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Research article

Toward autism spectrum disorders and Williams-Beuren syndrome co-occurrence condition in Tunisian patients: Genetic insights

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Abstract: Introduction: Williams-Beuren syndrome (WBS) is a rare genetic disorder characterized by congenital heart defects, dysmorphic features, intellectual delay, and a distinctive social behavioral profile. This highly recurrent and homogeneous phenotype has been curiously reported to be associated with autism spectrum disorders (ASD). Both genetic and environmental origins have been implicated. This study aimed to describe Tunisian patients associated with WBS and ASD and explore the underlying etiologies. **Methods:** Thirty-one clinically suspected WBS were referred for genetic exploration. A comprehensive evaluation using karyotyping, fluorescence in situ hybridization (FISH), and array comparative genomic hybridization (array-CGH) was performed. **Results:** All patients were clinically diagnosed and confirmed to have WBS through karyotyping and FISH analysis. Notably, six patients with complex or atypical clinical presentations underwent array-CGH. Two of these patients presented with ASD. Array CGH showed microdeletions ranging from 1.4 to 1.7 Mb in the 7q11.2 region. Further analysis of the extended region deletion identified a gene closely located in the deleted region, the HIP1 gene, involved in the central nervous system trafficking protein. **Discussion:** The recurrent deletion in WBS, as well as the mirror duplication, may contribute to ASD development in some cases, suggesting a potential involvement of the ASD genes pathway in this region. However, recessive genetic origins should also be considered, particularly in consanguineous families.

Furthermore, our findings highlight the potential role of genetic factors and regulatory elements within the deleted region in modulating gene expression, notably the HIP1 gene. This underscores the implications of gene dosage and environmental factors in the broader WBS region, notably with language and social development. **Conclusion:** The presence of ASD in WBS patients emphasizes the need to investigate all WBS patients for autistic traits to establish a better genotype–phenotype correlation. We underline the utility of array-CGH as a valuable genetic diagnostic tool for characterizing WBS cases, and we shed light on the complex interplay of behavioral disorders in the 7q11.2 region rearrangements.

Keywords: CGH-array; Williams Beuren-syndrome; autistic traits; HIP1

1. Introduction

Williams-Beuren syndrome (WBS; OMIM #194050) is a contiguous gene deletion syndrome caused by 1.5–1.8 Mb microdeletion on chromosome 7q11.23 [1]. WBS is distinguished from other neurodevelopmental disorders by a particular behavioral and cognitive profile characterized by hyper-sociability contrasting with a mental and psychomotor delay, a specific dysmorphic face called "elfin face", and congenital heart disease, which occurs in 75% of cases, including supra valvular aortic stenosis and pulmonary artery branch stenosis [2]. The cardiac phenotype is mainly due to the *ELN* haploinsufficiency, which codes for elastin, a component of the extracellular matrix and the main protein of the elastic fibers of arteries, veins, lungs, and skin [3]. Reducing the quantity or absence of elastin induces excessive smooth muscle cell proliferation in the vascular walls, leading to obstructive vascular disease [4].

The WBS deletion is usually described as sporadic, but familial cases have been observed. The same deletion size of 1.55 Mb was seen in 95% of patients, while 5% of patients exhibited a larger deletion of 1.84 Mb [5] Two low copy repeat (LCR) sequences with very high homology are at the origin of non-allelic homologous recombination (NAHR) during meiosis, resulting in deletions and duplication rearrangements.

The 7q11.23 deletion is usually detected by molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), and array comparative genome hybridization (array CGH). The latter technique provides more information about the size of the deletion and the genes implicated. The included genes count for at least 29 genes; CLIP1, GTF21, NCF1, and LIMK1 are known to contribute to the WBS neurophysiological features. In this paper, we present findings from 31 additional WBS Tunisian cases and comprehensively review the literature. We underline the potential association between WBS and autism, proposing a candidate locus at 7q11.23.

