6-9 points, p=0.005) and higher approachability to strangers. A functional SNP at the *BDNF* gene was also associated with IQ (corrected p=0.01) with positive interaction with *GTF2IRD2* (33 points, p=0.03), while SNPs at *HTR2A* and *ADORA2A* showed nominal association with anxiety, affective problems and the presence of attention deficits. *Gtf2i* therapy has been shown to normalize Bdnf levels in *Wbs* mice with beneficial effects in motor coordination, sociability, and anxiety. Therefore, our data indicate that both, genetic variation at the deletion breakpoint interacting with GTF2I as well at candidate genes in related developmental pathways contribute to the clinical variability of the WBS neurocognitive profile.

INTRODUCTION

Williams Beuren syndrome (WBS) is a neurodevelopmental disorder with an estimated prevalence of 1/7500 newborns (Strømme et al., 2002) characterized by specific facial features, supravalvular aortic stenosis, hypertension, hyperacusis and endocrinological problems (Pober, 2010). The neuropsychological profile involves mild to moderate intellectual disability, with a small proportion of cases reported with intellectual quotient (IQ) higher than 70 (Martens et al, 2008). The most prevalent psychiatric problems in WBS are Attention Deficit Hyperactivity Disorder (ADHD) and anxiety disorders (Lefter et al., 2006; Woodruff-Borden, et al., 2010). Other main features syndrome specific are very poor visuospatial skills and hypersociability (Mervis & Klein-Tasman, 2000; Jones et al., 2000)

WBS is caused by a recurrent heterozygous 1,55 Mb deletion (90% of patients) that includes 25-27 protein-coding genes on chromosome band 7q11.23, mediated by unequal recombination between misaligned segmental duplications (Bayes et al, 2003). A larger deletion of 1,83 Mb mediated by different blocks of segmental duplications and harboring the same 27 protein-coding genes and additional multi-copy transcriptional units occurs in around 10% of patients. Hemizygosity for the elastin gene (*ELN*) is responsible of the cardiovascular phenotype of WBS (Ewark et al. 1993). Studies of individuals with atypical deletions in the WBS region and in mouse models have implicated two related transcription factor genes, General Transcription factor 2 I (*GTF2I*) and GTF2I Repeat Domain containing protein 1 (*GTF2IRD1*), with the majority of symptoms of the cognitive and behavioral phenotypes (Antonell et al, 2010; Dai et al, 2009; Osborne, 2010; Borralleras et al, 2015).

Despite similar or identical size deletions and a global general pattern of the neurobehavioral phenotype, there is remarkable clinical variability among individuals with WBS. That is not surprising, since intelligence, behavior and every trait of the neuropsychological profile are strongly influenced by multiple environmental or biological factors. An example of a reported environmental factor that is a modifier of verbal intelligence in WBS is maternal education (Mervis

et al., 2012). Until now, other possible biological modifiers, like deletion size, have shown no association with the clinical profile. A maternal origin of the deletion was related in one study with more severe growth retardation and microcephaly, but the findings were not replicated in a larger sample (Pérez-Jurado et al., 1996). However, deletion of a functional NCF1 (Neutrophil Cytosolic Factor 1) gene copy at the deletion breakpoints was reported to act as a relevant modifier of the cardiovascular phenotype by protecting a proportion of individuals against hypertension (Del Campo et al., 2006). Another gene that can be affected by the breakpoint is *GTF2IRD2* (GTF2I repeat domain containing 2). *GTF2IRD2* is present with two functional copies in the human genome and the encoded protein is thought to interact and regulate the structurally related proteins GTF2I and GTF2IRD1. A possible role of the *GTF2IRD2* gene on executive function profile in the syndrome has already been suggested (Porter et al., 2012).

Understanding the genetic modifiers of the neurobehavioral phenotype of WBS may also help to better define the molecular pathways implicated in the deficits and design therapeutic interventions. The aim of this study was explore possible molecular modulators of the neuropsychological profile in WBS. We have analyzed differences between deletion size (1.55 vs 1.83 Mb), parental origin of the deletion (maternal vs paternal), different molecular variants depending of breakpoint, along with genetic variation in the non-deleted allele and elsewhere in the genome with a candidate gene approach. We selected a few single nucleotide polymorphisms (SNPs) have been associated to neuropsychiatric disorders and neurocognitive variation in general population, related with serotonin, norepinephrine and dopamine pathways and neural plasticity (Harrisberger et al., 2015).

METHODS

Subjects

Participants were 114 children and adults (66 males, 48 females) from 5-47 years old with mean age 17.19 (SD 9.96) with a diagnosis of WBS confirmed by the finding of a typical (1.55-1.83 Mb) heterozygous

deletion at 7q11.23. The study was approved by the Institutional Review Board and Ethics Committee, and informed consent was obtained from parents or caregivers. All participants were evaluated in a one day assessment. Medical history and records were recruited. Individuals with severe medical problems and/or perinatal complications considered that could interfere with the neuropsychological profile were excluded from the study. In addition, individuals meeting criteria for Autism Spectrum Disorder (ASD) by clinical evaluation and by scores in the Autism Diagnosis Interview-Revised (ADI-R; Rutter, Lecouteur & Lord, 2003) (n=6) were also excluded for this study due to the poor .

In order to control for some environmental influences on the profile, socioeconomic status (SES) was measured according to Hollingshead (1975). The sample distribution of SES was: high 15.8 %, medium high 22.8%, medium 18.4 %, medium low 19.3% and low 17.5%.

Neuropsychological instruments

Taking into account the main aspects and problems specific of the neuropsychological profile, a protocol with different instruments was performed with the aim to cover as many aspects as possible of the profile. Wechsler Intelligence Scales (WPSSI III; WISC-R; WAIS-III; Wechsler, 2002, 1974, 1997) were used to assess intelligence quotient (IQ). Score on Verbal IQ (VIQ) and Performance IQ (IQ) and global IQ were used. Executive function and behavior problems were measured using questionnaires answered by the principal caregiver (mother or father). Spanish version of the Behavior Rating Inventory of Executive Function Parent form (BRIEF-P, Gioia, Isquith, Guy & Kenworthy, 1996) was used to assess executive function. Inhibit, Shift, Emotional Control, Initiate, Working Memory, Plan/Organize, Organization of Materials, Monitor are the clinical scales of the instrument. Two indexes based on clinical scales are provided; Behavioral Regulation Index (BRI) which rates child ability to shift cognitive set and modulate emotions and behavior via appropriate inhibitory control (Inhibit, Shift and Emotional control scales), and Metacognition (MI) that rates the ability to initiate, plan, organize, self-monitor, and sustain work (composed of Initiate, Working Memory,

Plan/Organize, Organization of Materials and Monitor scales). Global Executive Composite scale (GEC) is the global score for all scales. T scores above 65 are considered clinical.

Behavior Problems were assessed using the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001). The questionnaire assesses eight empirically based scales based on the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV). Empirically-based scales are grouped into three higher-order factor scales: Internalizing Problems (Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints), Externalizing Problems (Rule-breaking Behavior, Aggressive Behavior) and Total Problems scale. T scores above 65 are considered subclinical and above 70 for empirically based scales. For analysis using the scales as a categorical variable, subclinical and clinical scores were computed together.

Social judgment (approachability to strangers) was evaluated by a modified version (Jones et al., 2000) of the Approachability task (Adolphs et al., 1998). Positive and negative faces were rated with five response options of approachability (yes, maybe, don't know, probably not and no). Total scores ranked from -2 (all responses are negative) to 2 (all responses are positive).

Visuospatial skills were measured by copy condition of the *Rey*-Osterrieth Complex *Figure* Test (ROCF; Rey, 1941, 1987). Raw scores were used.

Molecular analyses

DNA from all probands with WBS and both parents was isolated from peripheral blood cells using standard protocols and used for all molecular analyses.

Size, parental origin of deletion and deletion breakpoint mapping

Characterization of the size and parental origin of the 7q11.23 deletions was done by the analysis of single and multiple-copy microsatellites. To further define the breakpoint of the deletions and detect those mediated by inversions, site-specific nucleotides (SSNs) or Paralogous sequence variants (PSVs) were genotyped as previously

described in detail (Bayés et al, 2003, del Campo et al. 2006). In brief, by PCR amplification followed by digestion with restriction enzymes and size fractioning in agarose gels, the relative intensities of the SSN products were quantified and a dosage quotient was calculated to determine a gain or loss of specific blocks.

Specific SSNs from the originally described in the blocks B of segmental duplications (Bayés et al, 2003) were genotyped to identify the site of strand exchange in each deletion (Figure 1). These include SSN2 located at exon 21 of *GTF2I*, SSN4 to estimate the copy number of *NCF1*, SSN7 and SSN9 to infer the molecular variants of *GTF2IRD2*, and SSN11 to determine whether the deletion had been mediated by an inversion polymorphism in the transmitting parent.

