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Deletions of the Elastin Gene at 7q11.23 Occur in ~90% of Patients with Williams Syndrome

Elizabeth Nickerson,[1] Frank Greenberg,[1,2] Mark T. Keating,[3] Christopher McCaskill,[1] and Lisa G. Shaffer[1]

Departments of [1]Molecular and Human Genetics and [2]Pediatrics, Baylor College of Medicine, Houston; and [3]Howard Hughes Medical Institute, Eccles Program in Human Molecular Biology and Genetics, Department of Medicine and Human Genetics, University of Utah, Salt Lake City

Summary

To investigate the frequency of deletions of the elastin gene in patients with Williams syndrome (WS), we screened 44 patients by both FISH and PCR amplification of a dinucleotide repeat polymorphism. FISH was performed using cosmids containing either the 5′ or the 3′ end of the elastin gene. PCR analysis was performed on the patients and their parents with a $(CA)_n$ repeat polymorphism found in intron 17 of the elastin locus. Of the 44 patients screened, 91% were shown to be deleted by FISH. Using the DNA polymorphism, both maternally (39%) and paternally (61%) derived deletions were found. Four patients were not deleted for elastin but have clinical features of WS. Since deletions of elastin cannot account for several features found in WS, these patients will be valuable in further delineation of the critical region responsible for the WS phenotype. Although PCR can be useful for determining the parental origin of the deletion, our results demonstrate that FISH analysis of the elastin locus provides a more rapid and informative test to confirm a clinical diagnosis of WS. The presence of two copies of the elastin locus in a patient does not, however, rule out WS as a diagnosis.

Introduction

Williams syndrome (WS) is a genetic disorder that occurs in 1/20,000–1/50,000 live births (Greenberg 1990). Cases are generally sporadic, although familial cases are known and follow an apparent autosomal dominant pattern of inheritance (Morris et al. 1993). Chromosome abnormalities in WS were investigated previously; however, no consistent deletion or rearrangement was detected (Greenberg and Ledbetter 1988). Clinical features of WS include characteristic "elfin" facies, mental retardation, growth deficiency, gregarious personality, intermittent hypercalcemia, and hypertension. Cardiovascular abnormalities are prevalent in WS patients (80%), with the specific anomaly being supravalvular aortic stenosis (SVAS) in 84% of these cases (Morris et al. 1988).

SVAS, a congenital narrowing of the ascending aorta, can be asymptomatic in some patients but leads to sudden death in others (Giddins et al. 1989; Conway et al. 1990). Although sporadic cases exist, SVAS can also segregate in families as an autosomal dominant disorder (Chiarella et al. 1989; Ensing et al. 1989). Dominant SVAS was formerly linked to chromosome 7 (Ewart et al. 1993*b*; Olson et al. 1993), and, recently, investigation of a t(6;7) balanced translocation carried by a family with SVAS demonstrated that the translocation disrupted exon 28 of the elastin gene (Curran et al. 1993). Furthermore, a 100-kb deletion in the 3′ end of the elastin gene was found in another SVAS family (Ewart et al. 1994), strongly indicating a critical role for elastin in SVAS and proper vascular development. Although these individuals had only isolated SVAS and not WS, given the frequency of SVAS in WS patients, a role for elastin in WS was hypothesized. Southern blot analysis and FISH showed hemizygosity at the elastin locus in four familial and five sporadic cases of WS (Ewart et al. 1993*a*).

Elastin is a component of elastic fibers that gives elasticity to vertebrate tissues such as the lungs, the dermis, and the larger blood vessels. The gene encoding elastin has been mapped to 7q11.23 (Fazio et al. 1991; Foster et al. 1993). The gene spans ~45 kb and encodes a mature mRNA of ~3.5 kb. The intron:exon ratio is high, 19:1, as most of the gene's 34 exons are small in size. The 3′ end of elastin is rich in *Alu* sequences, containing four times as much *Alu* as would be predicted for most genes (Uitto et al. 1991). Elastin has been shown to exhibit several alternatively spliced isoforms (Indik et al. 1989).

We report the study of 44 patients with WS analyzed for hemizygosity at the elastin locus. A $(CA)_n$ repeat polymorphism, found in intron 17 of the elastin gene

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Address for correspondence and reprints: Dr. Lisa G. Shaffer, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Room 15E, Houston, TX 77030.

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(Foster et al. 1993), was used to assess the deletion status and parental origins of deletions in patients by PCR. Two cosmids, cELN272 and cELN11-D, containing the 5′ and 3′ end of the elastin gene, respectively, were used for FISH analysis of metaphase chromosome spreads. We present the results on these 44 patients and correlate deletion status with the variability of the phenotypic features.

