Williams-Beuren syndrome: phenotypic variability and deletions of chromosomes 7, 11, and 22 in a series of 52 patients

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Abstract

Fluorescence in situ hybridisation (FISH) and conventional chromosome analysis were performed on a series of 52 patients with classical Williams-Beuren syndrome (WBS), suspected WBS, or supravalvular aortic stenosis (SVAS). In the classical WBS group, 22/23 (96%) had a submicroscopic deletion of the elastin locus on chromosome 7, but the remaining patient had a unique interstitial deletion of chromosome 11 (del(11)(q13.5q14.2)). In the suspected WBS group 2/22 (9%) patients had elastin deletions but a third patient had a complex karyotype including a ring chromosome 22 with a deletion of the long arm (r(22)(p11→q13)). In the SVAS group, 1/7 (14%) had an elastin gene deletion, despite having normal development and minimal signs of WBS.

Overall, some patients with submicroscopic elastin deletions have fewer features of Williams-Beuren syndrome than those with other cytogenetic abnormalities. These results, therefore, emphasise the importance of a combined conventional and molecular cytogenetic approach to diagnosis and suggest that the degree to which submicroscopic deletions of chromosome 7 extend beyond the elastin locus may explain some of the phenotypic

variability found in Williams-Beuren syndrome.

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Key words: Williams-Beuren syndrome; 11q; 22q.

Williams-Beuren syndrome (WBS), first described by Williams *et al*¹ in 1961 and independently by Beuren *et al*² in 1962, is a congenital developmental disorder involving the vascular, connective tissue, and central nervous systems. It occurs with a frequency of 1 in 20 000 livebirths and is almost always sporadic, although there are a few reports of familial cases.³⁻⁵

WBS is characterised by a distinct facial appearance, including broad forehead, bi-temporal narrowness, periorbital fullness, wide mouth, broad nasal tip, long philtrum, prominent ear lobules, full cheeks, and micrognathia (fig 1); mental retardation, mean IQ of 58; cardiovascular anomalies, characteristically supravalvular aortic stenosis (SVAS), which can also be inherited as an autosomal dominant trait, and peripheral pulmonary stenosis (PPS); infantile hypercalcaemia and hyperacusis. Other features of WBS include feeding difficulties during infancy, a gregarious personality together with "cocktail party speech", poor visual motor integration, hoarse voice, and joint limitation.⁶⁻¹⁰

Hemizyosity of the elastin gene (ELN) on chromosome 7 has been shown to occur in the vast majority of WBS patients and mutations at the 3' end of the ELN in some cases of autosomal dominant SVAS.¹¹⁻¹⁹ It is now thought that WBS is a contiguous gene syndrome with the submicroscopic deletion at 7q11.23 spanning at least 250 kb²⁰ and presumably including additional as yet unidentified genes.

In the present study, we report the conventional and molecular cytogenetic (FISH) investigation of a series of 52 patients: 23 classical WBS cases, 22 suspected WBS cases, and seven patients referred with SVAS or PPS. The results were used to establish phenotype-genotype correlations, which indicate a broad range of phenotypes associated with WBS.

Methods

PATIENTS

Our study population comprised three categories: (1) 23 patients who, on the basis of their clinical features, had already been diag-

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Figure 1 Patient 1 (A) at 14 months, (B) at 17 years.

nosed as having WBS. Of these, 21 were known to the Clinical Genetics Service in Wessex and the remaining two were referred to us independently by a paediatrician and a cardiologist; (2) 22 suspected WBS cases, of which six were referred by clinical geneticists, and the remainder referred by paediatricians and cardiologists within Wessex; (3) seven patients referred primarily with SVAS or PPS or both, including two patients from autosomal dominant SVAS families.

A clinical assessment of the patients was carried out with emphasis on 18 features of development and dysmorphology and independent of the molecular cytogenetic results. Patients already known to the clinical genetics service were visited at home or seen at hospital clinic appointments. For those who were referred to us by independent paediatricians or cardiologists, clinical details were obtained either directly from the referring clinician or from the patients' notes.

CYTOGENETIC AND MOLECULAR CYTOGENETIC STUDIES

In all patients, GTL banded chromosomes were prepared from peripheral blood after

semisynchronisation with FdU and release with thymidine.²¹ These were then analysed at the 550 band level for structural chromosome abnormalities.