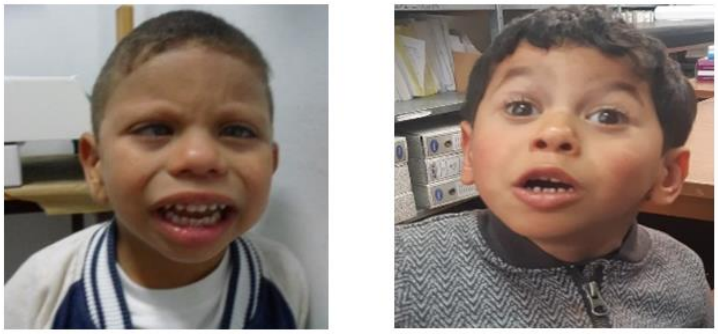
2. Materials and methods

2.1. Patients

2.1.1. Study design and participants

Thirty-one patients were referred to the Genetics Department of the Farhat Hachad Hospital in Sousse for genetic analysis. Figure 1 presents the clinical dysmorphology of Patients 5 and 6. The

clinical descriptions of these two patients with ASD, confirmed by a pedopsychiatrist using the Childhood Autism Rating Scale (CARS), are detailed in the results section below.



Patient 5 Patient 6

Figure 1. Photographs of Patients 5 and 6 at the age of consultation (4 years and 2 years).

2.1.2. Ethics approval of research

The local Ethics Board of the University Teaching Hospital Farhat Hached approved the present study (CER:14-2022), and informed written consent was obtained from the parents of all the children. Consent is an informed choice by the parent or legal guardian (either in writing or verbally). A separate consent form was completed for each child participant.

2.2. Methods

2.2.1. Karyotype

For all patients included in this study, a conventional R-band karyotype at a 450-band resolution was systematically performed. According to a standard protocol, metaphase chromosome spreads were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. Cell cultures were incubated for 72 hours. At least 25 mitoses were studied for each sample using Cytovision® karyotyping software version 4.0.

2.2.2. FISH analysis

FISH was carried out on metaphase spread chromosomes with commercial probes. The patient's DNA and the probe are denatured at 75 °C, which allows the specific hybridization of the probe with its target sequence in a second step. After overnight hybridization at 37 °C and washing, chromosomes were counterstained with 4,6 diamino-2-phenylindole (DAPI), and hybridizations were observed using an Axioskop Zeiss® fluorescent microscope.

2.2.3. Array CGH

Agilent® oligonucleotide array was performed with Agilent Human Genome CGH Microarray kit 44 K according to the manufacturer's instructions (Feature Extraction 9.1, CGH Analytics 4.5, Santa Clara, California, United States). A copy number variation was detected when at least three contiguous oligonucleotides had an abnormal ratio higher than 0.58 or lower than −0.75. In silico analysis of the unbalanced regions was made using the UCSC Genome Browser **(https://genome.ucsc.edu/),** the Database of Genomic Variants **(DGV: http://dgv.tcag.ca/dgv/app/home),** and the Online Mendelian Inheritance in Man database **(OMIM: https://omim.org/).**

2.2.4. Childhood autism rating scale (CARS)

The CARS test compares a child's behaviors, characteristics, and abilities to a typical child's expected developmental growth by evaluating its relationship to people, emotional response, body use, object use, visual response, listening response, fear and nervousness, verbal and nonverbal communication, activity level, level and consistency of intellectual response, and general impressions. These parameters are scored to diagnose the presence or absence of ASD and the severity of the condition. CARS total scores of less than 30 indicate that a person is non-autistic, while scores of 30 or higher indicate that a person is autistic. Individuals with scores of 30–36.5 are considered to have mild to moderate autism; scores of 37–60 define severe autism [6].

3. Results

The clinical and cytogenetic findings of the present cohort study are shown in Table 1. All the patients had a specific WBS dysmorphic face. The diagnosis age average was 6 years. The two major signs noted in this cohort were mental deficiency (83%) and cardiac defects (55%). The characteristic pulmonary artery stenosis was seen in 88% of cases, while 11% of cases presented an interventricular septal defect. Psychomotor delay was observed in 20% of the cases. Ahead of the known sociable and cooperative characteristic attitude in WBS, the clinical diagnosis was made mainly within the age of 5 years based on learning difficulties in the preparatory class. A language delay with or without behavioral disorders was noticed in 25% of the cases. Microcephalia and short stature were noted in 48% and 16% of the cases, respectively. Less frequently, gait disorders, scoliosis, and kidney defects were also observed. Notably, and contrasting with the well-described characteristic WBS, autism was noted in two patients (Patients 5 and 6). An evaluation using the pedopsychiatric CARS test was established. A score of 41, followed by a score of 37 eight months later, was assigned to patient 5, revealing severe autism. For patient 6, a score of 36 was assigned, indicating moderate autism.