Patients, Material, and Methods

Patient Samples

Forty-four individuals with WS were ascertained through the genetics clinic at Texas Children's Hospital (n = 30) or other referring medical genetics centers (n = 14). In every case, the patient was personally examined by one of us (F.G.) and had carried the diagnosis of WS for years, or the patient's medical records were reviewed by F.G. and/or discussed with the referring physician. Patients who did not meet the clinical criteria for WS, by history and physical findings, were excluded from the study. All patients had received either routine cytogenetic analyses (~550 band resolution) or high-resolution analyses (~850 bands) prior to entering the study. For the patients studied in the Kleberg Cytogenetic Laboratory at Baylor College of Medicine (n = 30), the karyotypes were reevaluated by one of us (L.G.S.) for deletions of 7q11.23.

Molecular Analyses

DNA was extracted from peripheral blood of the proband with WS and of each parent, using standard methodology. Three highly polymorphic dinucleotide repeat polymorphisms were used in a multiplex reaction to detect deletions and to determine the parental origins of the deletion when present. Markers D7S476 and D7S488, mapping outside the elastin locus on chromosome 7 and at 7p15-p21, respectively, were identified through the Genome Data Base. A $(CA)_n$ repeat polymorphism found in intron 17 of the elastin gene was amplified using primers from exon 17 and exon 18 (Foster et al. 1993). The markers were synthesized in the sequencing core within the Department of Molecular and Human Genetics, Baylor College of Medicine. Multiplex PCR was performed using previously published methods (Shaffer et al. 1993), producing nonoverlapping products that could easily be distinguished on a sequencing gel. Deletions were detected when the proband failed to inherit an allele from one of the parents at the elastin locus while inheriting one allele from each parent for at least one of the other markers.

FISH

FISH was performed on metaphase chromosomes from each proband, using cosmid cELN272, containing the 5′ end of the elastin gene, or cosmid cELN11-D, containing the 3′ end of the elastin gene (Ewart et al. 1993*a*). The cosmids were labeled with digoxigenin and were detected with antidigoxigenin conjugated to rhodamine. For both hybridizations, a biotin-labeled chromosome 7 alpha-satellite centromere probe (ONCOR) was used as a control to identify the chromosomes 7. The centromere probe was detected using avidin conjugated to fluorescein isothiocyanate. The slides were counterstained with DAPI and were viewed with a Zeiss Axiophot fluorescent microscope equipped with a triple-band pass filter that allows one to visualize multiple colors simultaneously. Digital images were captured using a PSI Powergene 810 probe system and were printed using a Tektronix Color/Monochrome Phasar II SDX printer. Metaphase chromosomes were prepared with standard protocols, and FISH was performed as described elsewhere (Shaffer et al. 1994), with the following modifications: each cosmid was diluted in a 65% formamide hybridization solution with Cot-1 DNA, for a final concentration of 35 ng/μl for cELN272, 120 ng/μl for cELN11-D, and 0.5ng/μl for the centromere probe.

Results

Cytogenetic Analyses

Routine or high-resolution cytogenetic analyses had been performed on all 44 patients prior to entering the

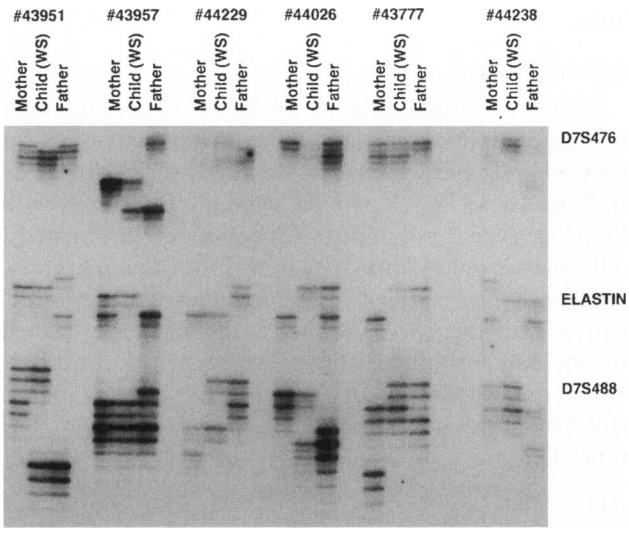

Figure 1 Representative molecular results for six WS patients and their parents, using a multiplex PCR assay for identification of deletions and parental origins. Each WS patient shown demonstrated a deletion for the elastin marker. For example, in family 43951 and markers D7S476 and D7S488, the child inherited an allele from each parent. However, for the elastin marker, the child failed to inherit an allele from the father. Therefore, this is a deletion of paternal origin. Likewise, patients 43957 and 44229 have deletions of paternal origin. The remaining patients inherited an allele from only the father and therefore have deletions of maternal origin.