SNPs analyses

A review of the relevant SNPs associated with neuropsychiatric disorders was performed with especial interest in those that had been related to cognition and the two most common psychopathological problems in WBS, anxiety and attention deficits. We selected SNPs in two genes implicated in serotonin activity; rs6295 in the 5-hydroxytryptamine receptor 1A (*HTR1A*) and rs6313 in the 5-hydroxytryptamine receptor 2A (*HT2AR*), responsible for post-synaptic activation upon serotonin transmission. Another SNP (rs6265: Val66Met) was on the *BDNF* gene, encoding a protein involved in neuro-genesis and neuroplasticity of the brain, previously implicated in depression, bipolar disorder and schizophrenia, among others (Harrisberger et al., 2015). Another SNP (rs4680) was in Catechol-O-methyltransferase gene (*COMT*), involved in metabolic de gradation of catecholamines. Finally, rs5751876 is in the Adenosine A2a receptor gene (*ADORA2A*), a CNS modulator that controls neuronal excitability, modulates neurotransmitter release and regulates ion channel function. It is expressed in brain, where it plays an important role in the regulation of glutamate and dopamine release.

For the analysis of genetic variants in the non-deleted allele as possible modifiers, we selected a total o five SNPs each located in an independent block of linkage disequilibrium according to Hapmap data (www.hapmap.org): rs799160, rs4717803, rs4717820, rs6460068

and rs2528997. SNP genotyping was done using the Sequenom Massarray iPLEX platform.

Statistical analyses

Different UNIANOVA analyses were performed for all quantitative neuropsychological variables with molecular variants. Logistic regressions were performed for categorical variables. For each variable different covariables (such as age, SES or gender) were taking into account. All possible genetic models were analyzed (dominant, recessive, codominant and overdominant) for each SNPs. In addition to regular comparisons, correction for multiple testing was also performed.

RESULTS

Molecular characterization

Of the 114 patients studied, 20 (17.5%) presented a 1.83Mb deletion and 94 (82.5%) a 1.55 Mb deletion. Regarding parental origin, 46 deletions were on the paternal chromosome (40,4%), 62 on the maternal (54,4%) while it could not be defined in 6 cases.

The 1.55 Mb deletion can occur at different breakpoints along the blocks B of segmental duplications depending of the site for chromosomal exchange during NAHR, resulting in variable functional copies of *NCF1* and *GTF2IRD2*. By mapping deletion breakpoint between two specific SSNs, 47 patients (41.2%) maintained the normal pattern of *GTF2IRD2* functional gene copies (2T+2M: two telomeric and two medial), 16 (14%) showed breakpoints within *GTF2IRD2* generating a chimeric copy (2T+1M+1C: two telomeric, one medial and one chimeric) and 27 (23.7%) had inversion-mediated deletion that resulted in the gain of a telomeric copy of and the loss of one medial (3T+1M: three telomeric and one medial). In the 1.83 Mb deletions, the crossover occurred between the centromeric and medial A blocks, resulting in a loss of the medial copy of *GTF2IRD2* (1M+2T) (and *NCF1)* both located in the deleted block B (Figure 1).

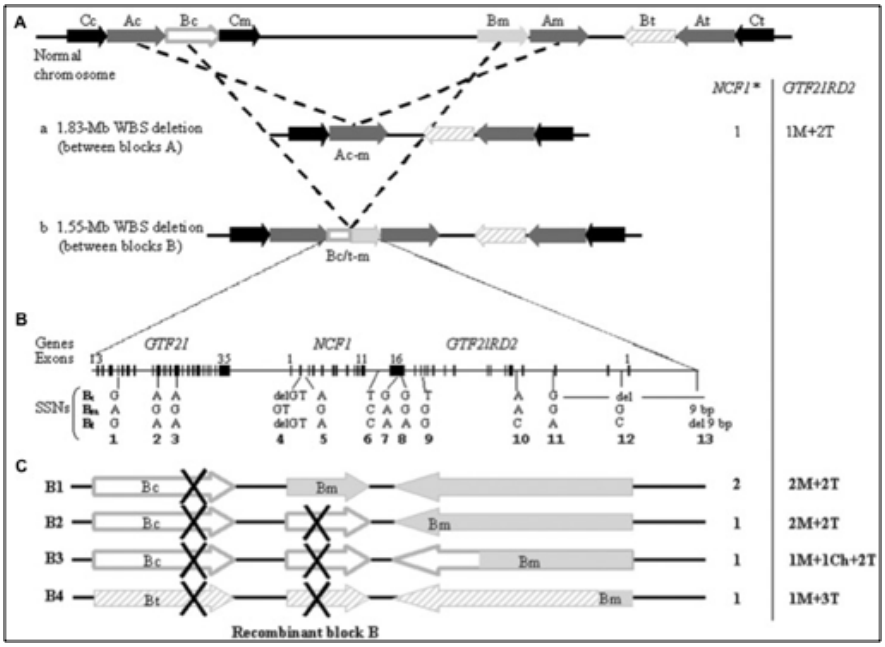

FIGURE 1. Schematic representation of the 1.55 Mb and 1.83 Mb deletions and breakpoint characterization**. A.** Representation of the 7q11.23 region, in black are represented the blocks C, in grey the A blocks and in white the B blocks. The top of the figure depicts the 1.83 Mb and 1.55 Mb deletions. **B.** Representation of the genomic content of the B blocks, as well as the location of the 13 genotyped site-specific nucleotides (SSNs) to refine the breakpoints of the deletion. **C.** Scheme of the different locations of the 1.55 Mb deletion. In breakpoint 1 (B1) the crossover occurs at *GTF2I*, therefore *NCF1* gene content and the functional copy of *GTF2IRD2* are not affected. In B2, the breakpoint is located at *NCF1*, therefore the patient presents with only one functional copy but *GTF2IRD2* remains the same. In B3, the breakpoint occurs at *GTF2IRD2* generating a chimeric copy with the final exons belonging to the centromeric block and the initial exons belonging to the medial block. Finally B4, which is the product of the inversion-mediated deletion, has a loss of *NCF1* gene and a gain of the telomeric *GTF2IRD2* copy.

Regarding the *NCF1* gene, the loss of a functional copy with a single gene copy remaining on the non-deleted allele was documented in 56 patients (49.1%), while two copies were present in 46 patients (40.4%). Ten patients had three gene copies (8.8%) and one four copies (0.9%) likely due to gene conversions, as previously reported (del Campo et al. 2006). Since sample size of individuals with 3 or 4 copy number variants was low, only participants with 1 or 2 copies were considered for analyses.

Clinical-molecular associations

*NCF1*copy number variants and the parental origin of deletion did not show any significant effect on the neuropsychological tests assessed. We did not detect any correlation of the clinical variables assessed also with any of the 5 SNPs distributed on the non-deleted allele or with those on the *HT2AR* and *COMT* genes.

Significant differences in overall IQ, verbal IQ and performance IQ were found between groups depending on *GTF2IRD2* variants related to deletion breakpoints (table 1). Individuals with a chimeric *GTF2IRD2* form showed significant higher overall IQ than individuals with inversion-mediated and larger 1.83 Mb deletion. On verbal IQ individuals with a chimeric *GTF2IRD2* form displayed higher verbal IQ than all the other three groups. Significant differences on performance IQ were only found between the group of chimeric *GTF2IRD2* and the group with inversion-mediated deletions. Rule breaking problem scale presented some significant differences between groups, with individuals with bigger deletion presenting with fewer problems but being a scale with low mean scores group 1 and 2. Same situation was found with two scales of executive function instrument with 1.83 Mb deletion presenting lower scores on org. materials and metacognition. Mean score of org materials scale for all groups are far away of being in the clinical rage /above 65) so differences found in this scale do not seem relevant to the profile. Finally, individuals with a chimeric form rated positive faces (faces that have being already selected as the most approachable faces on a general population sample) as more approachable than individuals with deletion mediated by inversion.

The analyzed SNP on BDNF also showed significant influence on cognitive abilities, especially on a recessive model. Individuals A/A (or met/met) scored higher on verbal IQ, performance IQ and overall IQ than individuals G/G or A/G (table 2). We also tested for possible interaction. A significant interaction (p=.038) was found between *GT2IRD2* and *BNDF* for IQ (table 3). Although the sample size was quite small, the two individuals that were homozygous A/A

(met/met) and had a chimeric *GT2IRD2* form showed significantly higher IQ than other conditions (table 3).

Nominal signification was found with the *BDNF* SNP for visuospatial skills when dominant and overdominant models were analyzed (table 4). As with visuospatial skills, ADH problems showed nominal signification on an overdominant model (table 5).

Significant associations were found for some SNPs and some behavior problems, for DSM oriented scales. For DSM-ADH problems scales for *BDNF* and *ADORA2A*, in the last case specially a dominant model. A dominant model on Conduct problems presented significance difference with higher number of individuals on a clinical score.