Conventional cytogenetic analysis of all the patients indicated a normal karyotype in all metaphases. FISH analysis based on the suggestive WBS clinical phenotype confirmed the deletion of the ELN gene (kreatech ® specific probe) in all 31 patients. To better understand the 7q11.2 implication in ASD, we investigated the potential association between WBS and ASD, a condition rarely described in WBS patients. Individuals with WBS without ASD traits were compared with those presenting both WBS and ASD (WBS+ASD).

The six patients who benefited from the array CGH presented severe or atypical WBS features: two types of congenital heart defects (lung and aortic stenosis, Patient 1), a familial case (Patient 2), a heart ultrasound without defects (Patient 3), the presence of renal agenesis and hypothyroidism (Patient 4), and, notably, the presence of ASD in Patients 5 and 6.

Array CGH analysis displayed a partial deletion on the long arm of chromosome 7, involving almost the same 7q11.2 region for the six patients. The deletion size ranged from 1.43 to 1.7 MB. Interestingly, the two patients with autism presented the common deleted region encompassing 1.7 Mb.

3.1. Clinical description of Patients 1–4

The patients who benefited from an array CGH presented atypical clinical features compared to the other WBS patients. The first patient, in addition to the facial dysmorphia and the hyper-sociable compartmental profile, presented an association of two congenital heart defects: pulmonary and aortic stenosis. Patient 2 was a familial case. Patient 3 did not show the typical WBS phenotype, and no heart defects were found. Patient 4 presented, in addition to the WBS typical phenotype, renal agenesis and hypothyroidism. Detailed clinical information of patients is mentioned in Table 1.

3.2. Clinical description of Patient 5

Patient 5 is a 4-year-old boy, suffering from speech delay. In addition, he presents specific dysmorphic features as well as strabismus, moderate to severe intellectual disabilities, and autism spectrum disorders. The cardiac ultrasound showed the presence of pulmonary narrowing. The patient is the offspring of a consanguineous marriage.

3.3. Clinical description of Patient 6

The first consultation for this patient was at the age of 4 months, during which a specific facial dysmorphia was noted as well as a supravalvular aortic stenosis identified by cardiac ultrasound. At the age of 2 years, this patient was reconvened to study his clinical evolution. Mental and psychomotor retardation were noted as well as a behavioral profile consistent with autism.

Table 1. Clinical features of the 31 patients.

Patient	Age	Sex	FD	Cranial perimeter (cm)	Weight (neonate) (g)	Weight (consultation) (kg)	Height (cm)	Clinodactyly 5	Gait disorders	Cardiac defect	Scoliosis	Kidney defect	Strabismus	Developmental delay/ID	Behavioral Profile	Language delay	Array CGH
1	4Y	M	+	49	22	22	105	-	-	APS	-	-	-	+	HS	+	Del7q (1.49MB)
2	2Y	F	+	45.5	9.5	9.5	77	+	-	PS	-	-	-	-	HS	+	Del7q (1.49MB)
3	7Y	F	+	*	*	*	*	-	-	-	-	-	-	+	Ag/ASD	-	Del7q (1.4MB)
4	10Y	F	+	50	21.5	21.5	129	-	-	*	-	+	+	+	Ag/ASD	+	Del7q (1.39MB)
5	4Y	M	+	50.5	16.5	16.5	99	-	-	PS	-	-	-	+		+	Del7q (1.7MB)
6	2Y9M	M	+	48	*	*	*	-	-	SVAS	-	-	-	+	NP	+	Del7q (1.7MB)
7	11Y	F	+	*	*	*	*	-	-	AS	-	-	-	+	Hs	+	NP
8	8Y	F	+	49	30	30	119	-	-	NP	-	-	-	+	NP	+	NP
9	3Y	F	+	*	*	*	*	-	+	-	-	-	-	+	Hs	+	NP
10	5Y	M	+	51	16.5	16.5	102	-	-	VSD	-	-	-	+	NP	+	NP
11	7Y	M	+	*	*	*	*	-	-	-	-	-	-	+	Hs	+	NP
12	6Y	M	+	50	18	18	115	-	-	-	-	-	-	+	NP	+	NP
13	5Y	F	+	*	*	*	*	-	-	-	-	-	-	+	NP	+	NP
14	5Y	F	+	*	*	*	*	-	-	-	-	-	-	+	Hs	+	NP
15	30Y	M	+	*	*	*	*	-	-	APS	-	-	-	+	Hs	+	NP
16	5Y	F	+	49	18	18	115	-	-	SVAS	-	-	-	+	Hs	+	NP
17	9Y	F	+	*	*	*	*	+	-	APS	-	-	-	+	Hs	+	NP
18	6Y	F	+	51	21	21	110	-	-	-	-	-	-	+	NP	-	NP
19	9Y	F	+	50	20	20	123	-	+	-	-	-	+	+	NP	-	NP