Table 1

FISH and PCR Results in 44 Patients with a Clinical Diagnosis of Williams Syndrome

No. of Patients	FISH		PCR
	cELN272 (5′)	cELN11-D (3′)	
4	del	del	mat del
3	del	del	pat del
13	del	del	U
5	del	NS	pat del
8	del	NS	U
2	del	del	NA
3	del	NS	NA
1	del	NS	NS
1	NA	NA	mat del
2	no del	no del	no del
1	no del	no del	U
1	no del	NS	U
44			

NOTE.—del = deletion; NS = not studied; NA = not available; mat del = maternal deletion; pat del = paternal deletion; U = uninformative; and no del = no deletion detected.

study. For those patients whose chromosomes were prepared in the Kleberg Cytogenetics Laboratory at Baylor College of Medicine, their karyotypes were reevaluated by one of us (L.G.S.). Only one patient (34704) was felt to have a subtle deletion of 7q11.23. The remaining 29 patients' chromosomes 7 appeared to be within normal limits.

Molecular Analyses

DNA was obtained from 39 WS patients and their parents. Samples were not obtained from the parents for five probands and were therefore not available for study by PCR. Thus, 39 WS patients were tested by PCR with the $(CA)_n$ repeat polymorphism contained in intron 17 of the elastin gene. Approximately 40% of patients analyzed were informative by this method. Of the 15 informative patients, 13 (87%) were shown to be deleted for one parental allele. There was no significant difference in the distribution of paternally ($n = 8$) and maternally ($n = 5$) derived deletions ($.50 < P < .25$) (fig. 1) (table 1).

FISH

To further evaluate hemizygosity at the elastin locus in patients with WS, we performed FISH using a cosmid spanning either the 5′ or the 3′ end of the elastin locus. Forty-three of 44 patients were screened by FISH using cosmid cELN272 containing the 5′ end of the elastin gene (fig. 2). For one patient, only DNA was available, and therefore this patient was not tested by FISH. Approximately 91% (39/43) of patients were shown to be deleted for the 5′ probe.

Twenty-five of the 43 available patient samples were screened with cosmid cELN11-D containing the 3′ end of the elastin gene. This cosmid proved problematic as a FISH probe, resulting in high-background hybridization. This may be due to the inordinately high amounts of *Alu*-repetitive sequence in the 3′ end of the gene (Indik et al. 1989). Therefore, 25 patients were initially tested with the 3′ cosmid, and, when all patients who were found to be deleted for the 5′ cosmid were also deleted for the 3′ cosmid, testing of the 3′ cosmid was discontinued. For the patients tested, 88% (22/25) were deleted for the 3′ probe (table 1).

Clinical Correlation

Of the 40 patients who were deleted for the elastin markers, 16 had SVAS, 1 had coarctation of the aorta,

