

CLINICAL SCIENCE

Detection of deletions at 7q11.23 in Williams-Beuren syndrome by polymorphic markers

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INTRODUCTION: Williams-Beuren syndrome (WBS; OMIM 194050) is caused by a hemizygous contiguous gene microdeletion at 7q11.23. Supravalvular aortic stenosis, mental retardation, overfriendliness, and ocular and renal abnormalities comprise typical symptoms in WBS. Although fluorescence *in situ* hybridization is widely used for diagnostic confirmation, microsatellite DNA markers are considered highly informative and easily manageable.

OBJECTIVES: This study aimed to test the microsatellite markers for the diagnosis of Williams-Beuren syndrome, to determine the size and parental origin of microdeletion, compare the clinical characteristics between patients with different sizes of the deletion and parental origin.

METHODS: We studied 97 patients with clinical diagnosis of Williams-Beuren syndrome using five microsatellite markers: D7S1870, D7S489, D7S613, D7S2476 and D7S489_A.

RESULTS AND DISCUSSION: Using five markers together, the result was informative in all patients. The most informative marker was D7S1870 (78.4%), followed by D7S613 (75.3%), D7S489 (70.1%) and D7S2476 (62.9%). The microdeletion was present in 84 (86.6%) patients and absent in 13 (13.4%) patients. Maternal deletions were found in 52.4% of patients and paternal deletions in 47.6% of patients. The observed size of deletions was 1.55 Mb in 76/84 patients (90.5%) and 1.84 Mb in 8/84 patients (9.5%). SVAS as well as ocular and urinary abnormalities were more frequent in the patients with a deletion. There were no clinical differences in relation to either the size or parental origin of the deletion.

CONCLUSION: Using these five selected microsatellite markers was informative in all patients, thus can be considered an alternative method for molecular diagnosis in Williams-Beuren syndrome.

KEYWORDS: Williams-Beuren syndrome; microsatellite markers, parental origin; size deletion; molecular diagnosis.

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INTRODUCTION

Williams-Beuren syndrome (WBS; OMIM 194050) is a neurodevelopmental disorder described independently,^{1,2} as a syndrome involving facial appearance characteristics, supravalvular aortic stenosis (SVAS) and mental retardation. In fact, WBS presents a wide collection of symptoms affecting blood vessels, growth, intelligence, and behavior. Children with this condition have distinctive “elfin” facial features, a hoarse voice associated with growth, mental retardation and an “engaging” personality;^{3,4} hyperacusis, infantile hypercalcemia, prematurely wrinkled skin and SVAS are also common symptoms.⁵⁻⁸

WBS is generally sporadic with an incidence of 1/13,700–1/25,000 live births with no ethnic or sex preference, although familial cases have been reported with apparent autosomal dominant inheritance.⁹⁻¹¹ Despite the consistency of the overall clinical features, the broad spectrum of anomalies and phenotypic variability frequently lead to a significant difference in the number of patients diagnosed.^{12,13}

The most common deletion, found in 90% to 95% of WBS patients, spans a genomic region of ~1.55 Mb and is the result of mispairing between the centromeric and medial LCR blocks B (Bcen and Bmid).¹⁴⁻¹⁶ In 5% to 10% of cases, the breakpoints are within the centromeric and medial LCR blocks A (Acen and Amid), leading to an ~1.84 Mb deletion.^{17,18} Atypical (~0.2 Mb to ~2.5 Mb) deletions may be the leading cause of the substantial phenotypic variability among WBS patients.

Duplication of the WBS region occurs at half the frequency of deletions with less distinctive and somehow

opposite clinical features, such as deficits of social interaction and an autistic-like phenotype.¹⁹⁻²⁰ Autism was also described in an atypically large deletion (2.4 Mb to 3.4 Mb) telomeric to the WBS critical region.²¹ The presence of inversions in WBS patients has also been reported.^{22,18}

The origin of deletion occurs at the same frequency, and there is no clear-cut clinical association between parental imprinting and phenotype differences, except for hypertension and some aspects of growth, such as height, weight and head circumference.^{17,13,23,24}

Although fluorescence *in situ* hybridization (FISH) is widely used and considered the gold standard for WBS molecular diagnosis, the use of microsatellite DNA markers has also been widely used and is considered highly informative and easily manageable.^{15,17,25,26,27} Confirmation of clinical suspicion is essential for clinical monitoring of the patient and genetic counseling of the family.