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Patient	Age	Sex	FD	Cranial perimeter (cm)	Weight (neonate) (g)	Weight (consultation) (kg)	Height (cm)	Clinodactyly 5	Gait disorders	Cardiac defect	Scoliosis	Kidney defect	Strabismus	Developmental delay/ID	Behavioral Profile	Language delay	Array CGH
20	7Y	F	+	*	*	*	*	-	-	MV	-	-	-	+	+	-	NP
21	3Y	F	+	48	15	15	100	-	-	PS	-	-	-	+	+	+	NP
22	6Y	F	+	47	17	17	118	-	-	*	-	-	-	+	+	-	NP
23	4Y	M	+	49	17	17	108	-	+	*	-	-	-	+	+	-	NP
24	5M	F	+	*	*	*	*	-	-	PS	-	-	-	-	Hs	-	NP
25	36Y	M	+	*	*	*	*	-	-	*	-	-	-	+	-	NP	NP
26	8M	F	+	44.5	9	9	68.5	NP	NP	AS	-	-	-	NP			NP
27	5Y	M	+	50	*	*	*	+	-	*	-	-	-	+	Hs	+	NP
28	1Y5M	F	+	44	8.8	8.8	72	-	-	PS	+	-	-	-	Hs	+	NP
29	2Y	M	+	47	10	10	84	-	-	VSD	-	-	-	+	NP	-	NP
30	3Y	M	+	46.5	11	11	82	-	-	APS	-	-	-	+	-	+	NP
31	5M	M	+	*	*	*	*	-	NP	APS	-	+	-	NP	NP	+	NP

Note: Y: years; M: months; +: present; −: not present; F: female; M: Male; *: data not collected or not mentioned; NP: not performed; FD: facial dysmorphia (broad front, bitemporal narrowing, periorbital fullness, iris with star and/or lace patterns, short, and snub nose with a bulbous tip, long philtrum, wide mouth, full lips, micrognathia); ID: intellectual disability; AS: artery stenosis; VSD: ventricular septal defect; APS: artery pulmonary stenosis; SVAS: supravalvular aortic stenosis; MV: mitral valvopathy; PS pulmonary stenosis; Hs: hyper-sociability, Ag: aggressivity; ASD: autistic spectrum disorder.

Table 2. Comparison of the clinical features of our patients with autism with those of the literature with 7q11.2 deletion and duplication.

	Age of diagnosis	Behavioral profile/ clinical autism test	Mental delay	Environment exposure	Language disorders	Growth delay	Heart defect	Dysmorphic face	Strabismus	Consanguinity	Transmission	Deletion/duplication	Genes
Patient [7]	6.6	Aggression, deficits in the comprehension of simple language, articulation, reciprocal conversation, attention to voice, empathy and socializing with peers, weakness in visuospatial construction	+	-	-	-	-	Elfin face	+	-	De novo	Deletion of 3.3 Mb	*ELN*, *IMK1*, *CLIP2*, *F2I*, *GTF2IRD*, *NCF1*, *IP1*, *YHWAG*
Patient [8]	12.5	Hypersensitivity to noise, stereotypic behavior, hyperactivity, exhibited inappropriate behavior toward strangers, aggression, paroxystic episodes	+	-	-	+	-	Dysmorphic features	-	-	De novo	Duplication of 1.4 MB	*FKBP6*, *ZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *GTF2I*, *GTF2IRD RFC2*, *LN2*
Patient 1 [9]	3.5	Anxiety, aggression, attention deficit, hyperactivity, stereotypy.	+	Not mentioned	+	+		Dysmorphic features	-	-	De novo	Duplication of 1.4 Mb	*ELN*, *LIMK1*, *CLIP2*, *TF2I*, *GTF2IRD*

