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Short Communication

# Fluorescent *in situ* hybridization (FISH) as a diagnostic tool for Williams-Beuren syndrome

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## Abstract

Fluorescent *in situ* hybridization (FISH) with commercial probes covering the elastin gene (*ELN*) was used to determine the frequency of the 7q11.23 deletion in 18 children clinically diagnosed with Williams-Beuren syndrome (WBS). A *de novo* deletion was detected in 15 of the children (83%). Diagnostic investigation for WBS started late in childhood (median = 5.8 years). All the children showed facial features typical of the syndrome, mental retardation and developmental delay. Over-friendliness was observed in the majority of cases. Clinodactyly of the 5th finger (n = 13), cardiovascular disease (n = 9), loquacity (n = 9), low birthweight (n = 8), and failure to thrive (n = 9) were observed only in those children with the deletion. Respiratory problems (n = 9), though not previously reported in the literature, was a common finding in the group studied. Our results confirmed that FISH is useful in identifying 7q11.23 deletions in cases of WBS. Clinical manifestations were more evident in the deletion-positive children

*Key words:* 7q11.23 deletion, *ELN* , FISH, Williams-Beuren syndrome.

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Williams-Beuren syndrome (WBS) comprises characteristic "elfin" facies (Figure 1), mental retardation, failure to thrive, congenital heart disease, unusual neurobehavioral features, learning disabilities and infantile hypercalcemia, with the clinical phenotype changing with advancing age so that its clinical diagnosis may not be suspected during early infancy (Williams *et al*., 1961; Beuren *et al*., 1962; Black *et al*., 1963; Kelly *et al*., 1975; Burn, 1986). The incidence of WBS has been estimated to be 1 in 20,000 to 50,000 live births. Cases are generally sporadic, though autosomal dominant inheritance has been reported (Cortada *et al*., 1980; Morris *et al*., 1993).

The multisystem phenotype of WBS is associated with a 1.5 Mb deletion at 7q11.23 that includes the *elastin* gene (ELN) and at least 21 other genes (Merla *et al.* 2002). The contribution of each of these genes to the WBS phenotype is not known, but *ELN* has been proven to be associated with congenital heart disease (Ewart *et al*., 1993; Lowery *et al*., 1995; Anon., 2001; Sugayama *et al*, 2003; Heller *et al*., 2003). Even though at least 95% of WBS individuals with the classical phenotype carry an apparently identical 1.5 Mb deletion, several individuals presenting classical WBS and a smaller deletion have been described, suggesting that the critical WBS region is smaller than 1.5 Mb (Osborne, 1999; Heller *et al*., 2003)

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The purpose of this study was to establish the frequency of the 7q11.23 deletion in children with clinically suspected WBS, who were referred to the Genetic Counseling Service of the Botucatu Institute of Biosciences, Brazil, between 1986 and 2002. The research protocol was approved by the Botucatu Medical School Research Ethics Committee - São Paulo State University, and parental consent was obtained.

All the children were clinically examined using a checklist of the most typical WBS features (Morris *et al*., 1988; Jones and Smith, 1975). They were submitted to neurological, ophtalmological and cardiological (echocardiography and measurement of serum calcium levels) evaluations. Parents provided information on the children concerning pregnancy, birth conditions and developmental milestones. Data from previous medical records were obtained. Post-natal growth was classified according to the National Center for Health Statistics Charts (2000) and head circumference according to Nellhaus (1968).

Of the 25 children referred with the diagnostic hypothesis of WBS, 18 were selected for the study based on the checklist of WBS features. The inclusion criteria were the presence of two of the three most frequent WBS features (typical facial features, heart disease and develop-

mental delay/behavioral problems) associated with a least two other less common features (failure to thrive, hypercalcemia, gastrointestinal, genitourinary, ophthalmologic, auditory, dermatologic, musculoskeletal and dental problems). All the 18 children showed typical facial features (broad forehead, wide mouth, full prominent lips, periorbital fullness), mental retardation and behavioral problems, principally over-friendliness.