In our previous study,²⁸ we assessed the use of three microsatellite markers (D7S1870, Hei and ELN 17/ exon 18) in the confirmation of the diagnosis of 32 WBS patients. They were informative in 78% of patients and uninformative in 22%. The most informative marker was D7S1870. In the present study, we maintained only the D7S1870 marker and added four other microsatellite markers to determine the size and parental origin of the microdeletion in the 7q11.23 region.

MATERIALS AND METHODS

Subjects

A total of 97 patients with a clinical diagnosis of WBS (61 boys and 36 girls) were ascertained through clinical

evaluation by geneticists of the Unit of Clinical Genetics of the Instituto da Criança, Hospital das Clínicas, Faculdade de Medicina, Universidade (ICr-HC-FMUSP) de São Paulo, Brazil.

The clinical diagnosis was made between the ages of 1 and 16 years, with an average of 6 y 2 mo. The mean maternal and paternal ages were 24 y 2 mo and 27 y, respectively.

Methods

The study was approved by the Institutional Review Board, and written consent was obtained from all participants. The molecular study was performed in the Laboratório de Investigação Médica do Instituto da Criança (LIM-36).

Among the 97 patients, DNA from both parents was obtained in 82 cases; in 13 cases, the molecular analysis was performed only with maternal DNA and in two cases, only with paternal DNA.

DNA was isolated from peripheral blood lymphocytes using a salt precipitation technique²⁹ and quantified in a Nano Vue Plus spectrophotometer (GE, New Jersey, USA).

The five microsatellites markers used were D7S1870, D7S489, D7S613 and D7S2476 inside the common 1.55-Mb deletion; D7S489A was used to distinguish deletions of 1.84 Mb (Fig. 1).

Polymerase chain reaction. PCR reactions were carried out in a total volume of 25 µL containing 50 ng of genomic DNA, 0.2 mM of dNTP mix, 0.4 µM of the each oligonucleotide, and 0.7 units of EasyTaq DNA Polymerase (LGC, São Paulo, Brazil). Samples were initially denatured for 5 min at 94°C; followed by 35 cycles of 1 min at 94°C, 1 min at the specific annealing temperature of each primer pair and