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	Age of diagnosis	Behavioral profile/ clinical autism test	Mental delay	Environment exposure	Language disorders	Growth delay	Heart defect	Dysmorphic face	Strabismus	Consanguinity	Transmission	Deletion/duplication	Genes
Patient 2 [9]	11	Self-injurious behavior, aggression, hyperactivity, repetitive play/behavior	+	Not mentioned	+	+	-	Dysmorphic features	-	-	De novo	Duplication of 1.4 Mb	*ELN*, *LIMK1*, *CLIP2*, *GTF2I*, *GTF2IRD*
Patient 3 [9]	4.5	Anxiety, stereotyped movements, repetitive play/behavior.	+	Not mentioned	+	+	-	Dysmorphic features	-	-	De novo	Duplication of 1.4 Mb	*ELN*, *LIMK1*, *CLIP2*, *GTF2I*, *GTF2IRD*
Patient 4 [9]	3	Self-injurious behavior, Stereotyped movements.	+	Not mentioned	+	-	-	Dysmorphic features	-	-	De novo	Duplication of 3.5 Mb	*ELN*, *LIMK1*, *CLIP2*, *GTF2I*, *GTF2IRD*, *HSPB1*, *YWHAG*, *SRCRB4D*, *ZP3*, *DTX2*
Patient 5 [9]	7	Self-injurious behavior, stereotyped movements.	+	Not mentioned	+	+	-	Dysmorphic features	-	-	Same maternal duplication	Duplication of 1.4 Mb	*ELN, LIMK1, CLIP2, TF2I, GTF2IRD*
Patient 1 (WBS179) [10]	5.5	Anxiety and attention deficit hyperactivity disorder, aggression, autistic traits, adaptive abilities.	+	Not mentioned	-	+	-	Elfin face	+	-	De novo	Deletion of 3.5 MB	*ELN*, *LIMK1*, *CLIP2*, *TF2I*, *GTF2IRD*, *NCF1*, *HIP1*, *YHWAG*

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	Age of diagnosis	Behavioral profile/ clinical autism test	Mental delay	Environment exposure	Language disorders	Growth delay	Heart defect	Dysmorphic face	Strabismus	Consanguinity	Transmission	Deletion/duplication	Genes
Patient 2 (WBS160) [10]	14.6	Epilepsy, anxiety, and obsessive behavior.	+	Not mentioned	-	-	SVAS	Elfin face	-	-	De novo	Deletion of 3.5 MB	*ELN*, *LIMK1*, *CLIP2*, *TF2I*, *GTF2IRD*, *NCF1*, *HIP1*, *YHWAG*
Patient 1 [11]	13	Emotional dysregulation, short and discontinuous attention span, motor restlessness, anxiety.	-	-	+	-	-	Elfin face	-	-	Maternal	Deletion of 1.3 MB	*GTF2IRD1*, *GTF2I*, *CF1*, *GTF2IRD2*
Patient 5 (Present study)	4	ASD (anxiety, hyperactivity, aggressivity). CARS: 47 (severe autism)	+	Prolonged exposure to television	+	-	Pulmonary stenosis	Elfin face	+	+	De novo	Deletion of 1.7 MB	*ELN*, *IMK1*, *LIP2*, *TF2I*, *GTF2IRD*, *NCF1*
patient 6 (present study)	2	ASD (anxiety, hyperactivity, aggressivity). CARS: 47 (severe autism)	+	Screen dependency	+	-	SVAS	Elfin face	-	-	De novo	Deletion of 1.7 MB	*ELN*, *MK1*, *LIP2*, *TF2I*, *GTF2IRD*, *NCF1*

Note: Abbreviations: +: presence; -: absence; SVAS: supravalvular aortic stenosis; ASD: autism spectrum disorder.