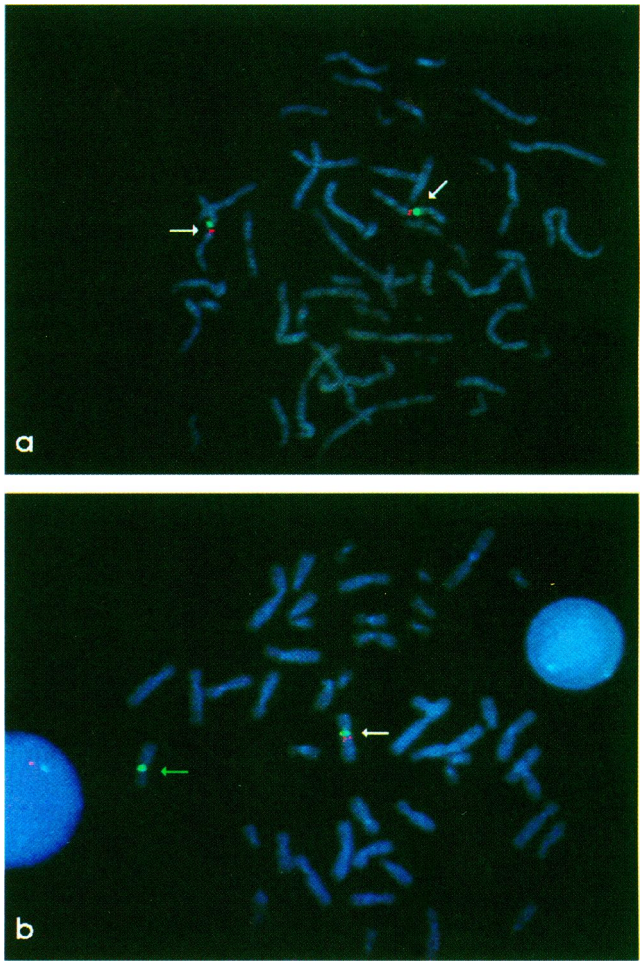

Figure 2 Representative FISH results using cosmid cELN272 containing the 5′ end of the elastin gene and a chromosome 7 alpha-satellite centromere probe as a control. *a*, Normal control sample showing two normal chromosomes 7, each with the cosmid signal (*red*) and the control signal (*green*). *b*, Deletion sample (45229) showing one normal chromosome 7, as described above (*white arrow*), and one deleted chromosome 7 with only the control signal (*green arrow*).

3 had peripheral pulmonic stenosis, 1 had documented hypertension of undetermined etiology, and 12 had no cardiac anomaly. The cardiac status is unknown for seven of the patients.

Two families with dominant transmission of WS were studied. Patients 43949 and 43950 are mother and daughter. Both were hemizygous for elastin and have typical facial dysmorphism, mental retardation, and normal cardiac evaluations. These patients have been reported previously as representing a family with autosomal dominant transmission (family C) (Morris et al. 1993) and as demonstrating hemizygosity of elastin in familial WS syndrome (family K2042) (Ewart et al. 1993*a*). On the basis of PCR analysis of her parents, the mother has an apparently de novo deletion that was transmitted to her daughter. The second family consists of a mother and son (45140 and 45141), both with classical WS features, mental retardation, and normal cardiac evaluations. The mother's parents were not studied, so it is unknown whether her deletion was inherited or de novo.

Four of 44 patients were not deleted for the elastin locus. Patient 44005 presents with typical WS but does not have a heart defect. Patient 44743 also has typical WS facies with neither a heart defect nor hypercalcemia. Patient 43918 has typical WS. Her cardiac status is unknown. Patient 44216, also not deleted, has typical WS facies and had severe infantile hypercalcemia but has neither a heart defect nor mental retardation (fig. 3).

Figure 3 Nondeletion patient 44216. Note the typical Williams syndrome "elfin" facies.

Discussion

Screening by PCR and FISH of 44 patients diagnosed with WS showed that 91% of the patients were hemizygous for the elastin gene mapped to 7q11.23. In those cases informative by PCR analysis with a $(CA)_n$ repeat polymorphism, approximately equal numbers of paternally and maternally derived deletions were found. All patients had a cytogenetic evaluation, and only one patient (34704) was felt to have a subtle deletion of 7q11.23. Therefore, the deletion in WS represents a true microdeletion, undetectable in the majority of cases, even at the 850-band resolution.

The $(CA)_n$ repeat polymorphism utilized in this study is found in intron 17, very near exon 18 of the elastin gene (Foster et al. 1993). The cosmids utilized in the FISH analysis contain the 5′ and 3′ ends of the gene and span, 5′–3′, a total of 114 kb of DNA (Ewart et al. 1993*a*). In all cases investigated by PCR and by FISH, complete concordance between results was found. Therefore, our results suggest that the entire elastin gene is most probably deleted in the hemizygous patients studied. These results also indicate that the elastin gene is not interrupted by a deletion breakpoint in most patients. The mechanism(s) resulting in deletions in these patients is currently unknown; however, it is unlikely that the extensive *Alu* sequences located at the 3′ end of the gene contribute to the mechanism of deletions in these patients. Our results demonstrate that the use of the 5′ cosmid cELN272 is most likely sufficient to determine hemizygosity for the entire elastin locus and to detect deletions in the majority of WS patients, since all patients studied with the 5′ and 3′ ends were deleted for both. However, since it is possible that the deletion breakpoints may vary among WS patients, it may be useful to develop a more reliable probe for detecting deletions of the 3′ end of the elastin gene.

Several features of the WS phenotype are attributable to haploinsufficiency of elastin. Elastin is the main protein found in the aorta (>50%). SVAS, a congenital narrowing of the aorta, is the most significant cardiac finding in WS patients. SVAS is progressive, and it is known that the amino acid composition of elastin in the aorta changes with age, as do the splice variants seen (Indik et al. 1989). Decreased elastin in the larger blood vessels potentially contributes to the hypertension found in the majority of WS patients, as well. Many WS patients have hoarse voices, and males may present with small genitalia. Elastin is an important component of the vocal cords and of the penis. Atypical facies and

premature aging of the skin could also be attributed to an elastin deficiency.

