Atypical Deletion of Williams-Beuren Syndrome Reveals the Mechanism of Neurodevelopmental Disorders

Jianrong Zhou
Guangdong Cardiovascular Institute
Ying Zheng
Guangdong Provincial People's Hospital
Guiying Liang
Guangdong Provincial People's Hospital
Xianyu Qin
Guangdong Provincial People's Hospital
Hang Yang
Shantou University Medical College
Shaoxian Chen
Guangdong Provincial People's Hospital
Tongkai Ge
Guangdong Provincial People's Hospital
Shufang Huang
Guangdong Provincial People's Hospital
Kai Zhang
Guangdong Provincial People's Hospital
Xianwu Zhou
Guangdong Provincial People's Hospital
Xiaoli Xu
Southern Medical University
Pengju Weng
Guangdong Provincial People's Hospital
Yong Zhang
Guangdong Provincial People's Hospital
Ping Li
Guangdong Provincial People's Hospital
Shushui Wang
Guangdong Provincial People's Hospital
Jian Zhuang
Guangdong Provincial People's Hospital
Yueheng Wu
Guangdong Provincial People's Hospital
Jimei Chen (chenjimei@gdph.org.cn)
Guangdong Academy of Medical Sciences: Guangdong Provincial People's Hospital

Research

Keywords: atypical deletion, Williams-Beuren syndrome, genotype–phenotype correlation, mental retardation, growth restriction

Posted Date: October 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-94534/v1

Abstract

Background The Williams-Beuren syndrome (WBS) is a multiple phylogenetic disorder, caused by the hemizygous deletion of 1.55 to 1.84 Mb on chromosome 7q11.23, which encodes a fragment of 26 to 28 genes. Among these genes, the deletion of the elastin (ELN) gene haplotype is the main cause of cardiovascular abnormalities. Other genes, such as CLIP2, GTF2IRD1, and GTF2I, may be associated with specific cognitive and craniofacial features. However, genes associated with specific neurocognitive phenotypes are still controversially discussed. The purpose of this study is to further explore the mechanism of neurodevelopmental disorders in patients with Williams-Beuren syndrome.

Patients and methods Patients who had been diagnosed with WBS were recruited. The deletion was precisely defined by chromosome microarray analysis and the clinical phenotype was evaluated.

Results This study identified nine patients with atypical deletions from 111 patients with WBS. One patient had normal neurodevelopmental with deletion of Williams-Beuren syndrome chromosomal region (WBSCR) telomere side genes, including GTF2I and GTF2IRD1, while another patient retained these genes but showed neurodevelopmental abnormalities. Seven of the eight patients with the WBSCR22 gene deletion developed growth restriction.

Conclusions By comparing the genotype and phenotype of patients with typical deletions and atypical deletions, the deletion of GTF2I and GTF2IRD1 genes alone insufficient to induce typical neurocognitive phenotypes in WBS patients. The BAZ1B, FZD9, and STX1A genes may play an important role in the neurodevelopment of patients with WBS. Furthermore, the deletion of the WBSCR22 gene may be the main cause of physical growth restriction in WBS patients.

Introduction

The Williams-Beuren syndrome (WBS; OMIM number 194050), also known as the Williams syndrome, is a multiple phylogenetic disorder caused by the hemizygous deletion of 1.55 to 1.84 Mb on chromosome 7q11.23, a fragment containing approximately 26 to 28 genes(1). Genome rearrangement often occurs in this region because low-copy repeats are located on both sides of the common deletion region, which leads to non-allelic recombination during meiosis(2). This region is also referred to as the Williams-Beuren syndrome chromosomal region (WBSCR). It has been estimated that the prevalence of WBS is about 1/7,500 to 1/20,000(3). Although the phenotype features extensive heterogeneity in severity and performance, it usually shows facial deformities, cardiovascular abnormalities, mental retardation, specific cognitive characteristics, developmental limitations, hypothyroidism, infantile hypercalcemia, and other clinical symptoms that affect multiple organs and systems(4). However, it remains unclear how these gene deletions cause the characteristic phenotype of WBS, which may be related to the low expression of gene products.

Most patients have the same deletion span but few individuals have smaller or larger deletion fragments. To study the relationship between genotype and phenotype, patients with atypical deletions are promising research objects. However, since WBS is a rare disease and since the proportion of atypical deletions is low (about 2–5%)(1, 2), the number of subjects available for research is extremely limited. So far, the deletion of the elastin (ELN) gene has been identified as the main cause of cardiovascular disorders in WBS patients, especially arterial stenosis(5). Moreover, several researchers have used atypical deletion patients and animal experiments to show that the genes located on the telomere side of WBSCR (i.e.,GTF2I, GTF2IRD1, and CLIP2) are the main causes of patients' behavior and cognitive phenotypes(6, 7). Other scholars suggested that the deletion of WBSCR centromere-side genes also contributes to the specific phenotype of WBS patients(8, 9).

This study describes nine cases of Chinese WBS patients with atypical deletions, one of which showed normal neurocognitive development. The clinical phenotypic characteristics and genotypes of these atypical deletion patients are used to verify the findings of previous literature and expand these. At the same time, we try to discuss the contribution of several gene deletions in WBSCR to the symptoms of WBS patients. High-resolution molecular testing is recommended for WBS patients, especially those with non-classical clinical symptoms. In this way, more comprehensive and accurate genetic information can be obtained, which can enable accurate diagnosis and treatment.

Materials And Methods

Patient subjects

The research plan was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital [No. GDREC2019587H(R1)]. Informed written consent was obtained from the patients’ parents.

Patients who had been diagnosed with WBS or were clinically suspected by the Lowery scoring system(10) were recruited. Their clinical data, including medical records, gestational age, birth weight, birth length, echocardiography, heart catheterization findings, gene test reports, and family history, were reviewed.

Genetic testing

Chromosomal microarray analysis (CMA) was used to detect genes in patients with suspected WBS using the Lowery scoring system. Approximately 2.0 ml of peripheral venous blood was collected from patients and parents. Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions.