4. Discussion

WBS is thought to affect at least 1/7,500 to 1/20,000 individuals [1]. Most WBS patients have been described as showing recognizable facial features, known as *elfin face*, a mental delay, a particular behavioral and characteristic cognitive profile including hyper-sociability and a sympathetic personality, and, frequently, a cardiovascular disease. Cytogenetic and array CGH analysis revealed a different deletion size in the 7q region characterized by a partial loss and a variable breakpoint. On this basis, the study aims to establish a genotype–phenotype correlation in the largest Tunisian cohort of 31 WBS patients.

The commonly deleted region in all patients involves five important genes: ELN, CLIP2, LIMK1, GTF2I, and GTF2iRD, which are implicated in the development of the main characteristics of WBS. However, the different deletion sizes and thus the variable-implicated genes could play an important role in the WBS phenotype spectrum (Figure 2).

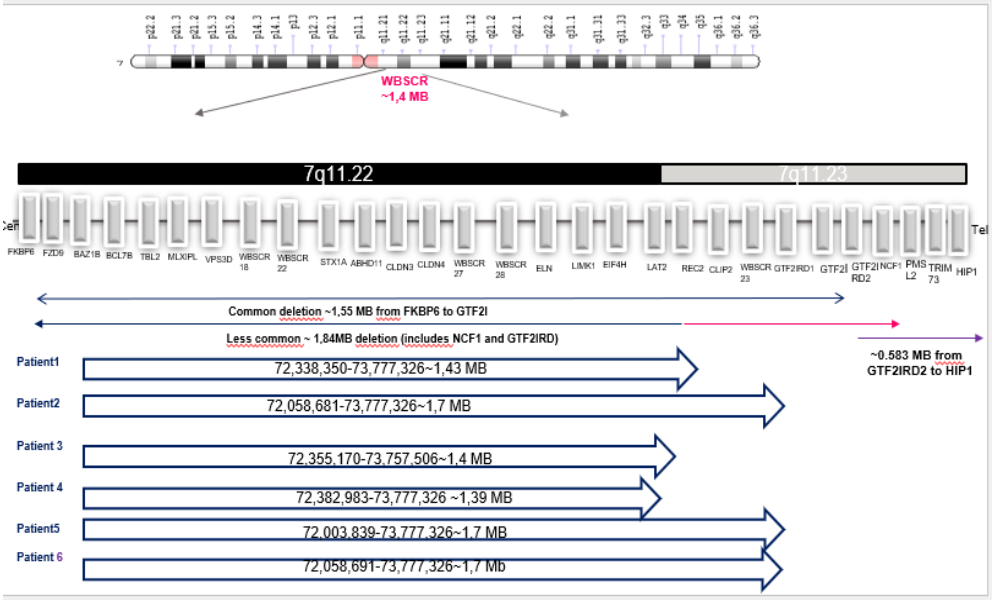

Figure 2. Summary of deletion mapping of WBS classical and atypical patients. Deletion mapping of WBS typical cases defines a critical region for the WBS visual-spatial construction, cognition, and social behavior (mentioned WBSCR). A schematic representation of the genes mapping at the 7q11.23 critical region is shown below the human chromosome pictogram. The typical 1.55 Mb WBS deletion interval is depicted below, also for the extended boundaries of the rarer 1.84 Mb deletion.

The ELN (Elastin) gene [OMIM #130160] in 7q11.23, coding for the elastin protein, has been described in autosomal dominant cutis laxa [OMIM #219200] and autosomal dominant supravalvular aortic stenosis [OMIM #185500]. Nevertheless, it has also been suggested to be associated with cardiac defects in WBS patients. The role of the protein in arterial development and disease has been established by generating knockout mice. Eln-/- mice die immediately after birth due to a vascular obstruction caused by enhanced smooth muscle cell proliferation. Additionally, elastin is a vital protein in the aorta and large arteries. supravalvular aortic stenosis constitutes the prototypical WBS

cardiovascular abnormality, found in approximately 70% of patients[12]. Pulmonary stenosis and aortic stenosis are seen together in 45% of WBS cases [13]; less frequently, in 30% of cases, ventricular septal defects were observed [2].