It is deducible from the above observations that elastin is most likely a dosage-sensitive gene that, when present at half the normal concentration, has an aberrant effect on several tissues. The fact that some systems, such as the pulmonary system, are not affected in the WS phenotype suggests that not all tissues are sensitive to the decreased dosage of elastin that results from hemizygosity at this locus. Dosage sensitivity has been attributed to several other genetic disorders, most remarkably in Charcot-Marie-Tooth disease type 1A (CMT1A) (Lupski et al. 1991, 1992). CMT1A is caused by a duplication of the PMP22 gene on 17p12, which causes distal muscle wasting and decreased nerve conduction. Hemizygosity of PMP22 results in a completely distinct phenotype, hereditary neuropathy with liability to pressure palsies, which presents as muscle weakness and nerve demyelination (Chance et al. 1993). Therefore, the molecular basis of these two diseases appears to be an alteration in the dosage of the PMP22 gene (Lupski et al. 1993).

Patients from whom samples were obtained, including those not deleted for elastin, were either personally examined by or had their records reviewed by one of us (F.G.). Patients were excluded from the study if they did not appear to have WS or if other chromosome abnormalities were identified prior to or during the investigation. While all WS patients diagnosed with SVAS were hemizygous for elastin, several patients not presenting with SVAS or other cardiac anomalies were also shown to be deleted for elastin. Therefore, deletions in elastin do not absolutely correlate with cardiovascular complications. Reasons for this nonconcordance may include modifier genes at other loci, an elastin polymorphism that affects specific allele expression, or stochastic processes.

The elastin locus was not deleted in four patients (~10%) tested. Of these, three had classical WS with facial dysmorphism and mental retardation. These patients suggest that, while hemizygosity at the elastin locus is required for vascular disease, it does not appear to be necessary for many other features of WS and that other genes most likely contribute to the phenotype. One nondeletion patient had the typical WS facial dysmorphism and severe infantile hypercalcemia but normal mentation (fig. 3). This patient is particularly interesting because of his milder findings and may be important in mapping this region. Three of the four nondeletion patients had normal cardiac evaluations. The cardiac status of one nondeletion patient is unknown. Therefore, the cardiac anomalies in patients with WS appears to be due to insufficient elastin.

There are several clinical features of WS that are not accountable by a lack of elastin. Hypercalcemia and mental retardation, for example, are conditions that cannot be explained by hemizygosity at the elastin locus alone. Furthermore, patients who have the elastin gene disrupted by a translocation (Curran et al. 1993) or who carry a small deletion within elastin (Ewart et al. 1994) present with isolated SVAS and do not have WS. This observation, as well as the four patients with WS who are not hemizygous for elastin, strongly suggests that WS is a contiguous gene deletion syndrome. The term *contiguous gene deletion syndrome* was first proposed by Schmickel (1986) and refers to a group of clinically recognized phenotypes described prior to the knowledge of a chromosomal basis. Such disorders include Prader-Willi syndrome (del 15q12), Miller-Dieker syndrome (del 17p13.3), and DiGeorge syndrome (del 22q11.2), among others. Additional features of a contiguous gene deletion syndrome may include mostly sporadic occurrence, although autosomal dominant transmission is observed; individuals with the syndrome may or may not demonstrate a deletion once the chromosome etiology is established, because of submicroscopic deletions; features of the syndrome may occur as individual Mendelian traits; and multiple, functionally unrelated loci located physically contiguous on the chromosome may be deleted resulting in a complex phenotype (Schmickel 1986; Schinzel 1988). On the basis of these criteria, WS is consistent with a contiguous gene deletion syndrome. Further identification of the extent of deletions in WS patients will prove valuable in delineating the critical region and genes responsible for the phenotypic features found in WS.

Our results demonstrate that the elastin gene is useful for identifying deletions in the majority of patients with WS. FISH of the elastin locus provides a noninvasive and rapid analysis to confirm a clinical diagnosis of WS, regardless of the presence of SVAS. Although PCR can be useful for determining the parental origin of the deletion, FISH is a more direct test for a deletion and allows for evaluation of parental chromosomes in cases suspected to be familial. The presence of two copies of the elastin locus in a patient does not, however, rule out WS as a diagnosis.

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