FISH with the elastin Williams-Beuren syndrome chromosome region (WSCR) probe (Oncor®) was essentially by the method recommended by the probe supplier. Hybridisation sites were detected using antidigoxigenin rhodamine and the chromosomes were counterstained with DAPI. Slides were viewed on a Zeiss Axiophot microscope and images captured using a Photometrics cooled CCD camera and enhanced using Digital Scientific Smart Capture software. A minimum of 10 metaphases were scored for a deletion of the 7q11.23 region in each case. Only cells with a clear signal from the control probe D7S427 at 7q36 on both chromosome 7 homologues and a clear signal at 7q11.23 on at least one chromosome 7 were scored.

Results

A summary of the clinical findings and laboratory results is presented in tables 1, 2, and 3 and in fig 2.

Table 1 Classical Williams syndrome category

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Developmental delay	SD	SD	SD	SD	SD	SD	SD	MD	SD	SD	SD	SD	MD	MD	SD	MD	SD	SD	MD	SD	SD	MD	MD
SVAS/PPS	SP	S	SP	S	—	SP	—	—	S	S	S	S	SP	S	SP	P	SP	—	S	S	P	S	—
Hypercalcaemia	+	—	+	—	—	—	—	—	—	+	+	—	+	+	+	—	+	+	—	—	+	+	—
WS personality	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hoarse voice	—	+	+	+	—	+	+	+	+	+	—	—	—	+	—	—	+	+	+	—	+	+	+
Feeding difficulties	+	+	+	—	—	+	+	+	+	+	+	+	—	+	+	—	—	+	+	+	+	—	—
Hyperacusis	+	+	+	—	—	+	—	+	+	—	+	+	—	—	+	—	—	+	+	—	+	—	—
Incoordination	+	+	—	+	—	+	+	+	+	+	+	+	—	+	+	+	+	+	+	—	+	+	+
Joint limitation	+	+	—	+	—	+	+	—	—	—	+	—	—	+	+	+	—	+	—	—	—	—	—
Full cheeks	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Wide mouth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Broad forehead	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Broad nasal tip	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prominent ear lobule	+	+	—	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Long philtrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bitemporal narrowness	—	+	+	+	—	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	—
Periorbital fullness	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Micrognathia	+	+	—	+	—	+	+	—	—	+	+	+	+	+	+	—	—	+	+	—	—	—	—
del(7)(q11.23)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Other abnormality	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	+

+ feature present.

— feature absent.

blank, information not available.

SD, severe developmental delay.

MD, moderate developmental delay.

S, SVAS.

P, PPS.

* 47,XXY.

† 46,XY,del(11)(q13.5q14.2).

Table 2 Suspected Williams syndrome category

	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Developmental delay	MD	MD	—	MD	MD	MD	—	—	MD	—	MD	MD	MD	—	—	SD	SD	—	MD	—	MD	MD
SVAS/PPS	—	S	P	—	P	—	—	—	—	+	+	P	—	—	—	—	—	—	—	—	—	—
Hypercalcaemia	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	+	—	—	—	+	—	—
WS personality	+	—	—	—	—	—	—	—	+	—	+	+	—	—	—	—	—	—	—	—	—	—
Hoarse voice	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Feeding difficulties	—	—	—	—	—	—	+	—	—	+	+	+	+	—	—	—	—	—	—	—	—	+
Hyperacusis	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—
Incoordination	—	—	—	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
Joint limitation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—
Full cheeks	+	+	+	+	—	—	—	—	—	—	—	+	—	—	—	—	—	—	+	—	+	—
Wide mouth	+	+	—	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Broad forehead	+	+	—	—	—	+	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—
Broad nasal tip	+	+	—	+	+	—	—	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—
Prominent ear lobule	+	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Long philtrum	+	+	—	+	—	+	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—
Bitemporal narrowness	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
Periorbital fullness	+	+	—	+	—	+	—	—	—	—	+	—	—	—	—	+	—	—	—	—	—	—
Micrognathia	+	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
del(7)(q11.23)	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Other abnormality	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Complex karyotype.