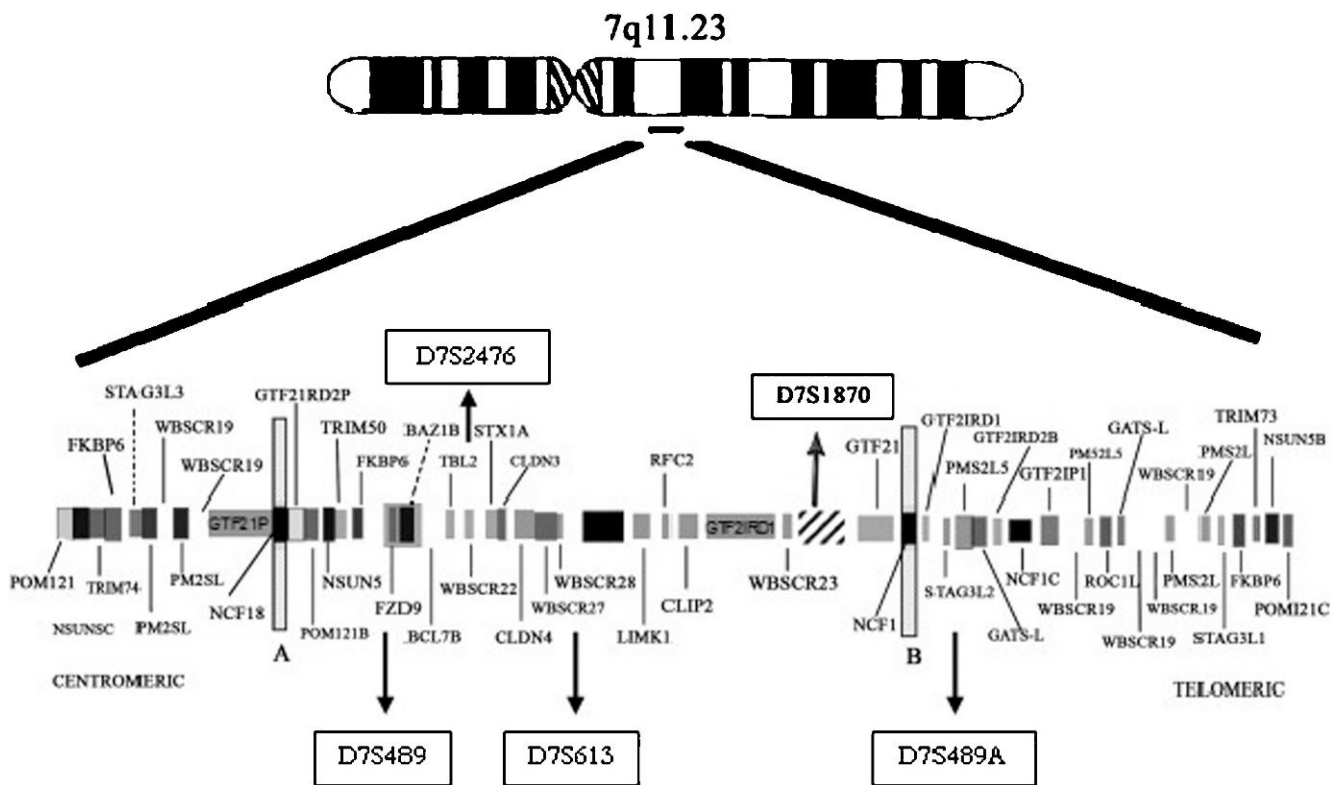


Figure 1 - Idiogram of chromosome 7 (7q11.23) illustrating the commonly deleted region and the relative localization of the tested markers.²⁹⁻³²

Table 1 - Primers sequences, amplicon sizes and PCR conditions for the five microsatellites markers used.

Markers	Primers	Amplicon size	MgCl ₂	UniSTS Accession number *	AT*
7q11.23					
D7S1870	F: TTCACTCAGGAAGTGGC R: TGGTGATGTGCTTTACTACG	108 pb	2,0 mM	Z51768	55°C
D7S489	F: CTGTTGACTTTCCACACTC R: GGCAACTCGAGACGTTAGTT	140-158 pb	1,5 mM	Z16646	56°C
D7S613	F: CAGCCTGGGTAACAAAAGC R: CCTCCCTCCTAATCCATG	85 pb	1,4 mM	L16300	55/60°C
D7S2476	F: GGGCAACATAGCAGCATT R: CAGGAGTCAGTTAGATAAGGTAC	128-160 pb	2,0 mM	Z53107	56°C
D7S489_A	F: GCACCTATGATCACAGCTTCTC R: ATGACATGAAGGTAAGTGGCCTT	419 pb	2,0 mM	BV097124	56°C

*Accession number in the GeneBank; AT – anelling temperature; pb – pairs base.

1 min at 72°C; and a final extension step of 15 min at 72°C. Primer sequences and PCR conditions used in this study are given in table 1. To check PCR amplification, 4 µL of the reaction products were analyzed on 2% agarose gel stained with ethidium bromide and visualized by UV exposure in an AlphaImager HP (Alpha Innotech, Corp. San Leandro, CA).

Polyacrylamide gel. The PCR amplification product was mixed in an equal volume of denaturing solution (99% formamide, 0.01% bromophenol blue; 0.01% xylene cyanol and 10 mM EDTA), denatured for 10 min at 95°C and separated by electrophoresis in a 7% denaturing polyacrylamide gel with urea at a final concentration of 7.5 M. Electrophoresis was performed in a Mini-Proteans III Cell apparatus (Bio-Rad®). The electrophoresis conditions were 105 V, 25 mA, and 2 W for two hours for the markers D7S1870, D7S489, D7S613 and D7S2476 and 230 V, 60 mA, and 5 W for four hours for marker D7S489A. The gels were fixed in 10% ethanol, silver stained and stored in 10% acetic acid until they could be photographed and interpreted.

The patient genotypes were compared with their parents. Deletions were diagnosed as maternal when the proband presented with gel-bands representing the allele marker inherited only from the father. When, by chance, both parents have the same alleles, the monoallelic inheritance of the corresponding microsatellite marker by the proband indicated an uninformative result.