Nominal significations were found for SNPs on the *ADORA2A* and *HTR2A* genes. Attention deficits and hyperactivity and affective problems were associated with *ADORA2A* and an overdominant model of *HTR2A* was linked to affective and anxiety problems.

TABLE 1:Association of neuropsychological data with GTF2IRD2 genotypes

	GTF2IRD2				**p**					
IQ	**1 (N=22)**	**2(N=36)**	**3(N=12)**	**4(N=15)**	**1-2**	**1-3**	**1-4**	**2-3**	**2-4**	**3-4**
IQ	52.69	55.99	61.75	53.60	.165	.005*****	.757	.050	.385	.018*****
VIQ	62.09	63.59	71.64	62.03	.562	.011*****	.986	.021*****	.630	.018*****
PIQ	53.12	56.42	58.84	54.77	.070	.018*****	.461	.279	.433	.118
Visuospatial skills	**1 (N=21)**	**2(N=29)**	**3(N=12)**	**4(N=13)**	**1-2**	**1-3**	**1-4**	**2-3**	**2-4**	**3-4**
Rey	7.07	7.17	10.08	6.61	.955	.201	.840	.195	.796	.184
Behavior problems	**1 (N=26)**	**2(N=44)**	**3(N=13)**	**4(N=17)**	**1-2**	**1-3**	**1-4**	**2-3**	**2-4**	**3-4**
Anxious/depressed	62.21	60.51	63.66	59.79	.420	.617	.364	.243	.771	.220
Withdrawn/depressed	59.32	58.39	60.27	60.17	.655	.739	.765	.479	.481	.958
Somatic complaints	62.25	64.04	62.71	62.25	.406	.877	.999	.626	.478	.888
Social problems	63.27	64.31	64.76	63.69	.596	.576	.870	.854	.781	.706
Thought problems	64.76	64.12	63.79	63.09	.725	.696	.465	.886	.626	.795
Attention problems	68.78	65.77	66.62	65.40	.183	.483	.235	.767	.887	.715
Rule breaking behavior	57.56	57.59	57.27	54.29	.981	.869	.046*****	.845	.030*****	.123
Aggressive behavior	57.93	58.01	60.38	56.51	.965	.340	.550	.322	.495	.168
Internalizing problems	63.02	61.54	64.04	61.89	.504	.735	.687	.375	.891	.514
Externalizing problems	57.30	56.99	58.99	54.45	.866	.503	.223	.395	.241	.101
Total problems	64.26	62.70	64.81	62.46	.364	.814	.405	.335	.903	.357
Affective problems	64.50	64.13	64.38	63.20	.849	.965	.593	.918	.678	.679
Anxiety problems	67.68	64.76	67.87	64.34	.115	.642	.153	.187	.848	.201
Somatic problems	57.98	60.41	58.86	59.91	.250	.760	.618	.563	.653	.887
ADHD	64.68	62.58	63.63	60.70	.213	.648	.063	.624	.340	.243
OPD	56.70	57.13	58.18	56.34	.804	.526	.866	.627	.693	.468

Conduct problems	55.04	55.90	57.25	53.25	.536	.246	.311	.445	.106	.056
Executive function	**1 (N=25)**	**2(N=42)**	**3 (N=13)**	**4(N=16)**	**1-2**	**1-3**	**1-4**	**2-3**	**2-4**	**3-4**
Inhibit	57.67	59.68	57.32	54.10	.489	.929	.329	.516	.103	.450
Shift	63.76	63.10	62.71	60.42	.832	.802	.399	.921	.468	.621
Emotional control	58.66	61.59	62.10	63.46	.305	.369	.182	.886	.574	.744
Initiate	68.18	66.18	67.49	62.91	.444	.854	.103	.678	.268	.217
Working memory	72.90	71.45	67.75	69.92	.634	.209	.436	.331	.666	.627
Plan/organize	67.36	66.07	65.45	62.74	.585	.547	.123	.834	.227	.431
Org. materials	58.74	57.30	54.26	46.48	.552	.170	.000*****	.316	.000*****	.031*****
Monitor	65.74	65.59	63.22	62.87	.958	.492	.405	.487	.395	.931
BRI	61.12	63.04	62.35	60.80	.505	.750	.929	.848	.506	.713
MI	69.84	67.85	66.27	63.12	.395	.254	.024*****	.584	.083	.354
GEC	68.32	67.37	65.75	63.01	.704	.441	.093	.599	.133	.450
Approachability task	**1 (N=20)**	**2(N=29)**	**3(N=12)**	**4(N=14)**	**1-2**	**1-3**	**1-4**	**2-3**	**2-4**	**3-4**
Positive faces	.925	1.29	1.54	1.13	.094	.034*****	.409	.328	.522	.190
Negative faces	-.356	-.062	.491	.280	.388	.061	.109	.166	.378	.662
Executive function	**1 (N=25)**	**2(N=42)**	**3 (N=13)**	**4(N=16)**	**1-2**	**1-3**	**1-4**	**2-3**	**2-4**	**3-4**
Inhibit	57.67	59.68	57.32	54.10	.489	.929	.329	.516	.103	.450
Shift	63.76	63.10	62.71	60.42	.832	.802	.399	.921	.468	.621
Emotional control	58.66	61.59	62.10	63.46	.305	.369	.182	.886	.574	.744
Initiate	68.18	66.18	67.49	62.91	.444	.854	.103	.678	.268	.217
Working memory	72.90	71.45	67.75	69.92	.634	.209	.436	.331	.666	.627
Plan/organize	67.36	66.07	65.45	62.74	.585	.547	.123	.834	.227	.431
Org. materials	58.74	57.30	54.26	46.48	.552	.170	.000*****	.316	.000*****	.031*****
Monitor	65.74	65.59	63.22	62.87	.958	.492	.405	.487	.395	.931
BRI	61.12	63.04	62.35	60.80	.505	.750	.929	.848	.506	.713

MI	69.84	67.85	66.27	63.12	.395	.254	.024*****	.584	.083	.354
GEC	68.32	67.37	65.75	63.01	.704	.441	.093	.599	.133	.450
Approachability task	**1 (N=20)**	**2(N=29)**	**3(N=12)**	**4(N=14)**	**1-2**	**1-3**	**1-4**	**2-3**	**2-4**	**3-4**
Positive faces	.925	1.29	1.54	1.13	.094	.034*****	.409	.328	.522	.190
Negative faces	-.356	-.062	.491	.280	.388	.061	.109	.166	.378	.662

*p<.05 . GTF2IRD2: 1 (3T+1M), 2 (2T+2M), 3 (2T+1M+1Q) & 4 (deletion 1.83Mb; 2T+1M). IQ analyze was adjusted by SES and age. Analyze with Rey and Approchability task was adjusted by IQ, age and gender. Behavior problems and executive function were adjusted by age and gender.

TABLE 2:Association between IQ and BDNF genotypes (rs6265 SNP) (adjusted by gender, age and SES)

VIQ						
Gene (SNP)	Model	Genotype	n	Response mean	Difference (95% CI)	P
BDNF (rs6265)	Codominant	G/G	41	63.71	11.97 (3.37-20.58)	.021*
		A/G	32	62.97		
		A/A	7	74.43		
	Recessive	G/G-A/G	73	63.38	12.03 (3.83-20.22)	.0053**
		A/A	7	74.43		
PIQ						
Gene (SNP)	Model	Genotype	n	Response mean	Difference (95% CI)	P
BDNF (rs6265)	Recessive	G/G-A/G	73	55.25	6.16 (0.73-11.58)	.029*
		A/A	7	60.57		
IQ						
Gene (SNP)	Model	Genotype	n	Response mean	Difference (95% CI)	P
BDNF (rs6265)	Codominant	G/G	41	54.83	11.06 (3.78-18.34)	.014*
		A/G	32	55.03		
		A/A	7	64.86		
	Recessive	G/G-A/G	73	54.92	10.78 (3.81-17.74)	.0034**
		A/A	7	64.86		

TABLE 3:Interaction of GTF2IRD2 and BDNF genotypes with IQ

	GTF2IRD2											
	1 (inversion mediated)			**2 (no changes)**			**3 (quimeric copy)**			**4 (1.83 Mb deletion)**		
	N	Mean	Diff	N	Mean	Diff	N	Mean	Diff	N	Mean	Diff
G/G	10	52.8	0.00	19	55.7	2.07	4	56.75	4.00	7	54.14	2.79
A/G	9	53.2	-0.05	11	56.6	3.44	6	57	3.30	6	52.83	1.63
A/A	1	55	1.56	3	60.7	6.14	2	85	**33.10**	1	47	-4.88

TABLE 4:Association of Rey figure test raw scores with BDNF genotypes (rs6265) (adjusted by gender, age and IQ)