In the present study, the cardiac phenotype was noted in 55% of cases. Additionally, 4 patients presented with a septal ventricular defect. Of note, cardiovascular complications are the leading cause of death in WBS patients [12]. Although the ELN gene deletion is responsible for the main phenotypic characteristics in WBS, the cardiac, neurodevelopmental, and dysmorphic phenotypes have not been linked to ELN. Considering that WBS is a multisystem disorder associated with multiple genes' loss of function, the phenotype varies accordingly.

In fact, additional genes such as CLIP2, LIMK1, GTF2I, and GTF2iRD have been linked to cognitive and craniofacial pathology. However, the contribution of a subset of the genes located within the deleted region still needs to be elucidated.

Interestingly, in this study, our findings describe new additional WBS patients associated with ASD. Contrasting with the hyper-sociability seen in the majority of WBS, Patients 5 and 6 presented ASD, as diagnosed by the CARS test. These results indicate that autistic spectrum disorder could be seen in WBS and, therefore, the 7q11.2 region could be considered as a genomic region implicated in the genetically heterogeneous disorder of autism. Therefore, some ASD common features, such as concentration difficulties, restrictive and repetitive behavior, musical affinity/sensitivity, language delay, and a higher level of anxiety, could notably exist in WBS, which is well-known by its hyper-sensibility phenotype traits [14] (Figure 3).

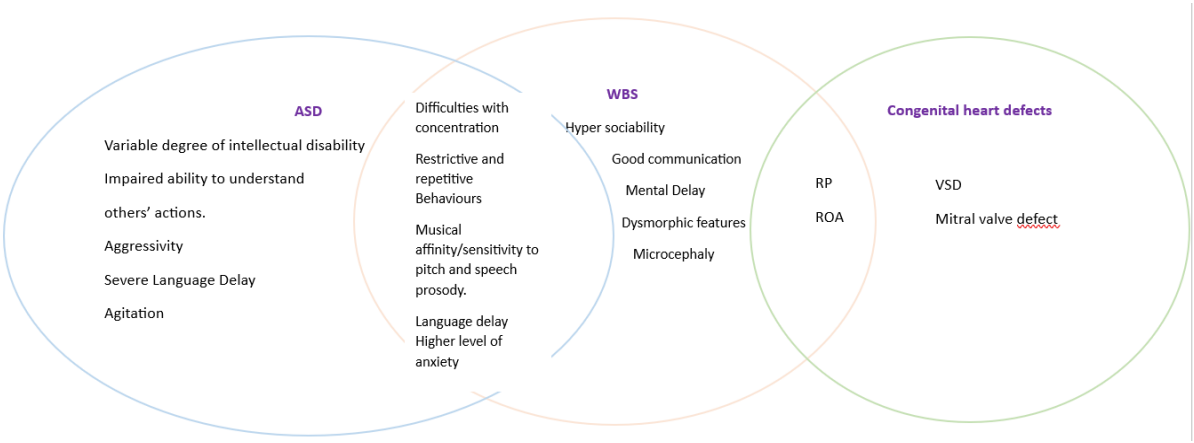

Figure 3. Shared and distinctive features of ASD and WBS in the domains of cognition, language, and social behavior [14].

Remarkably, a frequency as high as 12% of ASD is seen in individuals with WBS, which is considered higher than the frequency noted in the general population [15]. Indeed, recent studies reporting WBS associated with ASD [11,16] show atypical 7q11.2 deletions. When we focused on the genotype–phenotype correlation in both Patients 5 and 6, we noted a commonly deleted region. The Huntingtin-Interacting gene 1 (HIP1) [OMIM #601767], located upstream of the proximal limit of the commonly deleted region at 7q11.23, is strongly expressed in the brain. HIP1 and YWHAG have been proposed as the most compelling candidate genes for susceptibility to autistic traits for ASD in the 7q region [10]. Indeed, Hip1 /- model mice developed a neurological phenotype characterized by growth

retardation, gait ataxia, and epilepsy [16].