DNA samples (250 ng) were hybridized with Affymetrix Cytoscan 750 K array (Affymetrix, Santa Clara, CA, USA), which contains more than 750000 markers for copy number analysis and 200000 single nucleotide polymorphism (SNP) probes for genotyping. After hybridization, Chromosome Analysis Suite software (Affymetrix, USA) and human genome version GRCh37 (hg19) were used to analyze the results. The detected copy number variation (CNV) was compared with internal and national public CNV databases, such as the Database of Genomic Variants (DGV), the International Standards for Cytogenomic Arrays Consortium, and the Online Mendelian Inheritance in Man.

According to the latest standards and guidelines for sequence variations, as developed by the American College of Medical Genetics and Genomics, the CMA results were divided into five grades: "pathogenicity", "possible pathogenicity", "uncertain significance", "possible benign", and "benign".

Cardiovascular status assessment

Not only were clinical cardiovascular data collected from all treated hospitals, but also, WBS patients who voluntarily came to the center for physical examination, were subjected to cardiac ultrasound examination by pediatric cardiologists. Supravalvular aortic stenosis (SVAS) was diagnosed by echocardiography when the pressure gradient (PG) exceeded 10 mm Hg. Pulmonary stenosis (PS) was diagnosed if the main or branch of the pulmonary artery showed local stenosis or diffuse stenosis with a PG exceeding 10 mm Hg(11). Coarctation of the aorta (CoA) was defined by echocardiography as peak PG exceeding 40 mm Hg at the distal aortic arch(11).

Physical development assessment

The height (accurate to 0.1 cm) and weight (accurate to 0.1 kg) were measured by electronic height and weight meter without shoes and in light clothes. According to the patients' sex, date of birth, and date of the visit, the Z-scores of height-for-age (HAZ), weight-for-age (WAZ), and body mass index (BMI, i.e., the weight in kg divided by the height in m^2)-for-age (BAZ) were calculated by WHO Anthro software (https://www.who.int/childgrowth/software/en/). Based on the WHO 2006 and 2007 growth reference standards(12), stunting was defined as HAZ < -2; underweight was defined as WAZ < -2, and emaciation was defined as BAZ < -2.

Neurodevelopmental assessment

The Gesell development schedule (GDS) is one of the commonly used methods to assess the neurological and intellectual development of infants and children in China(13, 14). In the present study, a version of the GSD was used, that had been adjusted by the Chinese Pediatric Association(15), to evaluate the neurodevelopment of WBS patients. The assessment was conducted by trained rehabilitation doctors. The assessment contents included gross motor, fine motor, adaptive behavior, language, and social behavior. Each test obtained a development quotient (DQ) and the total average DQ was obtained by calculating the average of the five DQs. The correspondence between the individual neurocognitive development status and DQs is shown in Table 1.

Table 1 Definition of the individual neurocognitive development status

Development states	Definitions
Normal	85≤DQ<115
Borderline	75≤DQ<85
Mild retardation	55≤DQ<75
Moderate retardation	40≤DQ<55
Severe retardation	25≤DQ<40
Extremely severe retardation	DQ<25

DQ, development quotient

Statistical analysis and presentation

SPSS Statistics software 20.0 (IBM, Armonk, NY, USA) was used for all statistical evaluations. The collected data were expressed as mean ± SD. Figures were prepared by GraphPad Prism 8.0.1 (San Diego, CA, USA) and Adobe Illustrator CC 2019 (NY, USA).

Results

Deletion mapping in patients with atypical deletions

In this study, a total of 111 patients with WBS were recruited, including nine patients with atypical deletions. As shown in Figure. 1, all nine patients had heterozygous microdeletions in chromosome 7q11.23, ranging from 0.741 Mb to 4.06 Mb, and the deletion sites differed. By searching the database, all of the above CNVs were pathogenic and related to WBS.

The chromosome break sites of patients No. 1 and No. 3 were almost identical, and genes from BAZ1B to GTF2IRD1 were deleted in both patients. The deletion of patient No. 2 extended in centromeric direction, ranging from POM121 to GTF2IRD2. Although the deletion sites of patients No. 4 and No. 8 differed, the deleted genes ranged from FKBP6 to GTF2I. Compared with patient No. 4, the FKBP6 gene was not deleted in patient No. 5, while other gene deletions were identical. Patient No. 6 had deletions from the FKBP6 gene to the ELN gene, excluding WBSCR genes such as GTF2I and GTF2IRD1 on the telomere side. In contrast, patient No. 7 lost the gene related to the telomere side of ABHD11 while retaining its centromeric side. Moreover, the deleted fragment of patient No. 9 was the longest, and several genes in the 7q11.22 region were also deleted. No other chromosomal CNV or mutated gene related to clinical symptoms was found in any of these patients. All of them are *de novo* cases without family history.

Characteristics of participants

Table 2 lists the WBS patients with atypical deletions and their clinical characteristics. The age of these patients (two females and seven males) ranged from 11 months to 38 months (24.44 ± 9.85).

Table 2 WBS patients with atypical microdeletion in 7q11.23 and their clinical characteristics

Case No.	Sex	Age (month)	Gene tests	Deletion		Growth retardation	Cardiovascular diseases	Mental disability	Inguinal hernias	Endocrine abnormalities	Typical face
				Range(hg19)	size						
1	M	38	CMA	Del (7q11.23) (72,858,312-74,071,135) ×1	1.213 Mb	+	+	+	+	-	+
2	F	24	CMA	Del (7q11.23) (72,351,682-74,264,871) ×1	1.91 Mb	+	+	+	-	-	-
3	M	27	CMA	Del (7q11.23) (72,858,312-74,071,135) ×1	1.212 Mb	+	+	+	+	-	+
4	M	31	CMA	Del (7q11.23) (72,745,738-74,129,824) ×1	1.384 Mb	+	+	+	-	-	+
5	F	11	CMA	Del (7q11.23) (72,800,000-74,150,000) ×1	1.35 Mb	+	+	+	+	-	+
6	M	32	CMA	Del (7q11.23) (72,742,276-73,483,030) ×1	0.741 Mb	-	+	+	+	+	+
7	M	31	CMA	Del (7q11.23) (73,150,001-74,200,000) ×1	1.05 Mb	+	+	-	+	-	+
8	M	13	CMA	Del (7q11.23) (72,751,184-74,100,813) ×1	1.35 Mb	-	+	+	-	+	+
9	M	13	CMA	Del (7q11.23) (72,073,782-76,132,541) ×1	4.06 Mb	-	-	+	-	-	-

CMA, chromosomal microarray analysis; WGS, whole genome sequencing; WBS, Williams-Beuren syndrome.