		REY				
Gene (SNP)	Model	Genotype	n	Response mean	Difference (95% CI)	p
BDNF (rs6265)	Dominant	G/G	38	6.05	3.11 (0.45-5.78)	.025*
		A/G-A/A	34	9.16		
	Overdominant	G/G-A/A	45	6.26	3.23 (0.49-5.97)	.024*
		A/G	27	10.26		

*p<.05

TABLE 5:DSM scales (0: normal, 1: subclinical and clinical) and significant SNPs associated adjusted by gender and age

DSM- ADH Problems scale					
Gene (SNP)	Model	Genotype	Normal – subclinical/Clinical	OR (95% CI)	P
BDNF (rs6265)	Overdominant	G/G-A/A A/G	27 (54%) 32 (74.4%) 23 (46%) 11 (25.6%)	0.39 (0.16-0.98)	.041*
ADORA2A (rs5751876)	Codominant	C/C T/C T/T	24 (48%) 11 (25.6%) 21 (42%) 27 (62.8%) 5 (10%) 5 (11.6%)	3.30 (1.26-8.67)	.043*
	Dominant	C/C T/C-T/T	24 (48%) 11 (25.6%) 26 (52%) 32 (74.4%)	3.10 (1.23-7.85)	.014*
	Overdominant	C/C-T/T T/C	29 (58%) 16 (37.2%) 21 (42%) 27 (62.8%)	2.69 (1.11-6.48)	.025*
DSM- Conduct Problems scale					
Gene (SNP)	Model	Genotype	0 – 1	OR (95% CI)	P
BDNF (rs6265)	Codominant	G/G A/G A/A	42 (50.6%) 9 (90%) 33 (39.8%) 1 (10%) 8 (9.6%) 0 (0%)	0.13 (0.02-1.15)	.025*
	Dominant	G/G A/G- A/A	42 (50.6%) 9 (90%) 41 (49.4%) 1 (10%)	0.10 (0.01-0.87)	.0087**
DSM- Affective Problems scale					
Gene (SNP)	Model	Genotype	0 – 1	OR (95% CI)	P
ADORA2A (rs5751876)	Codominant	C/C T/C T/T	16 (33.3%) 19 (42.2%) 30 (62.5%) 18 (40%) 2 (4.2%) 8 (17.7%)	0.53 (0.22-1.30)	.032*
	Recessive	C/C-T/C T/T	46 (95.8%) 37 (82.2%) 2 (4.2%) 8 (17.8%)	5.23 (1.03-26.47)	.026*
	Overdominant	C/C-T/T	18 (37.5%) 27 (60%)	2.69 (1.11-6.48)	.037*

		T/C	30 (62.5%) 18 (40%)		
HTR2A (rs6313)	Overdominant	C/C-T/T T/C	30 (62.5%) 17 (37.8%) 18 (37.5) 28 (62.2%)	2.77 (1.18-6.51)	.018*
DSM- Anxiety Problems scale					
Gene (SNP)	Model	Genotype	0 – 1	OR (95% CI)	P
HTR2A (rs6313)	Overdominant	C/C-T/T T/C	22 (62.9%) 25 (43.1%) 13 (37.1) 33 (56.9%)	2.58 (1.05-6.33)	.035*

*p<.05 **p<.01

DISCUSSION

We have found a significant association of cognitive function in WBS with the molecular variants generated by deletion breakpoints. Individuals with WBS and a deletion mediated by an inversion, leading to the gain of a telomeric and likely functional copy of *GTF2IRD2* showed more affected cognitive abilities (lower IQ), especially verbal, and less sociable behavior compared to individuals in whom deletion breakpoints interrupted *GTF2IRD2* creating a chimeric and likely dysfunctional form of the coding protein. On verbal IQ individuals with the chimeric form presented higher scores than all the other molecular groups.

GTF2I encodes a phosphoprotein containing six characteristic repeat motifs that binds to the initiator element (Inr) and E-box element in promoters and functions as a regulator of transcription of several genes. The pathways of action are the assembly of the initiation of RNA polymerase II complex and the signaling by Akt, with actions in nucleus and cytoplasm (Porter et al, 2012). *GTF2IRD2* regulates *GTF2I* and also *GTF2IRD1*, and inhibits its function by direct interaction and by kidnapping inactive nuclear areas (Palmer et al., 2012). The cross of mice depleted of GTF2IRD1 with mice depleted of GTF2IRD2 restored the phenotypic alterations of the first ones in the cell morphology of the muscle (Palmer et al., 2012), demonstrating antagonistic effect.

Therefore, our findings are fully consistent with the crucial role of GTF2I dosage in the cognitive skills of WBS syndrome and the potential inhibitor role of GTF2IRD2 on GTF2I action. These results open the possibility of creating therapeutic targets inhibiting GFT2IRD2 in order to decrease its antagonistic effect and facilitate GTF2I bioavailability and action.

One of the most extensively examined markers in multiple neurocognitive phenotypes is the Brain-derived neurotrophic factor (*BDNF*). BDNF is a neurotrophin involved in neural growth, differentiation and synaptic plasticity, activated in cortical neurons and necessary for survival of striatal neurons. It is highly expressed in the hippocampus, cortex, and basal forebrain, areas vital for learning,

memory, and higher thinking. BDNF itself is important for long-term memory. Interestingly, patients with the WAGR syndrome (Wilms tumour, Aniridia, Genitorinary anomalies, mental Retardation) show lower adaptive behavior and reduced cognitive function when the *BDNF* gene is included in the deletion, implicating BDNF haploinsuffiency in cognitive abilities (Han et al., 2013).

A functional SNP comporting a missense change in the BDNF protein has been found associated with IQ in WBS. The same Met allele is also associated with higher cognitive function in schizophrenia (Vyas & Puri, 2012). Interestingly and despite a very small sample size, a significant interaction was found between *GT2IRD2* and *BNDF* genotypes for IQ (table 3). These data suggest that both genes may have some direct interaction or complementary functions. In this regard, mice with a deletion of the Wbs interval showed hypersociability, cognition deficits and altered synaptic plasticity, along with decreased BDNF levels. Replacement of GTF2I by intracisternal delivery resulted in normalization of BDNF levels and significant rescue of the phenotype (Borralleras et al. 2015). Since GTF2IRD2 is a negative regulator of GTF2I, it is logical to propose that the increased GTF2I bioavailability secondary to the hypofunctional GTF2IRD2 may be associated with higher BDNF levels with and positive effects on synaptic plasticity and cognition.

Therefore, our data indicate that both, genetic variation at the deletion breakpoint interacting with GTF2I as well at candidate genes in related developmental pathways contribute to the clinical variability of the WBS neurocognitive profile.

Acknowledgements

This work was supported by grants from the Spanish Fondo de Investigación Sanitaria (FIS PI1002512 and PI1302841 to LAP-J), the Catalan Government (SGR2009-1274, SGR2014-1468 and ICREA Acadèmia to LAP-J), and FIS and AGAUR predoctoral fellowships (FI11/00656 to DP-G and FI_B2 00137 to MGP-V).

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SUPPLEMENTAL MATERIAL

TABLE 1: Supplemental : Associations of neuropsychological data and parental origin of deletions (maternal vs paternal)

	Deletion origin		p
IQ	**Paternal (N=37)**	**Maternal (N=50)**	
IQ	54.77	55.53	.716
VIQ	64.08	63.70	.875
PIQ	55.33	55.20	.937
Visuospatial	**Paternal (N=32)**	**Maternal (N=45)**	
Rey	7.45	7.26	.908
Behavior problems	**Paternal (N=43)**	**Maternal (N=56)**	
Anxious/depressed	60.51	62.04	.407
Withdrawn/depressed	60.11	58.72	.428
Somatic complaints	63.35	62.84	.782
Social problems	64.20	63.87	.841
Thought problems	64.75	63.29	.362
Attention problems	66.44	67.00	.774
Rule breaking behavior	56.90	57.13	.843
Aggressive behavior	57.85	57.99	.928
Internalizing problems	62.32	62.58	.891
Externalizing problems	57.09	56.77	.837
Total problems	63.74	63.22	.727
Affective problems	64.91	63.79	.484
Anxiety problems	65.60	66.27	.682
Somatic problems	59.19	59.09	.954
ADHD	62.18	63.63	.320
OPD	57.42	56.46	.504
Conduct problems	54.90	55.69	.514
Executive function	**Paternal (N=43)**	**Maternal (N=53)**	
Inhibit	58.15	57.25	.707
Shift	62.47	61.76	.798
Emotional control	62.52	59.90	.287
Initiate	66.08	66.50	.847
Working memory	70.31	70.69	.881
Plan/organize	65.78	66.01	.908
Org. materials	54.44	56.68	.315
Monitor	65.21	64.81	.866
BRI	62.58	60.99	.511
MI	66.72	67.90	.560
GEC	66.44	66.57	.949
Approachability task	**Paternal (N=30)**	**Maternal (N=46)**	
Positive faces	1.19	1.19	.996
Negative faces	.059	.008	.856