Chromosome analysis was performed after high-resolution GTG banding (Yunis, 1976) in cultured peripheral blood lymphocytes. Fluorescent *in situ* hybridization (FISH) was performed using commercial Williams Syndrome Region probes encompassing *ELN*. The Vysis® probe (Cat No 32-190041, Vysis, USA) is approximately 180 kb in size and contains the *ELN* and *LIM-Kinase* 1 (*LIM-K1*) genes and the D7S613 locus, the control probe contains the D7S486 and D7S522 loci. The Cytocell® Williams-Beuren 450 kb probe (Cat No LPU 011, Cytocell, USA) consists of three non-overlapping clones from genes *FZD9* to *CYLN2*, does not include *ELN*, and contains the chromosome 7 centromere α-satellite D7Z1 probe for control. Whenever a deletion was detected, high-resolution GTG banding and FISH of the parents of the child were carried out.

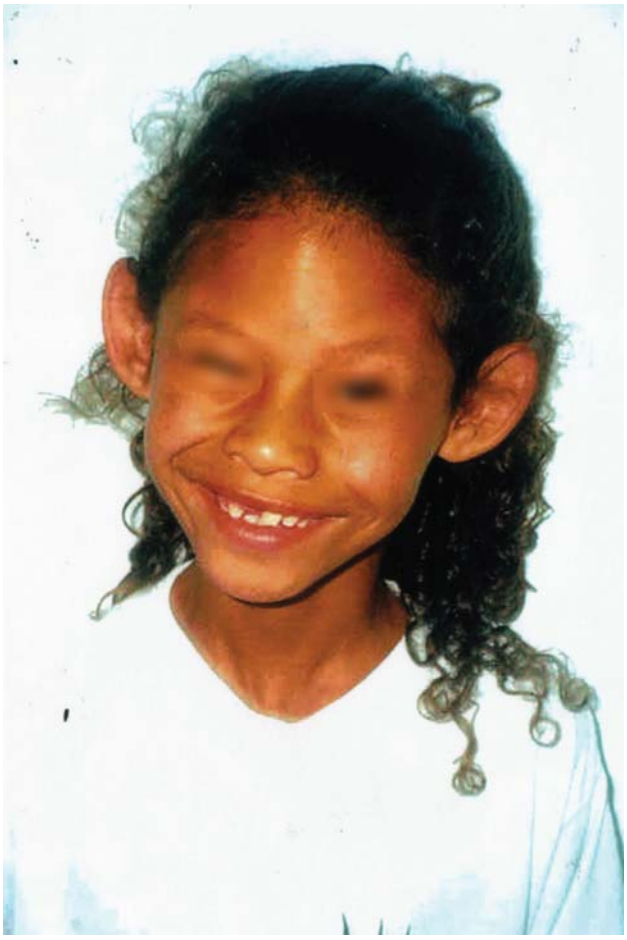

**Figure 1** - Typical facial appearance of individuals with Williams Beuren syndrome (WBS): broad forehead, large and anteverted ears, flat nasal bridge, wide mouth, full cheeks, full prominent lips, dental anomalies, small jaw.

Our GTG-banding analysis revealed normal karyotypes in the 18 children and their parents. Cytocell probe FISH analysis showed a 7q11.23 deletion in 15 children. The absence of a deletion in the three remaining children was confirmed by FISH with the Vysis probe, which was also used to retest 6 (Figure 2) of the 15 children identified as deletion-positive by the Cytocell probe and confirmed the diagnosis. This high deletion frequency is in agreement with previously reported data (Borg *et al*., 1995; Elçioglu *et al*., 1998; Sugayama *et al*., 2004a). Parents did not carry the deletion, thus confirming the *de novo* nature of this rearrangement.