The symbol used are "+" (exist) and "-" (not exist).

Growth status

By measuring the height and weight of patients, the Z-score analysis method and WHO Anthro software were used to calculate Z-score, as shown in Supplementary 1. According to the WHO growth standard reference, only three patients (No. 3, No. 6, and No. 7) showed normal growth and

development. Patients No. 3 and No. 5 were diagnosed as low-birth-weight children because their birth weight (2.1 kg for No. 3 and 1.88 kg for No. 5) was below 2.5 kg. All patients' gestational age was older than 37 weeks (37 to 40 weeks).

Cardiovascular phenotypes

Except for No. 9, all patients showed cardiovascular abnormalities. Among them, SVAS and PS were the most common cardiovascular diseases. Patient No. 3 had undergone surgical correction because of severe supravalvular pulmonary stenosis at five months. The details of cardiovascular diseases are shown in Table 3.

Neuropsychological testing

According to the information provided by the patient's parents, except for patient No. 7, all other patients suffered from significant delays in developmental milestones, such as standing and walking independently, saying the first word, and the first sentence.

Table 3 Type of cardiovascular diseases in nine WBS patients with atypical deletion

Case No.	Cardiovascular diseases							
	SVAS	PS	CoA	ASD	SVPS	PDA	PVS	MVP
1	+	+	+	-	+	-	-	+
2	+	+	+	+	-	-	-	-
3	+	+	-	+	+	-	+	-
4	+	-	+	-	-	-	-	-
5	+	+	-	+	-	+	+	-
6	+	-	-	-	-	-	-	-
7	+	+	-	-	-	-	-	-
8	+	+	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-

ASD, atrial septal defects; CoA, coarctation of the aorta; MVP, mitral-valve prolapse; SVAS, supravalvular aortic stenosis; PDA, patent ductus arteriosus; PS, pulmonary stenosis; PVS, pulmonary valve stenosis; SVPS, supravalvular pulmonary stenosis.

The symbol used are "+" (exist) and "-" (not exist).

According to their parents' recollection, most patients could walk without support at about 16 to 18 months, and they could only say a few simple words after 20 months, such as father and mother. They were not able to say a complete sentence consciously until the visit. However, the development of patient No. 7 was normal. He could sit at 7 months, walk alone at 12 months, and speak short sentences with 6-10 words when interviewed.

The patients' GDS scores are shown in Table 4. The results of 20 patients with typical deletion of WBS in the same age group are also shown for comparison. All patient test data were obtained before any medical intervention. The results of the assessment were almost consistent with the information provided by parents. Except for patient No. 7, the total average GDS scores of all patients remained below 85.

Table 4 Comparison of GDS between patients with typical deletions and those with atypical deletions

Case No.	GDS, DQ					
	Gross motor	Fine motor	Adaptive behavior	Language	Social behavior	Total average
1	53	67	70	54	78	64
2	60	57	66	61	58	60
3	75	75	69	60	55	67
4	65	60	69	60	55	62
5	58	47	53	50	60	54
6	76	58	55	57	67	63
7	108	90	83	83	93	91
8	70	40	55	39	50	51
9	47	33	45	34	40	40
Typical deletions*	61±14.5	60±20	59±14	54±18	58±18.5	60±15

GDS, Gesell Development Scale; DQ, developmental quotient. * Mean ± SD.

Facial features

Most of the nine patients with atypical deletions have distinct faces (see Supplementary 2), similar to those with typical deletions. However, patients No. 2 and No. 9 do not have specific facial features related to the syndrome. For example, patient No. 2 looked the same as ordinary people except for swelled tissue around the orbit. Unfortunately, the patients' parents did not agree to publish photos.

Other clinical symptoms

Five patients (No. 1, No. 3, No. 5, No. 6, and No. 7) developed inguinal hernia within six months of birth. Except for patient No.1, which required intervention by surgery, the other patients recovered. Patient No. 6 was diagnosed with subclinical hypothyroidism because of elevated Thyroid Stimulating Hormone (TSH) level (7.519 μ IU/mL, normal range, 0.560 to 5.910 μ IU /mL) with normal T3 and T4 levels. Since the blood calcium level was 2.81 mmol /L (normal range, 2.25 to 2.75 mmol/L), patient No. 8 was diagnosed with mild hypercalcemia.

Discussion

Nine WBS patients have atypical deletions, one of which shows normal neurocognitive development. These results show that if only the genes on the telomere side of WBSCR are deleted, especially the GTF2I and GTF2IRD1 genes, the effects are not sufficient to cause a neurodevelopmental delay in patients with WBS. This seems to contrast with previous reports(6, 7), which showed that the genes on the telomere side of WBSCR play a major role in the WBS phenotype. At the same time, according to patient No. 6, the deletions of BAZ1B, FZD9, and STX1A, located on the centromeric side of WBSCR, can also cause the typical neurocognitive phenotype of WBS. This suggests that the deletion of genes on the centromeric side of WBSCR also exerts an equally important effect on the phenotype of WBS. Furthermore, it can be inferred that the main target gene that causes growth retardation in patients with WBS is WBSCR22. WBS patients with smaller and larger deletions may not have a typical clinical phenotype, which detrimentally affects clinicians' precise diagnosis and treatment. At this time, complete genetic testing is particularly important. This is not only important for genetic counseling purposes, but also to avoid expensive and repetitive routine examinations, which can establish targeted follow-up. At the same time, atypical deletions play an important role in the study of gene function.