Table 3 SVAS/PPS category

	46	47	48	49	50	51	52
Developmental delay	—	—	—	—	—	—	—
SVAS/PPS	S	S	P	S	S	S	S
Hypercalcaemia	—	—	—	—	—	—	—
WS personality	+	+	—	—	—	—	—
Hoarse voice	+	+	—	—	—	—	—
Feeding difficulties	—	—	—	—	—	—	—
Hyperacusis	+	—	—	—	—	—	—
Incoordination	—	—	—	—	—	—	—
Joint limitation	—	—	—	—	—	—	—
Full cheeks	+	+	—	—	—	—	—
Wide mouth	—	—	—	—	—	—	—
Broad forehead	—	—	—	—	—	—	—
Broad nasal tip	+	+	+	—	—	—	—
Prominent ear lobule	—	—	—	—	—	—	—
Long philtrum	—	—	—	—	—	—	—
Bitemporal narrowness	—	—	—	—	—	—	—
Periorbital fullness	—	+	—	—	—	—	—
Micrognathia	—	—	—	—	—	—	—
del(7)(q11.23)	+	—	—	—	—	—	—
Other abnormality	—	—	—	—	—	—	—

CYTOGENETIC ANALYSIS

In the classical WBS category 21/23 patients had an apparently normal karyotype at the resolution available using conventional G banded analysis. Of the other two, one (patient 13) had an XYY sex chromosome complement in addition to an elastin gene deletion, and the other (patient 23) was found to have a de novo interstitial deletion in the long arm of one chromosome 11 (46,XY,del(11)(q13.5q14.2) de novo) (fig 3A).

In the suspected WBS category 21/22 patients had apparently normal karyotypes. The remaining case (patient 27) was shown by conventional and molecular cytogenetic investigations to have a complex karyotype with

a ring chromosome 22 resulting in monosomy for distal 22q (q13→qter) and a whole arm Y;21 translocation resulting in monosomy for the long arm of the Y (45,X,add(21p),r(22)de novo.ish 7q11.23(WSCR×2),der(Y;21)(p10;q10)(wcpY+,SRY+,Y190+,DYZ3+,D13Z1+,wcp21+),r(22)(wcp22+,cH748+,pH17—)) (fig 4A).

Finally, in the SVAS/PPS category no chromosome abnormalities were detected.

FISH ANALYSIS (FIG 5A, B)

A total of 22/23 (96%) classical WBS cases were shown to be hemizygous for the elastin locus. The only patient from this category who did not have a submicroscopic deletion at 7q11.23 was patient 23 who had been shown to have the interstitial deletion of 11q.

Of the suspected WBS cases, 2/22 (9%) were shown to be deleted at the elastin locus and 20 did not have a submicroscopic deletion. One of these 20 cases (patient 26) was initially thought to have a deletion at 7q11.23 in 5/30 (17%) cells examined from a peripheral blood sample. However, repeat investigations on blood lymphocytes and skin fibroblasts scored blind alongside normal control samples did not confirm the low level mosaicism originally detected and we therefore interpret the deleted cells as artefacts.

Among the patients referred with SVAS/PPS, one out of seven (14%) also showed hemizygosity at the elastin locus and the remaining six did not.

Figure 2 Phenotypic features of Williams syndrome and suspected Williams syndrome patients.

Figure 3 (A) GTL banded chromosomes 11 from patient 23 and schematic diagram showing interstitial deletion of 11q on one homologous del(11)(q13.5q14.2). (B) Patient 23 at 2 years 6 months. (C) Patient 23 at 5 years 6 months.

PHENOTYPE:GENOTYPE CORRELATIONS

In order to determine if there is a subset of WBS features which are present at a greater frequency in those patients where a deletion at the elastin locus has been shown, the phenotypic characteristics of our deleted cases from all three categories were compared with those of the non-deleted cases from the classical and suspected WBS categories (fig 2).

Of those patients with a submicroscopic deletion at 7q11.23, full cheeks and a broad nasal tip were observed in 100% and several other features were observed in 80% or more of patients, including developmental delay, SVAS/PPS, WBS personality, wide mouth, broad forehead, long philtrum, bitemporal narrowness, and periorbital fullness. In the non-deleted patients none of the listed features, with the exception of developmental delay, was observed in more than 50% of patients.