TABLE 2: Supplemental : Associations of neuropsychological profile data and NCF1 copy number (1 or 2)

	NCF1		p
IQ	**1 (N=44)**	**2(N=36)**	
IQ	55.51	54.32	.555
VIQ	64.23	62.17	.366
PIQ	55.46	54.94	.728
Visuospatial	**1 (N=43)**	**2(N=31)**	
Rey	7.95	6.47	.305
Behavior problems	**1 (N=50)**	**2 (N=44)**	
Anxious/depressed	62.46	60.37	.227
Withdrawn/depressed	60.68	57.78	.077
Somatic complaints	62.84	63.64	.636
Social problems	64.71	63.38	.393
Thought problems	64.51	63.83	.636
Attention problems	66.82	65.41	.440
Rule breaking behavior	57.03	56.88	.894
Aggressive behavior	58.74	57.16	.316
Internalizing problems	63.62	61.69	.256
Externalizing problems	57.42	56.43	.516
Total problems	64.37	62.40	.148
Affective problems	64.36	63.81	.711
Anxiety problems	67.08	65.29	.216
Somatic problems	58.92	60.05	.502
ADHD	63.08	62.16	.509
OPD	57.13	56.54	.673
Conduct problems	54.99	55.72	.546
Executive function	**1 (N=48)**	**2 (N=42)**	
Inhibit	57.53	58.08	.815
Shift	62.67	62.90	.933
Emotional control	61.75	59.24	.281
Initiate	66.67	65.07	.449
Working memory	69.65	71.16	.541
Plan/organize	66.29	64.39	.332
Org. materials	55.53	54.30	.575
Monitor	65.58	63.50	.350
BRI	62.14	61.32	.732
MI	67.70	66.00	.383
GEC	66.93	65.43	.465
Approachability task	**1(N=44)**	**2 (N=31)**	
Positive faces	1.18	1.27	.614
Negative faces	.087	.036	.848

DISCUSSION

Behavioral features of WBS compared to other subjects with ID

Behavioral and emotional profile of WBS is characterized by anxiety and attention problems with low prevalence of delinquent and aggressive behavior. Profile does not differ by gender unlike typical developing children were many differences between behavioral problems have been described (e.g. 133).

Although estimated prevalence of anxiety disorders in intellectual disability ranges from 3% (134) to 21.9% (135) (much lower than prevalence described in WBS (>60%)) no difference on anxiety between groups were found. Attention problems, as anxiety, didn´t differ between groups. Our findings reflect the possibility that these problems are not that syndrome specific and are secondary to intellectual disability. Maybe more differences would have been found by assessing symptoms or specific type of disorder.

On the other hand, aggressive and oppositional behaviors are less common in the syndrome than in other individuals with ID. Low externalizing problems do seem to by syndrome-specific of the profile. Probably the distinctive personality profile with high empathy and sensitivity to other people's emotions (36) is a protective factor for the appearing of these problems in WBS.

Finally, some associations between anxiety symptoms and IQ were found with higher levels of anxiety related to higher cognitive abilities. Thoughts and feelings present in anxiety disorders require cognitive abilities of understanding social roles and situations which would explain why individuals with higher cognitive skills show higher prevalence of anxiety disorders.

Executive function and personality influence in the behavioral and emotional profile

Although intellectual disability level has been associated to behavior problems in other neurodevelopmental disorders, our research showed no association of IQ with the behavioral profile of WBS. Probably due to the fact that stereotype and self injurious are the most common behaviors that have described related to ID level and

neither was measured with the instrument assessed or both has low prevalent in children with WBS.

Our results do show the important role that personality and executive function skills play an in the behavioral profile.

Emotional stability personality domain is associated with emotional problems and aggressive behavior in the syndrome. As described before in other studies, other personality dimensions do not show influence on behavior such as openness and agreeableness.

Dysfunctional executive functions are associated with behavioral profile of the syndrome mainly with ADHD and externalizing behaviors. ADHD has been related before with executive function deficits such as attentional and working memory deficits and difficulties with response inhibition (137). Maybe this fact makes ADH problems in WBS more complex because of more affected working memory and other executive function dimensions. Although low prevalent in WBS, aggressive behaviors seem related to negative emotionality and poor self-regulation.

Obtained results could be important for psychological therapy of behavior problem in WBS. If more knowledge of how executive function skills influence on behavior problems, clinicians would be able to create more effective therapy tools to improve certain executive function dimensions and therefore decrease severity of some behavioral problems.

Behavioral profile in children with WBS, cross-cultural similarities and differences

Differences found in several scales in raw scores but not found in T scores (when computed with normative data of each country), suggest that differences found by country in children with WBS are culture differences and not specific of WBS. Principally, the differences found in raw scores were related with internalizing problems. Cross cultural differences in emotional regulation have been reported before in typical developmental children (138) showing that the expression of emotions and its process and strategies of regulation differ across cultures (139). Therefore, our results reflect that behavioral and

emotional profile of children with WBS, as their typical development peers, is influenced by culture.

Even though studies have reported differences between countries and societies in behavior and emotional issues, another possible explanation for these differences could be related with parents of different cultures having different thresholds for reporting particular kinds of problems. Therefore, not only culture would influence in behavior but also parent's perception of what is considered a problem and how serious is that problem.

Our results could maybe be explained in terms of social behavior differences between societies and how high sociability behavior in children with WBS is fitted in each culture. Both countries compared in this study differ in how they are considered by their cultural social behavior or interaction. American society, is considered an individualistic culture, as Spanish society, where people are consider more interdependent and shape their behavior on the basis of in-groups norms (140). In Spain, as a collectivism culture, adolescents spend more evenings with their peers that in USA (Currie et al., 2008). Considering these issues, and that children with WBS present deficits in social skills; that would make Spanish children with WBS manage more social interactions than American children with WBS. Maybe having more social interactions makes Spanish children with WBS more vulnerable to suffer teasing from others than American. More research in this area should be considered because of the high levels reported by parents and the possible implications for internalizing problems and everyday life activities.

Lateral preference pattern and association with cognition and language

WBS, as other syndromes associated with intellectual disability, presents an atypical lateral preference with low prevalence of homogeneus lateral preference and higher prevalence of left and mixed lateral preference. Individuals with defined lateral preference (right or left) showed higher IQ and higher narrative abilities than those with mixed latera preference. Replication study also identified

differences for nonverbal reasoning, spatial, and receptive and expressive vocabulary.

Research on lateral preference has described handedness by a probable multifactorial model taking into account multiple genetic and environmental factors and interactions between them (142). Since lateral preference is defined before 5 years old, our results suggest that a high portion of children with WBS seem to have a poorer definition of laterality during brain development.

Lateral preference is shown to be related with brain asymmetry. 90% of individuals with right handedness show left hemisphere speech and language localization in while only 70% of left handers (143). Individuals with mixed handedness could maybe show a bilateral language representation that would affect language skills.

Other possible explanation is that WBS individuals show a smaller and morphologically different corpus callosum. Corpus callosum has been associated with functional language lateralization and visouspatial processing (144). This fact could maybe explain why mixed handedness children present lower language abilities and lower spatial and nonverbal reasoning.

Even thought we did not find correlation with molecular variants the increased prevalence of mixed handedness indicates that some of the dosage-dependent genes of the WBS deletion are probably required during development and lateral preference definition.

More morphological and functional brain studies and research on molecular mechanisms of laterality will help to understand the association of handedness with cognition and especially with language abilities.

GTF2IRD2 **and** ***BDNF*** **as modifiers of the neurospychological profile**

Although our understanding of WBS has significantly progressed during the last two decades, the molecular mechanisms underlying the WBS clinical phenotype (especially neuropsychological) and its variability are still awaiting clarification.

Since we wanted to analyze possible genetic modifiers and individuals with WBS have almost identical size deletions we considered other approaches for genotype-phenotype associations.

We found that chimeric form of *GTF2IRD2* with potential dominant-negative effect showed higher verbal and global IQ than other groups and higher approachability to strangers. A functional SNP at the *BDNF* gene was also associated with IQ with positive interaction with *GTF2IRD2*.

BDNF it is active in the hippocampus cortex, and basal forebrain and gives support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses. The same Met allele is also associated with higher cognitive function in schizophrenia.

Data suggest that both genes may have some direct interaction or complementary functions.

Our findings are fully consistent with the crucial role of GTF2I dosage in the cognitive skills of WBS syndrome and the potential inhibitor role of GTF2IRD2 on GTF2I action. These results open the possibility of creating therapeutic targets inhibiting GFT2IRD2 in order to decrease its antagonistic effect and facilitate GTF2I biodisponibility and action.

Finally, *NCF1* gene copy number, parental origin of the deletion and genetic variants on the non-deleted allele seem to not be related to this phenotypic variability.

CONCLUSIONS

1. Children and adults with WBS present with high prevalence behavior and emotional problems with no gender differences. Anxiety and attention deficits are the most common problems reported during their life span.