Nine (8%) out of 111 Chinese patients with WBS had atypical deletions, which exceeded the ratio of 2–5% reported in previous literature. Possible reasons include the advancement of genetic testing technology, which has increased the detection rate of atypical deletions. A further reason is the deepening of people's understanding of the disease, which has led to the diagnosis of many WBS patients with non-classical clinical phenotypes. Because of the economics of FISH technology, a number of laboratories still focus on this detection technology. However, with the development of sequencing technology and the reduction of price more modern technologies have become broadly available. For patients with suspected WBS, especially those with non-classical clinical phenotypes, the CMA or next-generation sequencing (NGS, i.e., high-resolution molecular testing, such as Whole Exome Sequencing (WES), Whole Genome Sequencing (WGS) and targeted region sequencing (TRS))(16, 17) is recommend to obtain more accurate and complete genetic information.

Neurologic and mental retardation is one of the most important and common features in patients with WBS(1, 18, 19). This study used GDS to assess patients' neurodevelopmental status, which includes the five main functional areas of the human body. All patients with atypical deletions (except patient No. 7) showed mild to moderate developmental retardation similar to those with typical deletions. The age and size of chromosome deletions were similar between patients No. 6 and No. 7, but their GDS results were significantly different (as shown in Figure. 2). This suggests that the difference between them is mainly caused by the different positions of chromosome breakage and gene deletions.

GTF2I and GTF2IRD1 belong to the transcription factor family. They interact with a variety of proteins and DNA to influence neurophysiology and developmental processes(20). Previously, the deletion of heterozygosity of GTF2I and GTF2IRD1 genes has been reported as the main cause of neurocognitive characteristics, special facial features, and motor dysfunction in WBS patients(7, 21, 22). However, the deleted genes of patient No. 7 in this study included these two genes but showed normal neurological development. As recently reported(23), the symptoms of neurodevelopmental delay in WBS cannot be explained by the deletion of functional heterozygosity of GTF2I and GTF2IRD1. In-depth study of the molecular and phenotypic characteristics of patient No. 7 showed that the language and adaptive development of the patient were in a marginal state. This may be because the genes GTF2I and GTF2IRD1 mainly affect the neurodevelopment related to these two functional regions(24, 25), but they are not sufficient to cause the neurodevelopmental retardation symptoms typical for WBS patients. However, it cannot be ruled out that CNV size-related position effects, non-deletion allele variants, epigenetic mechanisms, regulatory sequences, or other factors may affect the patient's phenotype(26).

In contrast, patient No. 6 mainly deleted genes from the centromere side of WBSCR (from FKBP6 to ELN) and showed typical WBS cognitive characteristics. Analysis of the molecular and phenotypic relationship of patient No. 6 as well as previous case reports(27-30) with similar deletion positions and mental retardation (as shown in Figure. 1) showed that the centromeric side gene of WBSCR also plays an important role in the phenotype of patients with WBS. BAZ1B, FZD9, and STX1A genes are particularly important in this regard, according to previous studies(9, 31-34) and the GeneCards Human Gene Database (https://www.genecards.org/).

BAZ1B (the bromodomain adjacent to zinc finger domain, 1B) gene, also known as Williams Syndrome Transcription Factor (WSTF), plays an important role in the differentiation and migration of nerve cells. It also participates in the neural crest specific transcription loop and remote regulation(31). Wnt signal plays an important role in the regulation of the balance between proliferation and differentiation of neural progenitor cells. Inhibition or overexpression of the Wnt signal function can lead to decrease or proliferation of neural progenitor cells, respectively. The BAZ1B gene is enriched in the Wnt signal transduction pathway, and this pathway is activated because of the deletion of this gene in WBS patients(35, 36). This affects the proliferation and differentiation of nerve cells in patients with corresponding neurocognitive phenotype. Studies(31, 37) have shown that the BAZ1B gene is associated with facial features and behavioral phenotypes of WBS patients.

Furthermore, a recent study(38) has suggested that through the PTEN-mediated pathway, the deletion of BZA1B gene heterozygosity reduces both the viability and survival of thyroid cells, thereby causing hypothyroidism in patients with WBS. At the same time, the BAZ1B gene is also involved in the development of sperm, and its deletion may be one of the influencing factors of infertility in WBS patients. Knockout of the BAZ1B gene can cause changes in the time of chromosome aggregation in cells and errors in the process. This may lead to delays in the prophase of mitosis, which may affect sperm development(39). Therefore, its deletion may be one of the influencing factors of infertility in WBS patients.

It should be noted that the FZD9 gene also plays a role in the Wnt signaling pathway(40). By increasing the doubling time and apoptosis of nerve cells, deletion of the FZD9 gene can affect the development of a patient's nervous system and cause cognitive impairment(9).

The STX1A gene encodes a neuronal soluble N-ethylmaleimide-sensitive fusion attachment protein receptor, which promotes the nerve function of the central nervous system by regulating the release of transmitters(41). Recent studies have shown that mutations or deletions of STX1A are related to human neuropsychological diseases, such as autism spectrum disorder and attention deficit hyperactivity disorder(42, 43). However, the contribution and underlying mechanism of its deficiency to the neurocognitive symptoms of WBS still remain unclear. These studies will enable a deeper understanding of the genotype-phenotype correlation of WBS microdeletion, and help to understand the molecular mechanisms of diseases and the human social brain.

Previous reports have shown that patients' clinical symptoms are also affected by the size of the deletion(6, 44). In comparison to other patients with atypical deletions, the chromosome deletions of patient No. 9 are larger and the neurodevelopmental delay is more severe, which is consistent with previous reports(44, 45). This suggests that this effect is related to the deletion of the extension genes HIP1 and YWHAG on the telomere side of WBSCR, which inhibits the patient's neurodevelopment(46). The data of this study also showed that patients with large deletions (such as patients No. 2 and No. 9) did not have classical facial features, which may be related to the size and position of the deleted fragments. This prompts to focus on the diagnosis of patients with non-classical atypical WBS.