The main clinical findings for the children are summarized in Table 1. Only deletion-positive children were small for gestational age (8 out of 15), and remained small with aging (7 out of 15). Head circumference was below the 2$^{nd}$ centile in 7 out of the 15 deletion-positive children and in one of the three deletion-negative children. There is a characteristic pattern of prenatal and postnatal growth deficiency in children with WBS, and weight and height are clearly below the normal range from birth to adulthood (Lashkari *et al*., 1999). In our series of cases, low stature and weight ( 5 centile) were detected in 7 out of 15 and 4 out of 15 deletion-positive children, respectively. Furthermore, feeding difficulties (11 out of 15), chronic constipation (12 out of 15), and failure to thrive (9 out of 15) were highly prevalent findings.

Nine of the 15 children (60%) with *ELN* deletion showed cardiovascular defects, and six of them had supravalvular aortic stenosis confirmed by echocardiogram. Other cardiopathies observed included aortic coarctation (1

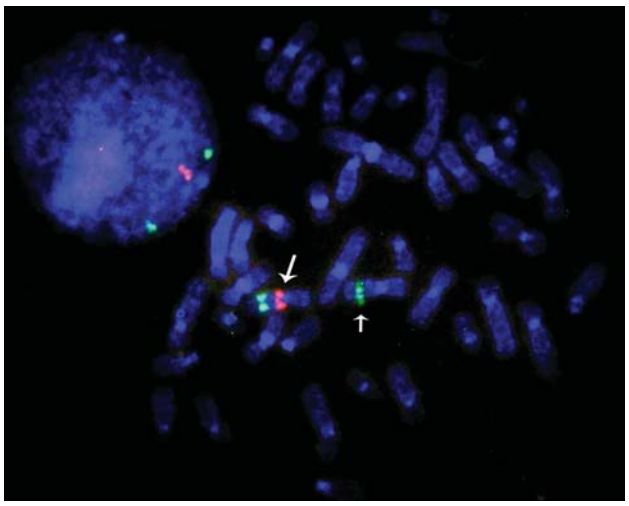

**Figure 2** - Metaphase after fluorescent *in situ* hybridization (FISH)with VYSIS® Williams Syndrome Region. The normal chromosome (long arrow) shows a pink signal at 7q11.23, and a green signal at the control segment. On the abnormal chromosome 7 (small arrow), only the control green signal is observed, indicating a deletion at 7q11.23.

**Table 1** - Clinical findings related with the Williams Beuren syndrome (WBS) children in our study with and without the 7q11.23 deletion compared with data from the literature*.

| | Deletion-positive | | Deletion-negative | |
|---|---|---|---|---|
| | Our study (n = 15) | Lit* (n = 379) | Our study (n = 3) | Lit* (n = 138) |
| Clinical feature | | % | | % |
| Broad forehead | 15 | 93 | 3 | 38 |
| Wide mouth | 15 | 93 | 3 | 40 |
| Full prominent lips | 15 | 94 | 3 | 42 |
| Periorbital fullness | 13 | 93 | 3 | 36 |
| Long philtrum | 12 | 91 | 1 | 36 |
| Malar hypoplasia | 12 | 100 | 2 | 92 |
| Flat nasal bridge | 11 | 91 | 3 | 90 |
| Dental anomalies | 10 | 64 | 2 | 32 |
| Full cheeks | 10 | 87 | 3 | 44 |
| Bitemporal narrowing | 8 | 88 | 2 | 32 |
| Strabismus | 8 | 51 | 1 | 7 |
| Anteverted ears | 7 | – | 1 | – |
| Microcephaly | 6 | 45 | 1 | 38 |
| Small jaw | 6 | 89 | 2 | 43 |
| Stellate iris | 3 | 39 | 0 | 23 |
| Clinodactyly of the 5$^{th}$ finger | 13 | 56 | 0 | 43 |
| Mental retardation | 15 | 86 | 3 | 66 |
| Over-friendliness | 13 | 83 | 3 | 35 |
| Small for gestational age | 8 | 69 | 0 | 17 |
| Stature | 7 | 65 | 0 | 40 |
| Weight | 4 | 50 | 0 | 35 |
| Feeding difficulties | 11 | 73 | 1 | 23 |
| Chronic Constipation | 12 | 57 | 2 | 24 |
| Hyperactivity | 11 | 75 | 3 | 44 |
| Inguinal/ umbilical hernia | 9 | 34 | 2 | 18 |
| Loquacity | 9 | 54 | 0 | 14 |
| Hoarse voice | 8 | 74 | 2 | 26 |
| Failure to thrive | 9 | 57 | 0 | 43 |
| Hypotonia | 7 | 72 | 1 | 57 |
| Respiratory problems | 7 | – | 2 | – |
| Supravalvular aortic stenosis | 6 | 60 | 0 | 10 |
| Other cardiopathy | 3 | 43 | 0 | 32 |
| Vomiting | 5 | 73 | 1 | 23 |
| Chronic otitis media | 4 | 29 | 0 | 0 |
| Renal problems | 4 | 18 | 0 | 8 |