2. The high prevalence of anxiety and attention problems in WBS does not seem to be syndrome-specific when measuring overall symptoms in comparison to individuals with intellectual disability of other causes. Individuals with WBS do differ on overall lower levels of externalizing problems, presenting less aggressive and oppositional behaviors.

3. Intelligence quotient does not influence most the behavioral profile of WBS although some associations have been found with specific anxiety symptoms. Individuals with higher IQ present higher levels of symptoms.

4. WBS is associated with a specific profile of executive functioning with poor abilities to initiate, plan, organize and sustain future oriented problem solving in working memory. This characteristic executive function is an important component of the behavioral profile related to the ADHD and externalizing behaviors. Different dimensions of executive functions also interfere in the main emotional problems.

5. The distinctive personality profile of individuals with WBS is associated with behavioral and emotional problems mainly during adulthood. In children, emotional instability shows a positive relation with emotional and behavior problems. The personality profile of WBS changes during aging.

6. The sociocultural environment influences the behavioral and emotional profile of WBS. When these influences are controlled, the parental description of the behavioral profile of children with WBS from two different countries (Spain and USA) shows no differences in emotional problems, with only social problems differing by country. There is higher vulnerability related to hypersociability and poorer social adjustment for WBS individuals in a "more collectivist environment" than those in a "more individualistic society".

7. WBS as other syndromes associated with intellectual disability presents an atypical lateral preference with low prevalence of homogeneus lateral preference and higher prevalence of left and mixed lateral preference. Individuals with mixed hand laterality present lower cognitive abilities and lower language performance than those with defined lateral preference (right or left). Nonverbal reasoning, spatial skills could also be affected.

8. The high frequency of atypical handedness in WBS indicates that some of the dosage-dependant genes included in the 7q11.23 deletion are required for the proper definition of laterality during development. The relationship between handedness, cognition and language abilities suggests a dysfunction of interhemispheric inhibition in WBS.

9. There is significant variability of the neuropsychological profile in WBS individuals with identical size deletions. *NCF1* gene copy number, parental origin of the deletion and genetic variants on the non-deleted allele seem to not be related to this phenotypic variability.

10. Depending on breakpoints, deletions affect differently *GTF2IRD2*, a gene with two functional copies only in humans whose product is an inhibitor of GTF2I action. Variation at *GTF2IRD2* correlates with global and verbal cognitive performance as well as sociability in WBS, further indicating that the GTF2I pathway is highly relevant for cognition in WBS and *GTF2IRD2* is a major modifier.

11. A functional SNP at the *BDNF* gene was also associated with IQ in WBS, with positive interaction with *GTF2IRD2*. Our data, along with previous work showing that *Gtf2i* therapy normalizes Bdnf levels with beneficial neurobehavioral effects in *Wbs* mice, indicate that the GTF2I and BDNF pathways are related and potential therapeutic targets to improve cognition in WBS.

12. Functional SNPs at *HTR2A* and *ADORA2A* showed nominal association with anxiety, affective problems and the presence of attention deficits in WBS, indicating also a role of these genes as modifiers of some the WBS neurocognitive profile.

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LIST OF ACRONYMS

ABHD11	Abhydrolase domain containing 11
aCGH	array Comparative Genomic Hybridization
ADH	Attention Deficit Hyperactivity
ADHD	Attention Deficit Hyperactivity Disorder
ANOVA	Analysis of the variance
ASD	Autism Spectrum Disorder
BAZ1B	Bromodomain adjacent to zinc finger domain, 1B
BCL7B	B-cell CLL/lymphoma 7B
BFQ	Big Five Questionnaire
BFQ-NA	Big Five Questionnaire- Children and Adolescents
BRI	Behavioral Regulation Index
BRIEF-P	Behavior Rating Inventory of Executive Function Parent form
CBCL	Child Behavior Checklist 4-18 & 6-18
CNVs	Copy Number Variants
CLDN3	Claudin-3
CLDN4	Claudin-4
CLIP2	CAP-GLY domain containing linker protein 2
DAS	Differential Ability Scales
DBD-NOS	Disruptive Behavior Disorder—Not Otherwise Specified
DNA	Deoxyribonucleic Acid

DNAJC30	DnaJ (Hsp40) homolog, subfamily C, member 30
DSM	Manual of Mental Disorders
Dup7	7q11.23 Duplication syndrome
EIF4H	Eukaryotic translation initiation factor 4H
ELN	Elastin
EVT	Expressive Vocabulary Test, second edition
FISH	Fluorescent In Situ Hybridization
FKBP6	FK506 binding protein 6, 36kDa
FZD9	Frizzled class receptor 9
GAD	Generalized Anxiety Disorder
GCA	General Conceptual Ability
GEC	Global Executive Composite
GTF2I	general transcription factor Iii
GTF2IRD1	GTF2I repeat domain containing 1
GTF2IRD2	GTF2I repeat domain containing 2
ID	Intellectual Disability
IQ	Intelligence Quotient
kb	kilobases
LAT2	Linker for activation of T cells family, member 2
LIMK1	LIM domain kinase 1
Mb	Million base pairs
MD	Mean differences
MI	Metacognition Index
MLPA	Multiplex Ligation-dependent Probe Amplification

MLXIPL	MLX interacting protein-like
MRI	Magnetic Resonance Imaging
MSCA	McCarthy Scales of Children's Abilities
NCF1	Neutrophil cytosolic factor 1
NAHR	Non-Allelic Homologous Recombination
ODD	Oppositional Defiant Disorder
PAS	Pulmonary Artery Stenosis
PCR	Polymerase Chain Reaction
PIQ	Performance Intelligence Quotient
PPVT-4	Peabody Picture Vocabulary Test, fourth edition
PSV	Paralogous sequece variants
Qpcr	Quantitative polymerase chain reaction,
RFC2	Replication factor C (activator 1) 2, 40kDa
ROCF	*Rey*-Osterrieth Complex *Figure* test
RRB	Restricted and Repetitive Behaviours
SD	Standard desviation
SES	Socioeconomis Status
SNC	Special Nonverbal Composite
SNPs	Single Nucleotide Polimorphisms
STRs	Short Tandem Repeats.
STX1A	Syntaxin 1 A
SVAS	Supra Valcular Aortic Stenosis
TBL2	Transducin (beta)-like 2
TRIM50	Tripartite motif containing 50
TSH	Thyroid-Stimulating Hormone
VIQ	Verbal Intelligence Quotient

VPS37D	Vacuolar protein sorting 37 homolog D
WAIS-III	Wechsler Adult Intelligence Scale III
WBSCR22	Williams Beuren syndrome chromosome region 22
WBSCR27	Williams Beuren syndrome chromosome region 27
WBSCR28	Williams-Beuren syndrome chromosome Region 28
WBS	Williams Beuren Syndrome
WBSCR	Williams-Beuren Syndrome Critical Region
WISC-R	Wechsler Intelligence Scale for Children-R
WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence III

ANNEX

Síndrome de Williams Beuren, 50 años tras su descripción

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El síndrome de Williams Beuren (SWB) fue descrito hace ya 50 años cuando de manera casi simultánea el cardiólogo neozelandés John Williams y el pediatra alemán Alois Beuren describieron varias personas con un cuadro clínico similar que incluía rasgos faciales comunes, discapacidad intelectual con un patrón asimétrico, problemas cardiacos y de los vasos sanguíneos y niveles elevados de calcio en sangre (hipercalcemia) (Pober, 2010). La prevalencia del SWB se ha estimado varias veces alrededor de 1 de cada 20,000 recién nacidos, si bien el único estudio prospectivo realizado en el año 2002 en Noruega estableció una mayor ocurrencia, de 1 cada 7.500 recién nacidos (Stromme et al., 2002). La causa molecular se empezó a conocer en 1993 cuando un grupo de la universidad de Utah, EEUU (Ewark et al., 1993), describió la pérdida (deleción) de una copia del gen *ELN* (que codifica para la proteína elastina) en varios pacientes con criterios clínicos SWB. Después de aquel descubrimiento, se ha ido esclareciendo el tamaño de la deleción (1.55 Mb -1.8 Mb), así como el número de genes de los que se pierde una copia (26-28) y su función.

Actualmente existen diferentes herramientas diagnósticas que nos permiten detectar y caracterizar en detalle la lesión molecular (deleción) desde material genético obtenido de cualquier grupo de células de las personas con SWB, y así realizar un diagnóstico certero y precoz. Estas técnicas incluyen la hibridación in situ fluorescente (FISH), los microarray de hibridación genómica comparada (aCGH), la amplificación múltiple dependiente de sondas ligadas (MLPA) y la

reacción en cadena polimerasa cuantitativa (qPCR). Además de demostrar el origen casi siempre *de novo* (no heredado) de la alteración, estos estudios permiten observar si existe variación en el material genético que se pierde, lo que puede ser importante para el correcto seguimiento de cada caso y el asesoramiento a las familias.