Growth restriction is another characteristic of WBS patients(4). The WBSCR22 gene encodes a putative methyltransferase protein that is strongly expressed in the heart, skeletal muscle, and kidney. Its hemizygous deletion may lead to growth retardation, myopathy, or premature aging(47). In this study, only patient No. 7 retained the WBSCR22 gene and showed normal physical development, while seven of the eight (87.5%) patients with deletion of this gene showed growth restriction, of which patient No. 3 with low-birth-weight. This suggests that this effect may be caused by the

deletion of the WBSCR22 gene. However, the growth of patients is affected by many factors such as diet, endocrine, function, and the environment. Therefore, more research is needed to verify the effect of the WBSCR22 gene on the growth and development of WBS patients.

The ELN gene encodes elastic fibers, which are essential elements of the extracellular matrix. Heterozygous deletion of the ELN gene is the main cause of cardiovascular abnormalities in WBS patients, especially SVAS and PS(5). All patients in this study have a deletion of heterozygosity in the ELN gene, and all patients (except for patient No. 9) developed cardiovascular disease, which is consistent with previous reports(48). The possible reason why patient No. 9 has not yet developed a relevant cardiovascular phenotype may be the young age of the patient. Alternatively, the patient's deletion of genes outside the WBSCR region may also be related to the cardiovascular phenotype.

Conclusions

The BAZ1B, FZD9, and STX1A genes may play an important role in the neurodevelopment of patients with WBS. Furthermore, the deletion of the WBSCR22 gene may be the main cause of physical growth restriction in WBS patients. Identifying patients with an atypical deletion of WBS has important clinical and scientific significance. To study the contribution of each gene to the patient's phenotype, more subjects with atypical deletions are need to better understand their molecular-phenotype relationship. Animal experiments can then be used to study and verify the function of relevant genes.

Abbreviations

Williams-Beuren syndrome (WBS), elastin (ELN), Williams-Beuren syndrome chromosomal region (WBSCR), chromosomal microarray analysis (CMA), single nucleotide polymorphism (SNP), copy number variation (CNV), Database of Genomic Variants (DGV), Online Mendelian Inheritance in Man (OMIM), American College of Medical Genetics and Genomics (ACGM), Coarctation of the aorta (CoA), Z-scores of height-for-age (HAZ), Z-scores of weight-for-age (WAZ), body mass index (BMI), Z-scores of BMI-for-age (BAZ), Gesell development schedule (GDS), development quotient (DQ), Williams Syndrome Transcription Factor (WSTF).

Declarations

Acknowledgments

We sincerely thank all of the patients and their family members for their enthusiasm and continued participation in this study. We would also like to thank the clinicians and physicians who involved in this study.

Funding: This work was supported by the National key Research and Development Program [2018YFC1002600], and the Science and Technology Planning Project of Guangdong Province, China [No.2017A070701013 and 2017B090904034,2017030314109, 2019B020230003], and the Guangdong peak project [DFJH201802].

Competing interests: None declared.

Ethics approval: The research plan was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital [No. GDREC2019587H(R1)].

Consent to participate: Informed written consent was obtained from the patients' parents.

Data availability statement: All data relevant to the study are included in the article or uploaded as supplementary information.

Consent for publication: Not applicable

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Jianrong Zhou], [Ying Zheng], [Guiying Liang]. The first draft of the manuscript was written by [Jianrong Zhou] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Pober BR. Williams-Beuren syndrome. N Engl J Med. 2010;362(3):239-52.
2. Bayes M, Magano LF, Rivera N, Flores R, Perez Jurado LA. Mutational mechanisms of Williams-Beuren syndrome deletions. Am J Hum Genet. 2003;73(1):131-51.
3. Meyer-Lindenberg A, Mervis CB, Berman KF. Neural mechanisms in Williams syndrome: a unique window to genetic influences on cognition and behaviour. Nat Rev Neurosci. 2006;7(5):380-93.