We first used a two-step algorithm to identify the most common 1.55-Mb deletion. We then tested the D7S489A marker either to identify the larger 1.84-Mb deletion (in those patients in which a deletion of at least one marker was detected in the first step) or to confirm the lack of a deletion.

Statistical Analysis

Pairwise comparisons between clinical features of WBS and the presence of deletion, clinical features and deletion size and clinical features and parental origin of deletion were tested for significance using two-tailed Fishers exact test. A 2x2 contingency table was used to compare clinical

features. P analysis was performed in SPSS 13.0 software and considered statistically significant when $p \leq 0.05$.

RESULTS

Using five markers (D7S1870, D7S489, D7S613, D7S2476 and D7S489A) were informative in all patients. The most informative marker was D7S1870 (78.4%), followed by D7S613 (75.3%), D7S489 (70.1%) and D7S2476 (62.9%) (Table 2).

The microdeletion was present in 84/97 (86.6%) patients and absent in 13 (13.4%) patients. A prototypic acrylamide gel showing some of the results is shown in Figure 2.

All deletions were *de novo*, and none of the available parents had any clinical manifestation of WBS. It was also possible to determine the presence of a deletion for the 15 patients for whom DNA was available from only one of the parents.

There was no difference in clinical features found in patients with and without a deletion except SVAS as well as ocular and urinary abnormalities, more frequent with statistically significant association with a deletion. (Table 3) Other cardiovascular diseases, such as pulmonary artery stenosis, mitral valve prolapse and ventricular septum defects, were similar between the two groups. Ocular abnormalities observed were strabismus, stellate irides, punctuate opacities in the lens, glaucoma and myopia. Urinary abnormalities present included enuresis, diverticulosis, asymmetric kidneys, narrowing of the renal artery and renal microlithiasis.

The observed size of deletions was 1.55 Mb in 76/84 patients (90.5%) and 1.84 Mb in 8/84 patients (9.5%). The parental origin of deletion was maternal in 44/84 patients (52.4%), and paternal deletions in 40/84 patients (47.6%). There was no clinical difference in relation to either the size of deletion or the parental origin of deletion.

Twenty-three patients were previously studied by FISH, and 21 proved to have a deletion (FISH positive); whereas 2

Table 2 - Results of the analysis of microdeletions by microsatellite markers.

	D7S1870	%	D7S613	%	D7S489	%	D7S2476	%
Positive	65/97	67,1	66/97	68,1	58/97	59,8	56/97	57,7
Negative	11/97	11,3	7/97	7,2	10/97	10,3	5/97	5,2
Uninformative	21/97	21,6	24/97	24,7	29/97	29,9	36/97	37,1
Informative	76/97	78,4	73/97	75,3	68/97	70,1	61/97	62,9

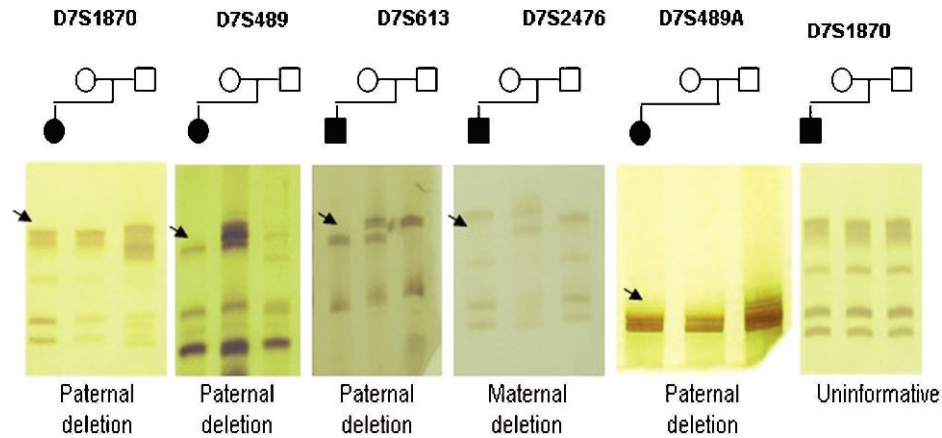


Figure 2 - Genotyping of the five microsatellites markers in WBS families. DNA fragments of those affected are always the first column of each gel followed by DNA from the mother and the last column the DNA of the father. Black arrows indicate allelic loss.

patients were FISH negative. Microsatellite marker analysis of these cases revealed microdeletions in all patients, including the two FISH negative patients.