En nuestra unidad realizamos investigación clínica, siguiendo en la actualidad a más de 250 personas (47.2% mujeres, 52.8% hombres), consistente en un examen médico completo con un protocolo común, una evaluación neurocognitiva exhaustiva, y la recogida de datos analíticos diversos y de imagen. En todos los casos se realiza además una caracterización molecular detallada.

La edad de diagnóstico media del SWB es de 5.7 años, aunque en los últimos cinco años está claramente bajando hasta los 2.5 años aproximadamente. En cuanto a los problemas médicos asociados al SWB, resumidos en la tabla 1, destacan los problemas cardiovasculares (siendo la estenosis aórtica supravalvular el problema más frecuente), los síntomas gastrointestinales, la disfunción de la vejiga urinaria, las hernias, y los problemas esqueléticos y de odontología. También hemos detectado recientemente alteraciones metabólicas asociadas. Algunas de ellas son el hipotiroidismo, que parece subclínico (40%) y no está claro si precisa tratamiento, la diabetes o intolerancia a la glucosa (6.5%), la disminución de los triglicéridos en sangre (35%) y la posibilidad de presentar niveles ligeramente altos de bilirrubina (15%). Estas alteraciones se corresponden con defectos de expresión de genes concretos afectados por la deleción y otros genes que regulan.

Edad gestacional >41s	31
Peso al nacer <p10	53
Talla <p3	64
OFC <p10	42
Rasgos faciales	100
WSCP / personalidad	100
Hiperacusia	96
Cardiovascular	75
Hipertensión	47
Hipercalcemia	15
Gastrointestinal	88
Anomalías tracto urinario	23
Disfunción vesical	85
Estrabismo / hipermetropía	33
Problemas esqueléticos	57
Hernias	50
Problemas ortodóncicos	75

Tabla 1: Datos clínicos (% ; n = 260)

Las evaluaciones neuropsicológicas muestran un cociente intelectual medio de 55 en las personas con SWB, indicativo de una discapacidad intelectual entre leve y moderada, pero con un rango que oscila entre 40 y 85. En la mayoría de los casos hay diferencias significativas entre el cociente intelectual verbal y el cociente intelectual manipulativo, con una preservación relativa de las áreas verbales y especial afectación del área visuoespacial dando lugar al perfil desigual descrito. Presentan rasgos de personalidad característicos, como una gran sociabilidad, empatía y excesiva ansiedad anticipatoria. Los problemas conductuales de mayor prevalencia son precisamente la ansiedad (trastorno de ansiedad generalizada y fobias), el déficit de atención con hiperactividad (especialmente en edades tempranas) y los trastornos afectivos que suelen comenzar por lo general a las edades entre 15 y 25 años (figura 1) (Pérez-García et al 2011).

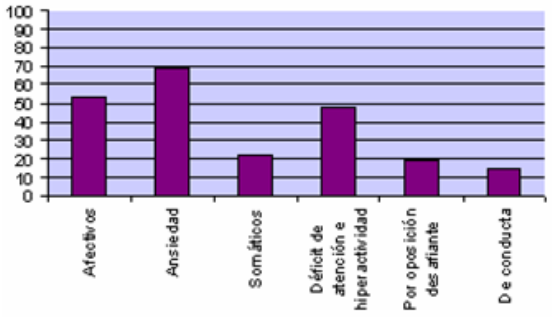

Figura 1: Prevalencia de problemas (n = 68)

Para definir mejor los mecanismos de la ansiedad y sociabilidad en SWB, hemos participado en otra investigación clínica colaborativa reciente utilizando sistemas de imagen cerebral. El objetivo del estudio ha sido conocer mejor las zonas del cerebro que intervienen en el funcionamiento social y la ansiedad del síndrome, de manera comparada a personas controles e individuos con trastorno de ansiedad social. Para ello se ha realizado una resonancia magnética funcional mientras el sujeto realiza diferentes tareas de reconocimiento

de caras y emociones además de una valoración (por entrevista y cuestionarios) sobre ansiedad y personalidad.

La existencia de un cuadro clínico con manifestaciones en diversos órganos y sistemas implica la conveniencia de que se realice siempre un seguimiento especializado integrado, idealmente en una unidad multidisciplinar en la que participen varios especialistas, idealmente con experiencia: genética, pediatría o médico de cabecera, ortopedia, nefrología, oftalmología, endocrinología, ortodoncia, cardiología, psicología y neurología.

Tras deteminar y hacer seguimiento de los problemas médicos y conductuales asociados, la investigación se enfoca en intentar definir el mapa fenotípico de la región delecionada, es decir, establecer la posible correspondencia entre genes y manifestaciones clínicas. En la figura 2 se muestran los genes que se pierden en las personas con SWB y la correspondencia con las manifestaciones en las que se cree están implicados. El conocer qué gen o genes son responsables de las diferentes manifestaciones es de vital importancia para entender el mecanismo de mala función del organismo y podrá conducir en un futuro a tratamientos más eficaces.

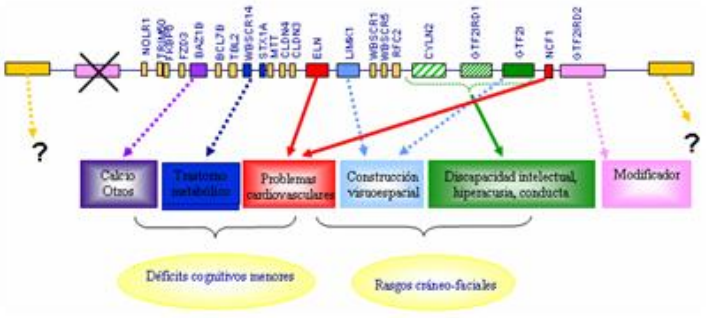

Figura 2: Mapa fenotípico de la región 7q11.23

Otra gran herramienta para definir los genes importantes y los mecanismos del cuadro es el trabajo con modelos animales y celulares. El ratón ha demostrado ser un modelo ideal para el estudio de diversos problemas en el ser humano. En el caso del SWB, existen diferentes modelos de ratón, varios de los cuales han sido generados por nosotros y seguimos estudiando. Hemos generado un modelo de

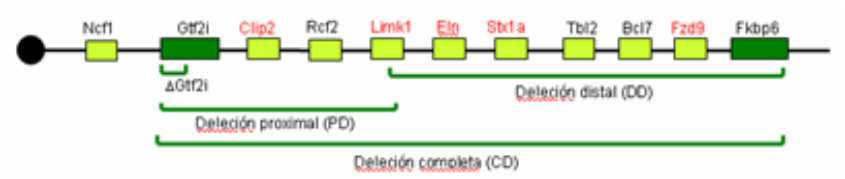

Figura 3: Modelos de ratón para el SWB

ratón con la deleción completa (la misma alteración que las personas con SWB), otros dos con deleciones parciales en la región y uno que sólo tiene afectado uno de los genes más relevantes (*Gtf2i*) (figura 3). Los principales resultados de los estudios ratones han demostrado el papel fundamental del gen *GTF2I* en el perfil neurocognitivo y el del gen *NCF1* como modificador de la severidad del cuadro cardiovascular (Antonell et al 2010, del Campo et al 2006). En los ratones se han podido realizar ensayos preclínicos con medicación, que documentan la utilidad de algunas medicinas para mejorar los problemas cardiovasculares (Losartán y Apocinina) (Campuzano et al 2012).

En cuanto a modelos celulares, además de disponer de células de la sangre y de la piel de muchos casos, estamos actualmente generando células iPSC (células pluripotenciales inducidas, reprogramables). Una de las limitaciones para el estudio de enfermedades del desarrollo neurológico humano es la imposibilidad de analizar en detalle el órgano directamente implicado, el cerebro. Una posible estrategia consiste en generar células con la alteración genética que se puedan reprogramar y diferenciar a la célula de interés, a neurona en este caso. La diferenciación a células neuronales se puede conseguir desde casi cualquier célula, siendo preferible el uso de células de la piel (fibroblastos de debajo de la epidermis). Para ello se han seleccionado personas con SWB y personas con el síndrome de duplicación 7q11.23 (que tiene una ganancia en lugar de pérdida de los mismos genes que el SWB), así como controles. Se pretende realizar estudios morfológicos de las células (cómo son), estudios electrofisiológicos (cómo funcionan) y estudios farmacológicos (ver si reaccionan a determinados fármacos).

Por último, se ha solicitado la posibilidad de realizar ensayos clínicos adicionales con productos aparentemente seguros para valorar efectos en función cognitiva, si bien no existe todavía financiación para su realización. La plasticidad sináptica, es decir, la capacidad que las neuronas tienen para alterar su capacidad de comunicación entre ellas, es uno de los mecanismos alterados en el SWB así como en otros cuadros como los síndromes de X Frágil o Down. Estudios previos muestran que los flavonoides modulan la estructura de las espinas dendríticas en la plasticidad sináptica, y lo hacen modulando una de las vías de señalización que podría ser común a varios cuadros. Por este motivo, se plantean como diana terapeútica para la mejora del rendimiento cognitivo.