∗ Modified from Sugayama *et.al.* (2004b); – not reported.
Lit: The literature.

out of 15) and atrio-ventricular communication (2 out of 15). Cardiovascular abnormalities are very frequent (53-80%) in WBS individuals, and *ELN* deletion has been associated with the typical vasculopathy of WBS, namely supravalvular aortic stenosis and pulmonary arterial stenosis (Ewart *et al.*, 1994; Eronen *et al.*, 2002; Sugayama *et al.*, 2003). However, a considerable number of the WBS-associated cardiovascular problems, including supravalvular aortic stenosis, may not manifest until adulthood and the symptoms might be missing or non-specific, misleading the diagnosis for years. The frequency of cardiovascular abnormalities depends on accurate cardiovascular evaluation (Ino *et al.*, 1988; Lowery *et al.*, 1995, Eronen *et al.*, 2002; Sugayama *et al.*, 2003).

Serum calcium was measured in all the children but hypercalcemia (11.2 mg $dL^{-1}$) was detected in only one child, who was under one year of age.

Most of the children showed respiratory problems, particularly bronchospasms, bronchitis and episodes of pneumonia. To our knowledge, these symptoms have not been previously reported. The possibility of a casual association cannot be ruled out because respiratory complaints are very frequent in pediatric visits. Nonetheless, closer attention should be paid to this feature in future studies.

The diagnosis of WBS is often late (Morris *et al*., 1988; Huang *et al*., 2002). This fact was confirmed in our series of cases, with age at first diagnosis ranging from 16 months to 12 years with a mean of 5 years. Only three children were diagnosed below the age of 3 years, and in nine children, the diagnosis was made after the age of 7 years, even though they met the Lowery criteria for classic WBS (Lowery *et al*., 1995), had developmental delay and learning difficulties and attended special schools. The delay in establishing WBS diagnosis may be explained by some of the following factors: (1) WBS physical manifestations are subtle and may not be apparent at an early age, making diagnosis difficult in infants and young children who lack classic manifestations such as supravalvular aortic stenosis and hypercalcemia; (2)infantile hypercalcemia is probably under-diagnosed as it manifests itself during the first year of life as failure to thrive, persistent vomiting, irritability, colic and intestinal constipation that are common problems during infancy (Martin *et al*., 1984; Morris *et al*., 1988; Lashkari *et al*., 1999; American Academy of Pediatrics, 2001; Carrasco *et al*., 2005); and (3) although these children exhibit developmental delay, variable degrees of mental retardation and visual spatial impairment, their hyperactivity, loquacity and over-friendliness may suggest that their intellectual ability is better than it actually is (Jones and Smith, 1975; Udwin *et al*., 1976; Morris *et al*., 1998; Carrasco *et al*., 2005).

In conclusion, this study confirmed the delay in clinical diagnosis and the usefulness of FISH in the detection of the 7q11.23 deletion in children with WBS.

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## Internet resources


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