4. de Sousa Lima Strafacci A, Fernandes Camargo J, Bertapelli F, Guerra Junior G. Growth assessment in children with Williams-Beuren syndrome: a systematic review. J Appl Genet. 2020;61(2):205-12.
5. Duque Lasio ML, Kozel BA. Elastin-driven genetic diseases. Matrix Biol. 2018;71-72:144-60.
6. Edelmann L, Prosnitz A, Pardo S, Bhatt J, Cohen N, Lauriat T, et al. An atypical deletion of the Williams-Beuren syndrome interval implicates genes associated with defective visuospatial processing and autism. J Med Genet. 2007;44(2):136-43.
7. Antonell A, Del Campo M, Magano LF, Kaufmann L, de la Iglesia JM, Gallastegui F, et al. Partial 7q11.23 deletions further implicate GTF2I and GTF2IRD1 as the main genes responsible for the Williams-Beuren syndrome neurocognitive profile. J Med Genet. 2010;47(5):312-20.
8. Chen P, Zhang T, Yuan Z, Shen B, Chen L. Expression of the RNA methyltransferase Nsun5 is essential for developing cerebral cortex. Mol Brain. 2019;12(1):74.
9. Chailangkarn T, Trujillo CA, Freitas BC, Hrvoj-Mihic B, Herai RH, Yu DX, et al. A human neurodevelopmental model for Williams syndrome. Nature. 2016;536(7616):338-43.
10. Lowery MC, Morris CA, Ewart A, Brothman LJ, Zhu XL, Leonard CO, et al. Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: evaluation of 235 patients. Am J Hum Genet. 1995;57(1):49-53.
11. Cha SG, Song MK, Lee SY, Kim GB, Kwak JG, Kim WH, et al. Long-term cardiovascular outcome of Williams syndrome. Congenit Heart Dis. 2019;14(5):684-90.
12. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ. 2007;85(9):660-7.
13. Cao X, Li J, Cheng L, Deng Y, Li Y, Yan Z, et al. The associations between prenatal exposure to polycyclic aromatic hydrocarbon metabolites, umbilical cord blood mitochondrial DNA copy number, and children's neurobehavioral development. Environ Pollut. 2020;265(Pt B):114594.
14. Huang X, Ye Y, Li Y, Zhang Y, Zhang Y, Jiang Y, et al. Maternal folate levels during pregnancy and children's neuropsychological development at 2 years of age. Eur J Clin Nutr. 2020.
15. Liu P, Wu C, Chang X, Qi X, Zheng M, Zhou Z. Adverse Associations of both Prenatal and Postnatal Exposure to Organophosphorous Pesticides with Infant Neurodevelopment in an Agricultural Area of Jiangsu Province, China. Environ Health Perspect. 2016;124(10):1637-43.
16. Sun F, Oristaglio J, Levy SE, Hakonarson H, Sullivan N, Fontanarosa J, et al. Genetic Testing for Developmental Disabilities, Intellectual Disability, and Autism Spectrum Disorder. AHRQ Comparative Effectiveness Technical Briefs. Rockville (MD)2015.
17. Le TN, Williams SR, Alaimo JT, Elsea SH. Genotype and phenotype correlation in 103 individuals with 2q37 deletion syndrome reveals incomplete penetrance and supports HDAC4 as the primary genetic contributor. Am J Med Genet A. 2019;179(5):782-91.
18. Mervis CB, John AE. Cognitive and behavioral characteristics of children with Williams syndrome: implications for intervention approaches. Am J Med Genet C Semin Med Genet. 2010;154C(2):229-48.
19. Royston R, Waite J, Howlin P. Williams syndrome: recent advances in our understanding of cognitive, social and psychological functioning. Curr Opin Psychiatry. 2019;32(2):60-6.
20. Bayarsaihan D, Dunai J, Greally JM, Kawasaki K, Sumiyama K, Enkhmandakh B, et al. Genomic organization of the genes Gtf2ird1, Gtf2i, and Ncf1 at the mouse chromosome 5 region syntenic to the human chromosome 7q11.23 Williams syndrome critical region. Genomics. 2002;79(1):137-43.
21. Howard ML, Palmer SJ, Taylor KM, Arthurson GJ, Spitzer MW, Du X, et al. Mutation of Gtf2ird1 from the Williams-Beuren syndrome critical region results in facial dysplasia, motor dysfunction, and altered vocalisations. Neurobiol Dis. 2012;45(3):913-22.
22. Schneider T, Skitt Z, Liu Y, Deacon RM, Flint J, Karmiloff-Smith A, et al. Anxious, hypoactive phenotype combined with motor deficits in Gtf2ird1 null mouse model relevant to Williams syndrome. Behav Brain Res. 2012;233(2):458-73.
23. Kopp N, McCullough K, Maloney SE, Dougherty JD. Gtf2i and Gtf2ird1 mutation do not account for the full phenotypic effect of the Williams syndrome critical region in mouse models. Hum Mol Genet. 2019;28(20):3443-65.
24. Laws G, Bishop D. Pragmatic language impairment and social deficits in Williams syndrome: a comparison with Down's syndrome and specific language impairment. Int J Lang Commun Disord. 2004;39(1):45-64.
25. Somerville MJ, Mervis CB, Young EJ, Seo EJ, del Campo M, Bamforth S, et al. Severe expressive-language delay related to duplication of the Williams-Beuren locus. N Engl J Med. 2005;353(16):1694-701.
26. Euteneuer J, Carvalho CM, Kulkarni S, Vineyard M, Grady RM, Lupski JR, et al. Molecular and phenotypic characterization of atypical Williams-Beuren syndrome. Clin Genet. 2014;86(5):487-91.
27. Howald C, Merla G, Digilio MC, Amenta S, Lyle R, Deutsch S, et al. Two high throughput technologies to detect segmental aneuploidies identify new Williams-Beuren syndrome patients with atypical deletions. J Med Genet. 2006;43(3):266-73.
28. Tassabehji M, Metcalfe K, Karmiloff-Smith A, Carette MJ, Grant J, Dennis N, et al. Williams syndrome: use of chromosomal microdeletions as a tool to dissect cognitive and physical phenotypes. Am J Hum Genet. 1999;64(1):118-25.