En resumen, los recursos generados durante muchos años por diversos grupos de investigación, las bases de datos y registros de personas con SWB, los modelos animales y las células derivadas de personas con SWB, permitirán entender mejor los mecanismos que funcionan mal en este cuadro e intentar otras aproximaciones a tratamiento que sean más específicas. La colaboración de distintos profesionales y asociaciones de pacientes, tanto a nivel nacional como internacional, es fundamental para contribuir a mejorar el conocimiento sobre la historia natural del cuadro, sus posibles complicaciones, y que la información pueda llegar a todas las familias de manera rápida cuando existan nuevas iniciativas o ensayos clínicos, y conseguir contribuir a mejorar la calidad de vida de las personas con SWB

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La comunicación del diagnóstico

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Revista Asociación Española síndrome de Willliams, 2015

La manera en que se produce la comunicación del diagnóstico nuevo de un niño/a con síndrome de Williams, así como el profesional que la realiza, tiene una gran importancia por su influencia en cómo los padres van a manejar y procesar la información que se les ha facilitado. Tanto el momento en el que se recibe la información del diagnóstico como el/los profesionales que transmiten dicha información serán siempre recordados por los padres. En la mayoría de casos, incluso se recuerdan las palabras textuales de cómo se les comunicó.

El impacto de recibir una noticia como el diagnóstico de un hijo/a, en este caso de una condición que les va afectar para toda la vida, produce que las personas pasen por un proceso denominado duelo. El duelo es el proceso mediante el que se requiere una adaptación a nuevas circunstancias inesperadas. Tras el diagnóstico, los padres tendrán que modificar la idea formada sobre cómo iba a ser su hijo/a. El proceso comienza con una sensación de shock o impacto inicial. En esos momentos existe duda y perplejidad ante la noticia, ante la cual suele haber una tendencia a negar lo que ha ocurrido y bloquear los sentimientos de dolor. Estas sensaciones dan paso a una segunda fase de ira o rabia por la incapacidad de modificar los hechos y una búsqueda del por qué. Existen sentimientos de desesperación y desorden, y se piensa que nada podrá ser como

antes. A partir de estas fases, comienza la fase de aceptación y a una reorganización de los planes, para finalmente dar paso a lo que se denomina la "nueva identidad", en donde hay una adaptación a los acontecimientos y donde existe un crecimiento personal como resultado del proceso.

Este proceso de duelo es variable en tiempo en función de diversos factores como el momento en el se produce la noticia, la red de apoyos de la familia o su nivel de recursos. También existen ciertos factores de riesgo que pueden complicar el proceso o que este se convierta en patológico: el momento vital, las vivencias personales, el estilo de afrontamiento del estrés, estilos básicos de gestión de conflictos, la ausencia de soporte familiar, la falta de red social, carecer de hobbies y práctica de eventos placenteros y la forma de comunicación del diagnóstico. En este último aspecto, durante los últimos años se han producido diversos estudios tanto a nivel nacional (diagnóstico de un hijo/a con discapacidad) como internacional (específicamente del síndrome de Williams-Beuren) analizando, a través de cuestionarios contestados por los padres, los aspectos positivos y negativos de aquel momento.

En España la comunicación suele hacerse en el 41.3 % de los casos por pediatras y en el 38.3% por médicos especialistas. A la hora de la comunicación del diagnóstico, ambos estudios muestran como en algunos casos, sólo un progenitor estaba presente. Este hecho debería ser evitado ya que es muy importante procurar que los padres puedan estar juntos. Cuando no es posible, sería conveniente que otro familiar cercano pudiera acompañar a la madre o padre en esos momentos.

En la mayoría de casos, la información era comunicada en un despacho. Algunos padres manifestaron el haber recibido la noticia en un pasillo, en presencia de desconocidos o por teléfono. El recibir el diagnóstico en estas circunstancias era considerado por los padres como muy negativo, así como que el profesional pasara poco tiempo con ellos una vez les hubiera comunicado el resultado de las pruebas. Es importante, y así lo valoran las familias, que todo el proceso no se produzca en un solo contacto. Los profesionales deben estar a disposición de los padres y ofrecerles suficientes oportunidades para aclarar sus dudas o derivarlas a otros servicios, y explicarles los recursos existentes. Muchas familias recalcaron la importancia de que se les pusiera en contacto con un médico con suficiente experiencia y conocimiento sobre el síndrome, así como con otra familia que hubiera pasado por el mismo proceso. Las familias que durante el proceso de diagnóstico tuvieron visita con un asesor genético tenían una mejor percepción del proceso. En España, los padres consideran un punto fuerte la derivación hacia los servicios de atención temprana.

Durante el proceso, puede existir una sobrecarga informativa que debe ser canalizada, teniendo en cuenta que se produce en un momento de gran carga emocional. Los sentimientos predominantes que relatan los padres son tristeza, confusión, duda, desorientación, desconcierto, inseguridad e inquietud, angustia, dolor y pérdida.

Aunque, como es lógico, es muy conveniente que el diagnóstico del síndrome de Williams sea lo más precoz posible, el proceso de duelo puede ser más difícil a edades tempranas. Cuanto mayor es la edad de la persona a la que se diagnostica síndrome de Williams,

aumenta la probabilidad de que la experiencia del diagnóstico sea considerada como positiva por parte de los padres. Cuando el diagnóstico se produce cuando la persona es adolescente o adulta, los padres pasan de manera diferente por el proceso comentado, en parte porque existe una tranquilidad relativa de conocer la causa de las dificultades que ya han sido asumidas.

Estos estudios enfatizan la importancia del momento de comunicar un diagnóstico y demuestran que existe cierta insatisfacción de algunas familias en la manera en que se produce. El conocimiento y difusión de estos deberían servir de aprendizaje para los profesionales e instituciones sanitarias, al objeto de que exista una mayor concienciación y se desarrollen mejores herramientas para la comunicación, así como que se establezcan protocolos hospitalarios para el acompañamiento de la familia durante el proceso.

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Emily Pearl Kingsley, guionista de Barrio Sésamo, escribió este texto en 1987, para intentar expresar, basada en su propia experiencia personal, los sentimientos y sensaciones de la comunicación de la noticia. Aunque ya se ha publicado en otras ocasiones, creemos que su lectura es muy apropiada y útil para

todas las familias que se tienen que enfrentar por primera vez a la noticia de un diagnóstico de síndrome de Williams.

Bienvenidos a Holanda

A menudo me piden que describa la experiencia de criar a un niño con una discapacidad, que intente ayudar a la gente que no han compartido esa experiencia única a imaginar cómo se sentirían. Es así...

Cuando vas a tener un bebé es como planear unas vacaciones fabulosas en Italia. Compras un montón de guías y haces tus maravillosos planes. El Coliseo. El David de Miguel Ángel. Las góndolas de Venecia. Puede que aprendas algunas frases útiles en italiano. Es todo muy emocionante.

Después de meses de ansiosa anticipación, finalmente llega el día. Preparas tus maletas y allá vas. Varias horas más tarde el avión aterriza. La azafata viene y dice: "Bienvenidos a Holanda".

- ¿Holanda? - dices -. ¿Cómo que Holanda? Yo me embarqué para Italia. Se supone que estoy en Italia. Toda mi vida he soñado con ir a Italia.

- Pero ha habido un cambio en la ruta de vuelo. Han aterrizado en Holanda y aquí se debe quedar.

Lo importante es que no te han llevado a ningún lugar horrible, sucio o pestilente, con hambruna y enfermedad. Simplemente es un sitio diferente.

Así que tienes que salir y comprarte nuevas guías. Y tienes que aprender una lengua completamente nueva. Y conocerás a un grupo entero de gente que nunca habrías conocido.

Simplemente es un sitio diferente. Camina a un ritmo más lento que Italia, es aparentemente menos impresionante que Italia. Pero cuando, después de haber estado un rato allí, contienes el aliento y miras alrededor, empiezas a notar que en Holanda hay molinos de viento. Holanda tiene tulipanes. Holanda tiene incluso Rembrandts y otras muchas obras de arte.

Pero todo el mundo que conoces está muy ocupado yendo y viniendo de Italia y todos presumen muy alto de qué maravillosamente se lo han pasado en Italia. Y, durante el resto de tu vida, dirás "Sí, ahí era donde se suponía que yo iba. Eso es lo que había planeado."

Y ese dolor nunca, nunca, nunca, se irá, porque la pérdida de ese sueño es una pérdida muy importante.

Pero si te pasas la vida quejándote del hecho de que nunca llegaste a Italia, puede que nunca tengas libertad para disfrutar de las cosas, muy especiales, maravillosas, de Holanda.