DISCUSSION

The five microsatellite markers (D7S1870, D7S489, D7S613, D7S2476, and D7S489A) used in our study were informative in all patients.

The D7S1870 microsatellite marker showed the highest power of detection, identifying 78.4% of the cases alone and confirming the results found by previous studies.^{17,18,26,28,34-37}

Another two markers (D7S613, D7S489) were also able to diagnose over 70% of our patients, while the D7S2476 marker showed the poorest results (62.9%). These results were similar to those found in the literature.^{17,25,26,37}

The two best markers (D7S1870 and D7S613) in our study were informative in 97.6% to the detect deletion when used together. When the third marker, D7S489, was included informative detection increased to almost 99%. To increase the detection closer to 100%, additional useful markers will need to be chosen.^{27,23,35-37}

The microsatellite marker D7S489A was effective in the analysis of the size of the deletion and in the confirmation of the absence of fragments in the region critical for WBS. The 1.55-Mb deletion was found in 76/84 (90.5%) patients, and the 1.84-Mb deletion found in 8/84 patients (9.5%); these

observed proportions are similar to those found in studies in the literature.^{18,21}

Clinical findings in our patients were similar in the groups with a deletion and without a deletion, except for SVAS and ocular and urinary abnormalities. There was also no relationship between the size of the deletion and observed clinical features. These results are similar to those found in literature,^{23,33} where the clinical features found in WBS patients were very similar with both the 1.55-Mb and 1.84-Mb deletions.

In the 13 cases without a deletion, there is the possibility of a deletion smaller than 1.0 Mb that is not detectable by microsatellite markers; thus, this diagnostic should not be ruled out.^{38,39}

There was no significant difference between the frequencies of maternal and paternal deletions (52.4% and 47.6% respectively). The literature^{18,23,28,36,37,40-42} is concordant with our findings, except for one study,²⁵ where the maternal deletion was more frequent (77.8%) than the paternal deletion (22.2%).

Despite the frequency of the paternal and maternal deletions being very close, some authors have suggested a slight tendency for deletion due to the effect of maternal genomic imprinting, when all data are combined.^{25,36,42}

The phenotypic aspects between the groups of the patients with paternally (n=44/84) and maternally derived (n=40/84) deletions were similar. Some reports in the literature, however, suggest a parent-of-origin effect on microcephaly and growth retardation in WBS.^{17,36}

The FISH technique is currently considered the “gold standard” for molecular diagnosis of WBS, but partial microdeletions can lead to false negative results depending on the sequence and composition of the probe used.^{25,30,38} In the present study, the results obtained by FISH were compared with microsatellite marker analysis. The results were concordant, except for two negative FISH.

Other techniques in molecular biology are also being used for the molecular diagnosis of WBS, such as quantitative real-time PCR, multiplex ligation dependent probe amplification (MLPA) and array comparative genomic hybridization (aCGH), however, many other factors should be considered in relation to the cost-benefit and practicality.⁴³⁻⁴⁵ In developed country, aCGH is currently the method of choice for genome-wide screening for chromosome, but in the developing country this technic is not in a disposal due to an expensive cost.^{46,47}

Table 3 - Clinical findings in patients with and without deletion.

Clinical Findings	With deletion	Without deletion	p
	Frequency %	Frequency %	
Typical facies	100.0	100.0	-
Mental retardation	100.0	100.0	-
Friendly personality	92.5	91.7	-
Hyperacusis	81.9	58.3	0.120
Height (<5%)	51.2	23.1	0.059
Microcephaly	24.7	23.1	-
Cardiovascular disease	77.5	61.5	0.297
SVAS	58.1	12.5	0.022
Ocular abnormalities	65.2	20.0	0.012
Urinary abnormalities	48.1	16.7	0.041

CONCLUSION

The diagnosis of WBS based on clinical assessments may be difficult because of the great variability of its manifestations. Laboratory tests to detect the microdeletion in 7q11.23 are essential to confirm the clinical diagnosis for WBS. Although fluorescence in situ hybridization (FISH) is widely used, microsatellite DNA markers are considered highly informative and easily manageable for diagnostic confirmation.

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