Notable exceptions were patients 23, 27, and 46. Patient 23, a 6 year old boy, had a deletion of chromosome 11 and no submicroscopic deletion at 7q11.23 despite displaying the majority of Williams-Beuren characteristics. He had originally been diagnosed as WBS by a clinical geneticist at 2 years of age. This diagnosis was fully accepted by the paediatrician over the next three years, and by the parents who met other children with WBS. He is, therefore, included in our classical WBS cat-

egory. However, when assessed by a clinical geneticist at 5 years of age, when the cytogenetic and elastin results were known, he was considered to be atypical in some respects. He has moderate developmental delay and a gregarious personality but does not have disproportionate verbal facility. His facial features include a broad forehead, periorbital fullness, wide mouth, full cheeks, prominent ear lobules, and a long philtrum (fig 3B, C).

Patient 27, a 22 month old boy referred to us by his paediatrician as ?Williams-Beuren syndrome, was also found to have no submicroscopic 7q11.23 deletion but was shown to have a complex karyotype with deletions of 22q and Yq. He has moderate developmental delay and poor growth. His motor development and performance are assessed to be at 15–16 months and language skills at 10–12 months. His facial appearance was initially suggestive of Williams-Beuren syndrome (fig 4B). He had periorbital fullness, light irides, full cheeks, prominent nasal tip, and a long, well defined philtrum. However, he had a high nasal bridge and a small mouth, not typical of WBS. Full details of this case will be presented elsewhere (Pike *et al*, in preparation).

Patient 46, a 6 year old girl, was originally referred to us with pulmonary stenosis as “? Turner syndrome, ?Noonan syndrome, ?22q deletion”. She was found to have an apparently normal female karyotype in 30 cells when examined using conventional cytogenetics and no evidence of a submicroscopic deletion using FISH with the H748 cosmid specific for the DiGeorge syndrome critical region in 22q11.2. However, a WSCR deletion was found when her cardiac diagnosis was altered to SVAS. She is short, unlike her two sibs in appearance, and has a gregarious personality, a hoarse voice, and hyperacusis. Mild developmental delay was reported early on but, while her development is slightly delayed compared with her sibs, her IQ is within the normal range and she is doing well in a normal school. Her facial features are limited to full cheeks and a broad nasal tip (fig 6).

Discussion

In our study, 96% of the classical WBS and 9% of suspected WBS patients were shown to have a submicroscopic deletion at 7q11.23. These figures are comparable with previous reports.^{15–19}

In this series, only full cheeks and a broad nasal tip were observed in all our patients with elastin gene deletions. However, other features frequently associated with a submicroscopic deletion included developmental delay (96%), SVAS/PPS (80%), WBS personality (88%), wide mouth (96%), broad forehead (92%), long philtrum (96%), bitemporal narrowness (88%), and periorbital fullness (92%). Hypercalcaemia, often regarded as a good indicator of WBS during early infancy, was almost as frequent in the non-deleted as in the deleted cases. Infantile hypercalcaemia was, therefore, the poorest indicator of WBS in this study population, consistent with other recent reports.^{15 16 19}

Figure 4 (A) Partial karyotype from patient 27 showing a ring chromosome 22 and a derived Y;21 chromosome from a whole arm translocation. (B) Patient 27 at 2 years.

The only patient in the classical WBS category who did not have a submicroscopic deletion on chromosome 7 (patient 23) was shown by routine cytogenetic analysis to have a *de novo* interstitial deletion in the long arm of one chromosome 11 resulting in monosomy for 11q14.1. Several chromosome abnormalities have previously been reported in isolated cases of WBS, including a 15p deletion,²² a balanced 9;17 translocation,²³ a deletion of the long arm of chromosome 4,²⁴ and an unbalanced 13;18 translocation.²⁵ A further patient with features of WBS was shown by Tupler *et al*²⁶ in 1992 to have a complex unbalanced chromosome rearrangement with 10 breakpoints and monosomy for the region 4q33→q35.1. Two of the breakpoints were on 11q (11q13.1 and 11q23), but neither of these correspond to our patient's breakpoints. We are unaware of any published cases with a similar deletion of 11q to our patient and, therefore, it is not yet possible to determine if a deletion of this region consistently results in a phenotype remarkably similar to that of WBS. Another interesting case in our series (patient 27) was found to have a complex rearrangement including deletions of 22q and Yq in association with a WBS-like phenotype. This 22 month old boy had some of the facial features reported in WBS including full cheeks, prominent nasal tip, and periorbital fullness. Overall, however, his phenotype was not typical of patients with classical WBS and he was too young to be assessed for the "Williams syndrome personality". Both these cases highlight the importance of routine cytogenetic analysis in conjunction with FISH investigations.