29. Gagliardi C, Bonaglia MC, Selicorni A, Borgatti R, Giorda R. Unusual cognitive and behavioural profile in a Williams syndrome patient with atypical 7q11.23 deletion. J Med Genet. 2003;40(7):526-30.
30. Korenberg JR, Chen XN, Hirota H, Lai Z, Bellugi U, Burian D, et al. VI. Genome structure and cognitive map of Williams syndrome. J Cogn Neurosci. 2000;12 Suppl 1:89-107.
31. Lalli MA, Jang J, Park JH, Wang Y, Guzman E, Zhou H, et al. Haploinsufficiency of BAZ1B contributes to Williams syndrome through transcriptional dysregulation of neurodevelopmental pathways. Hum Mol Genet. 2016;25(7):1294-306.
32. Ramirez VT, Ramos-Fernandez E, Henriquez JP, Lorenzo A, Inestrosa NC. Wnt-5a/Frizzled9 Receptor Signaling through the Galphao-Gbetagamma Complex Regulates Dendritic Spine Formation. J Biol Chem. 2016;291(36):19092-107.
33. Gao MC, Bellugi U, Dai L, Mills DL, Sobel EM, Lange K, et al. Intelligence in Williams Syndrome is related to STX1A, which encodes a component of the presynaptic SNARE complex. PLoS One. 2010;5(4):e10292.
34. Osborne LR, Soder S, Shi XM, Pober B, Costa T, Scherer SW, et al. Hemizygous deletion of the syntaxin 1A gene in individuals with Williams syndrome. Am J Hum Genet. 1997;61(2):449-52.
35. Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science. 2002;297(5580):365-9.
36. Zechner D, Fujita Y, Hulsken J, Muller T, Walther I, Taketo MM, et al. beta-Catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. Dev Biol. 2003;258(2):406-18.
37. Zanella M, Vitriolo A, Andirko A, Martins PT, Sturm S, O'Rourke T, et al. Dosage analysis of the 7q11.23 Williams region identifies BAZ1B as a major human gene patterning the modern human face and underlying self-domestication. Sci Adv. 2019;5(12):eaaw7908.
38. Allegri L, Baldan F, Mio C, De Felice M, Amendola E, Damante G. BAZ1B is a candidate gene responsible for hypothyroidism in Williams syndrome. Eur J Med Genet. 2020;63(6):103894.
39. Ohta S, Taniguchi T, Sato N, Hamada M, Taniguchi H, Rappsilber J. Quantitative Proteomics of the Mitotic Chromosome Scaffold Reveals the Association of BAZ1B with Chromosomal Axes. Mol Cell Proteomics. 2019;18(2):169-81.
40. Frost J, Estivill X, Ramsay M, Tikly M. Dysregulation of the Wnt signaling pathway in South African patients with diffuse systemic sclerosis. Clin Rheumatol. 2019;38(3):933-8.
41. Fujiwara T, Sanada M, Kofuji T, Akagawa K. Unusual social behavior in HPC-1/syntaxin1A knockout mice is caused by disruption of the oxytocinergic neural system. J Neurochem. 2016;138(1):117-23.
42. Reuter MS, Tawamie H, Buchert R, Hosny Gebril O, Froukh T, Thiel C, et al. Diagnostic Yield and Novel Candidate Genes by Exome Sequencing in 152 Consanguineous Families With Neurodevelopmental Disorders. JAMA Psychiatry. 2017;74(3):293-9.
43. Kofuji T, Hayashi Y, Fujiwara T, Sanada M, Tamaru M, Akagawa K. A part of patients with autism spectrum disorder has haploidy of HPC-1/syntaxin1A gene that possibly causes behavioral disturbance as in experimentally gene ablated mice. Neurosci Lett. 2017;644:5-9.
44. Lugo M, Wong ZC, Billington CJ, Jr., Parrish PCR, Muldoon G, Liu D, et al. Social, neurodevelopmental, endocrine, and head size differences associated with atypical deletions in Williams-Beuren syndrome. Am J Med Genet A. 2020;182(5):1008-20.
45. Marshall CR, Young EJ, Pani AM, Freckmann ML, Lacassie Y, Howald C, et al. Infantile spasms is associated with deletion of the MAGI2 gene on chromosome 7q11.23-q21.11. Am J Hum Genet. 2008;83(1):106-11.
46. Ramocki MB, Bartnik M, Szafranski P, Kolodziejska KE, Xia Z, Bravo J, et al. Recurrent distal 7q11.23 deletion including HIP1 and YWHAG identified in patients with intellectual disabilities, epilepsy, and neurobehavioral problems. Am J Hum Genet. 2010;87(6):857-65.
47. Doll A, Grzeschik KH. Characterization of two novel genes, WBSCR20 and WBSCR22, deleted in Williams-Beuren syndrome. Cytogenet Cell Genet. 2001;95(1-2):20-7.
48. Collins RT, 2nd. Cardiovascular disease in Williams syndrome. Circulation. 2013;127(21):2125-34.