Data described above suggest that ASD enlarges the WBS phenotypic spectrum and should, therefore, be screened in this condition. The HIP1 gene could be the 7q11.2 ASD candidate gene. Nevertheless, Patient 5, the offspring of a consanguineous marriage, provides an important context for understanding the observed WBS phenotypic variability. Consanguinity can increase the likelihood of inheriting homozygous variants from shared ancestors, which may contribute to the additional variability in symptoms observed among WBS patients. Indeed, mutations in the remaining alleles may lead to a loss of function or partial loss of function in key genes and epistatic interactions, which may modulate the phenotypic expression of WBS. The combination of WBS-related gene deletion and homozygosity for variants in other parts of the genome could result in nonlinear effects on gene expression and protein function, further modifying the phenotype.

Here, we highlight the role of gene networks in multisystem disorders like WBS, where both environmental and genetic factors may contribute to the severity and development of autism spectrum disorders. Specific factors that might relate to this genomic disorder include deletion size differences, the genes involved in the deletion, and the effect of modifying genes outside the deleted locus, such as other autism-related gene mutations or CNVs.

Interestingly, WBS region duplications have been associated with severe language delays, mild to moderate intellectual disability, and autism spectrum disorders, suggesting that specific genes within the WBS region can influence language and social development through gene dosage effects [8,9,18]. The two WBS patients showing autistic disorder with severe expressive language delay presented the common WBS deletion. This emphasizes the WBS common deletion region as a contributor to the autistic pathway, potentially influencing development through mechanisms beyond gene dosage. Certainly, genetic factors might be largely responsible for the occurrence of autism, but they cannot fully account for all cases, and it is likely that specific environmental factors, especially prolonged exposure and dependence on screens, might add to the risk and enlarge the WBS phenotypic spectrum to ASD. Despite the absence of HIP1 gene deletion, the patients in this study have a presumed phenotype similar to those reported in the literature (Table 2). Indeed, the ASD trait evolution in Patient 5 emphasizes the influence of the environmental component in ASD, underscoring the need for better disease management to reduce its severity.

These findings suggest that specific genes in the WBS region could influence social communication depending on genetic background interaction with other genes and/or environmental factors. We cannot ignore that WBS patients could develop ASD. Comprehensive array CGH analysis to define the deleted region and focus on the affected genes could help establish a better genotype–phenotype correlation, enabling improved management and early screening for autistic traits in WBS patients.

5. Conclusions

Our study reveals an intriguing association between Williams-Beuren syndrome (WBS) and autism spectrum disorder (ASD). Although rare, our findings demonstrate that ASD can occur in a subset of WBS patients, suggesting the involvement of the 7q11.2 genomic region in the genetic complexity of autism. These findings further support the involvement of the 7q11.23 region in social behavior development, driven by its genes and their close interactions. To improve clinical management and enhance screening for autistic traits in WBS patients, accurate delineation of deleted

regions through techniques such as array comparative genomic hybridization (array CGH) is highly recommended. Nevertheless, further investigations into genetic factors and environmental influences are essential. Continued functional studies and high-throughput technologies could elucidate the involvement of genes like HIP1 in ASD and shed light on epigenetic influences.

Statement of ethics

The local Ethics Board of the University Teaching Hospital Farhat Hached approved the present study (**CER:14-2022**) and **informed written consent was obtained from the parents of all the children.** Consent is an informed choice by the parent or legal guardian (either in writing or verbally). A separate consent form was completed for each child participant.

Consent for publication

The local Ethics Board approved the present study (**CER:14-2022**) and informed written and verbal consent was taken from the parents of all children for photo publication.

Data Availability Statement

Data and materials are available from the corresponding author and available upon request.

Declarations

We confirm that the study protocol is performed by the relevant guidelines and regulations.

Author contributions

Soumaya Mougou Zerelli: Conceptualization, Supervision, review & editing, medical consultation; Rim Khelifi: Experimental work, Analysis, Interpretation of data, Writing – original draft; Afef Jeloul, Wafa Slimani, Khouloud Rjiba: Experimentation, Partial analysis; Houda Ajmi, Manel Dardour: Patient referral, Clinical assessment; Sarra Dimassi: Medical consultation; Ali Saad, Moez Gribaa: Resources. All authors read and approved the final version of the manuscript for publication.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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