Proximal 7q deletions detectable by conventional cytogenetics are relatively rare. However, at least 21 cases have been reported to date and the clinical findings associated with these deletions have been reviewed by Zackowski *et al*²⁷ and Gillar *et al*.²⁸ Of these 21 cases, 16 might be expected to include a deletion of the elastin locus at 7q11.23. While WBS was not considered as a diagnosis in these 16 cases,

Figure 5 Representative FISH results using the WSCR probe (7q11.23) and D7S427 control probe (7q36). (A) Normal sample showing two normal chromosomes 7, each with a clear signal at 7q11.23 and at 7q36. (B) Deleted sample showing one normal chromosome 7 and one deleted chromosome 7 (arrowed) with only the control signal at 7q36.

Figure 6 Patient 46 at 5 years 6 months.

WBS clinical manifestations have been reported in many of them, including short bulbous nose, full cheeks, long philtrum, micrognathia, feeding difficulties in infancy, and cardiac defects. Notable patients with similarities to WBS include a 14 year old boy reported by Frydman *et al.*,²⁹ a 4 year old girl reported by Klep-de Pater *et al.*³⁰ (case 2), and a 10 year old girl reported by Young *et al.*³¹ (case 3). It would be of interest to reinvestigate these patients using FISH in order to determine how many of these proximal 7q deletions encompass the elastin locus and to what extent the phenotypic outcome is dependent on deletion of the WBS critical region.

Of our seven patients referred with SVAS/PPS where a diagnosis of WBS was not being considered, one girl (patient 46) was shown to be hemizygous at the elastin locus. As far as we are aware, this is the first reported case of a patient with normal development and an elastin gene deletion detected by FISH. In addition, patient 47, from an autosomal dominant SVAS family, did not have a WSCR deletion yet presented with some of the facial features typical of WBS, including full cheeks, a broad nasal tip, and periorbital fullness together with a hoarse voice. Subtle WBS facial features were present in her affected daughter. This is consistent with previous reports of some subjects in autosomal dominant SVAS families who showed minor features of WBS.^{31,32} This phenotypic overlap may not be unexpected if hemizygosity for, or mutations within, the elastin gene account for connective tissue abnormalities including SVAS and some of the dysmorphic facial features but not other aspects of WBS, such as the neurobehavioural features. If WBS is a contiguous gene disorder with deletion of genes other than elastin contributing to mental retardation and the WBS personality, variability in the extent of the deletion could be a significant factor in determining the considerable phenotypic variability seen in this

syndrome. In our series the extremes were represented by patient 46 (fig 6) who had a normal IQ and relatively few WBS features, and patient 1 (fig 1) who had severe mental retardation, no speech, and classical WBS features. Cases such as patient 32 and four of the patients reported by Nickerson *et al.*,¹⁸ who had many WBS features without SVAS and appeared normal with the WSCR probe, may turn out to have deletions within the WBS critical region which do not involve the ELN gene.

In conclusion, we have seen a wide range of phenotypes associated with a deletion at the elastin locus in this series. Since only full cheeks and a broad nasal tip were seen in all our deleted cases, the absence, even of developmental delay or the loquacious WBS personality, should not exclude a suspected WBS case from investigation with FISH especially if SVAS or PPS is present. Molecular studies to determine the size of deletions at the elastin locus correlated to the phenotypic features of patients should be the next line of investigation and may show the cause of the wide spectrum of severity observed in Williams-Beuren syndrome. The occurrence of three different cytogenetic abnormalities in this series, two of them apparently responsible for a WBS-like phenotype, is a reminder that conventional cytogenetics should not be omitted from investigations of WBS.

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