Figures

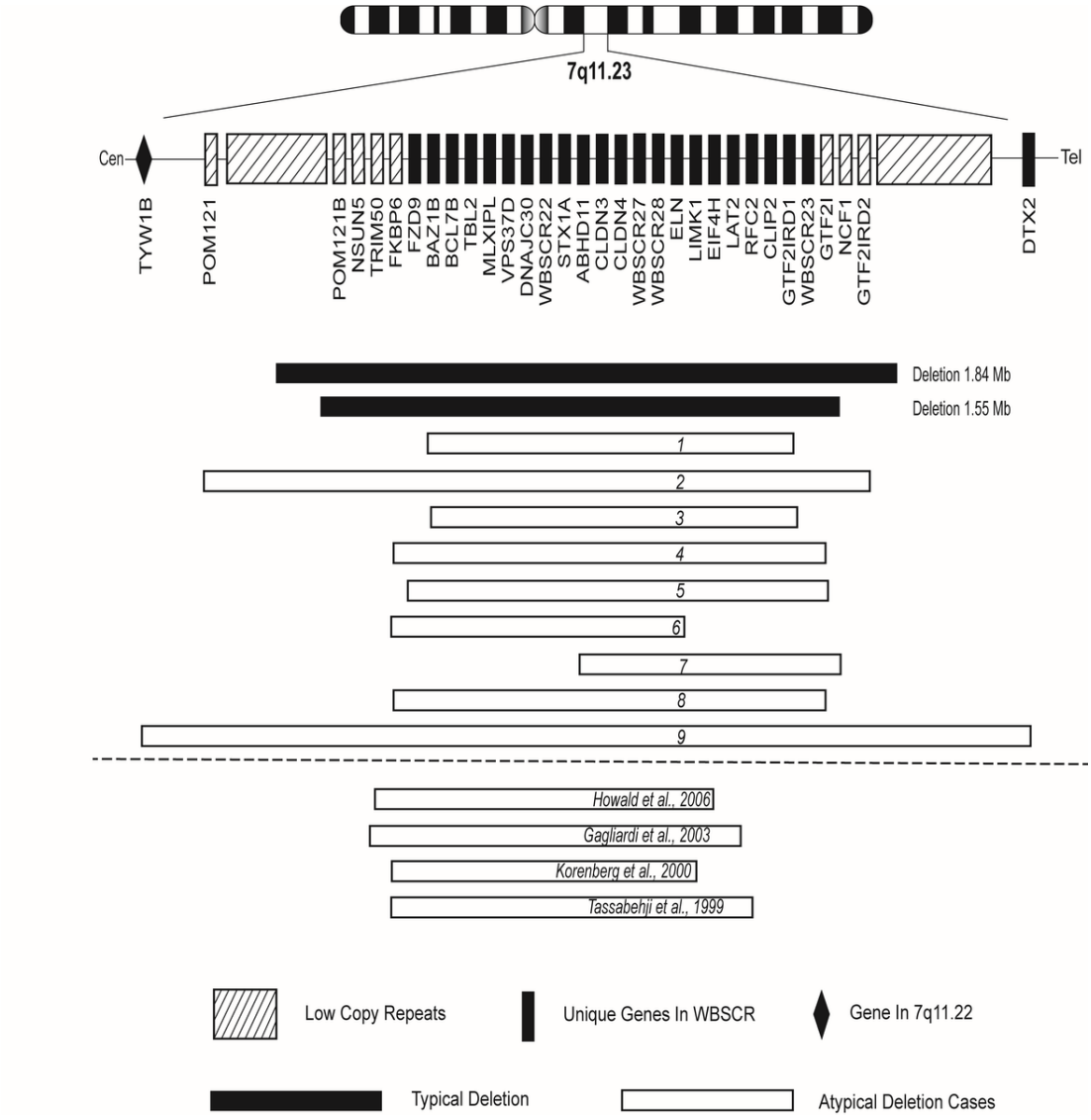

Figure 1

A pattern of atypical deletions detected in patients with WBS. The black long boxes indicate the degree of the deletion observed in patients with classic WBS (1.55 and 1.84Mb deletions). The gray band below represents the gene deletion fragments of patients with atypical deletion, including nine patients in this cohort and four cases of previously reported deletion genes that did not include WBSCR telomere-side genes such as GTF2I and GTF2IRD1.

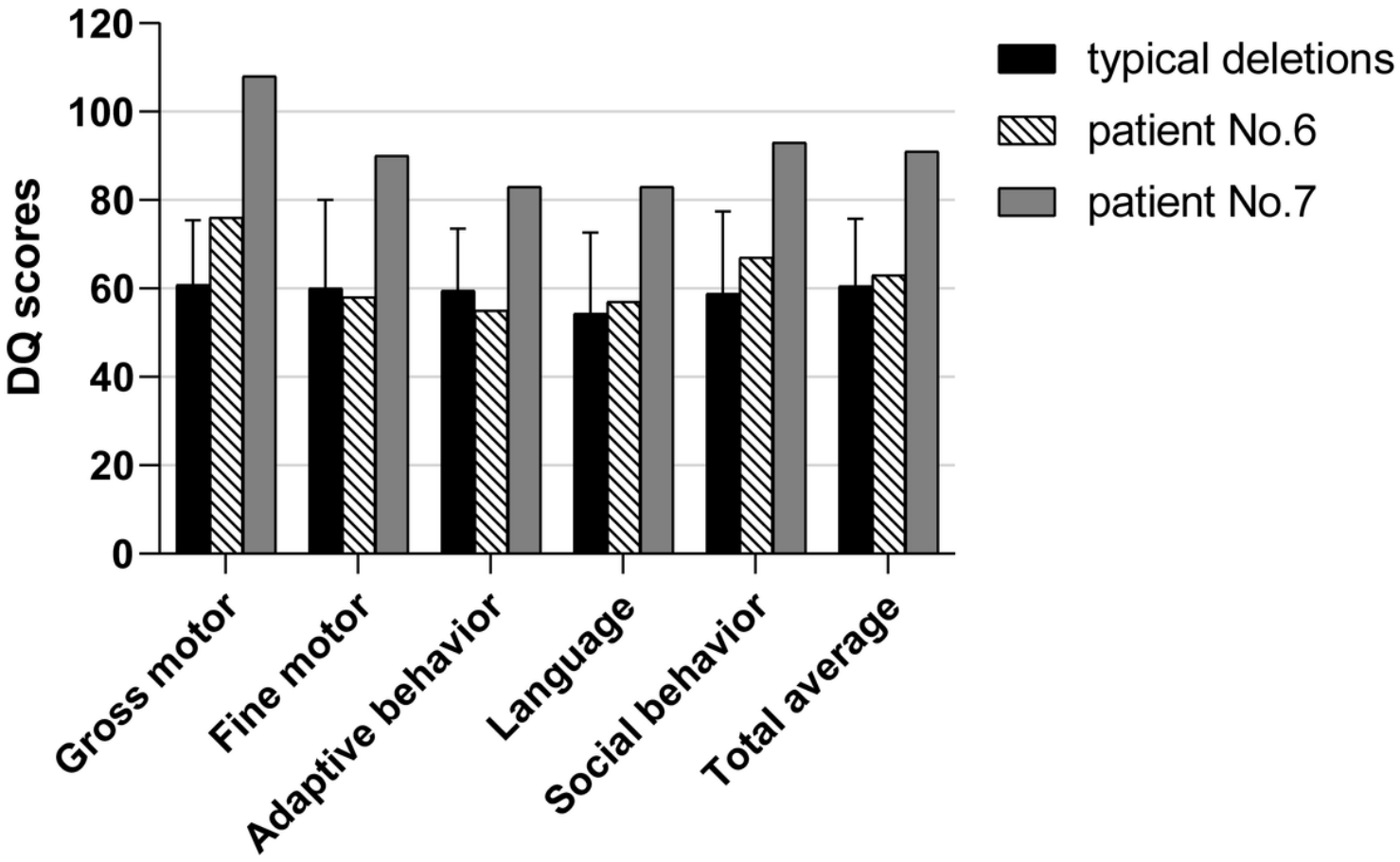

Figure 2

Neurocognitive development assessment scores of patients No. 6, No. 7, and 20 WBS patients with typical deletion. It Shows that the neurocognitive development of patient No. 7 is normal, while patient No. 6 and patients with typical deletions have developmental limitations. DQ, Development Quotient.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary2.docx
- Supplementary1.docx