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GTF2I Hemizygosity Implicated in Mental Retardation in Williams Syndrome: Genotype–Phenotype Analysis of Five Families With Deletions in the Williams Syndrome Region

Colleen A. Morris,[1]* Carolyn B. Mervis,[2] Holly H. Hobart,[3] Ronald G. Gregg,[4] Jacquelyn Bertrand,[5] Gregory J. Ensing,[6] Annemarie Sommer,[7] Cynthia A. Moore,[5¶] Robert J. Hopkin,[8] Patricia A. Spallone,[3] Mark T. Keating,[9] Lucy Osborne,[10] Kendra W. Kimberley,[3] and A. Dean Stock[3]

[1]*Department of Pediatrics, Division of Genetics, University of Nevada School of Medicine, Las Vegas, Nevada*
[2]*Department of Psychological and Brain Sciences, University of Louisville, Louisville, Kentucky*
[3]*Department of Pediatrics, Division of Genetics, Laboratory of Molecular Cytogenetics, University of Nevada School of Medicine, Las Vegas, Nevada*
[4]*Department of Biochemistry and Molecular Biology and Center for Genetics and Molecular Medicine, University of Louisville, Louisville, Kentucky*
[5]*National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia*
[6]*Department of Pediatrics and Communicable Diseases, University of Michigan Congenital Heart Center, Ann Arbor, Michigan*
[7]*Department of Pediatrics, College of Medicine and Public Health, Ohio State University, Columbus, Ohio*
[8]*Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*
[9]*Howard Hughes Medical Institute; Department of Cardiology, Children's Hospital of Boston, and Department of Cell Biology, Harvard Medical School, Boston, Massachusetts*
[10]*Department of Medicine, University of Toronto, Toronto, Ontario, Canada*

Most individuals with Williams syndrome (WS) have a 1.6 Mb deletion in chromosome 7q11.23 that encompasses the elastin (*ELN*) gene, while most families with autosomal dominant supravalvar aortic stenosis (SVAS) have point mutations in *ELN*. The overlap of the clinical phenotypes of the two conditions (cardiovascular disease and connective tissue abnormalities such as hernias) is due to the effect of haploinsufficiency of *ELN*. SVAS families often have affected individuals with some WS facial features, most commonly in infancy, suggesting that *ELN* plays a role in WS facial gestalt as well. To find other genes contributing to the WS phenotype, we studied five families with SVAS who have small deletions in the WS region. None of the families had mental retardation, but affected family members had the Williams Syndrome Cognitive Profile (WSCP). All families shared a deletion of *LIMK1*, which encodes a protein strongly expressed in the brain, supporting the hypothesis that *LIMK1* hemizygosity contributes to impairment in visuospatial constructive cognition. While the deletions from the families nearly spanned the WS region, none had a deletion of *FKBP6* or *GTF2I*, suggesting that the mental retardation seen in WS is associated with deletion of either the centromeric and/or telomeric portions of the region. Comparison of these five families with reports of other individuals with partial deletions of the WS region most strongly implicates GTF2I in the mental retardation of WS. © 2003 Wiley-Liss, Inc.

KEY WORDS: supravalvar aortic stenosis; Williams syndrome; 7q11.23 deletion; genotype–phenotype correlation; *ELN*; *LIMK1*; *GTF2I*

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*Correspondence to: Colleen A. Morris, M.D., University of Nevada School of Medicine, Department of Pediatrics, Division of Genetics, 2040 W. Charleston Blvd. #401, Las Vegas, NV 89102. E-mail: cam@unr.edu

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INTRODUCTION

Williams syndrome (WS) (MIM:194050) is a complex disorder characterized by elastin arteriopathy, connective tissue abnormalities (inguinal hernias, bladder and bowel diverticuli, hoarse voice, and soft skin), dysmorphic facial features, mental retardation in 75%, a specific cognitive profile with relative strength in auditory rote memory and deficit in visuospatial cognition, and a characteristic personality that includes generalized anxiety, empathy, and sociability [Morris et al., 1988; Mervis et al., 2000]. WS is caused by a 1.6 Mb deletion of chromosome 7q11.23 that includes the elastin (*ELN*) gene and at least 18 others [Ewart et al., 1993b; Francke, 1999; Osborne et al., 2001]. Haploinsufficiency of elastin clearly causes the arteriopathy observed in WS, since point mutations in *ELN* exons 1 through 28 have been identified in the autosomal dominant disorder, supravalvar aortic stenosis (SVAS) (MIM:185500) [Li et al., 1997; Metcalfe et al., 2000]. Point mutations in *ELN* exons 30 or 32 cause a different autosomal dominant disorder, cutis laxa [Tassabehji et al., 1998; Zhang et al., 1999]. *ELN* hemizygosity is also responsible for some of the other connective tissue manifestations of WS such as inguinal hernia, hoarse voice, stellate iris pattern, and soft skin, as these features have also been observed in individuals with SVAS caused by *ELN* point mutations. However, other components of the WS phenotype (infantile hypercalcemia, mental retardation, WS personality, growth deficiency) do not have an obvious link to *ELN* hemizygosity, and have not been observed in SVAS kindreds. For this reason, several investigators have searched for individuals/families with smaller deletions in the WS region to try to correlate genotype with specific aspects of the phenotype. In this report, we describe clinical, cognitive, and genetic characteristics of five families with SVAS (Fig. 1) who have deletions in the WS region ranging in approximate size from 84 to 850 kb. The five families have deletions that nearly span the classic WS region and have some features that overlap the WS phenotype, but none have classic WS. Two of the families (K1895 and K2049) were previously reported [Frangiskakis et al., 1996], but photographs and medical details were not published; they are included in this article for comparison.

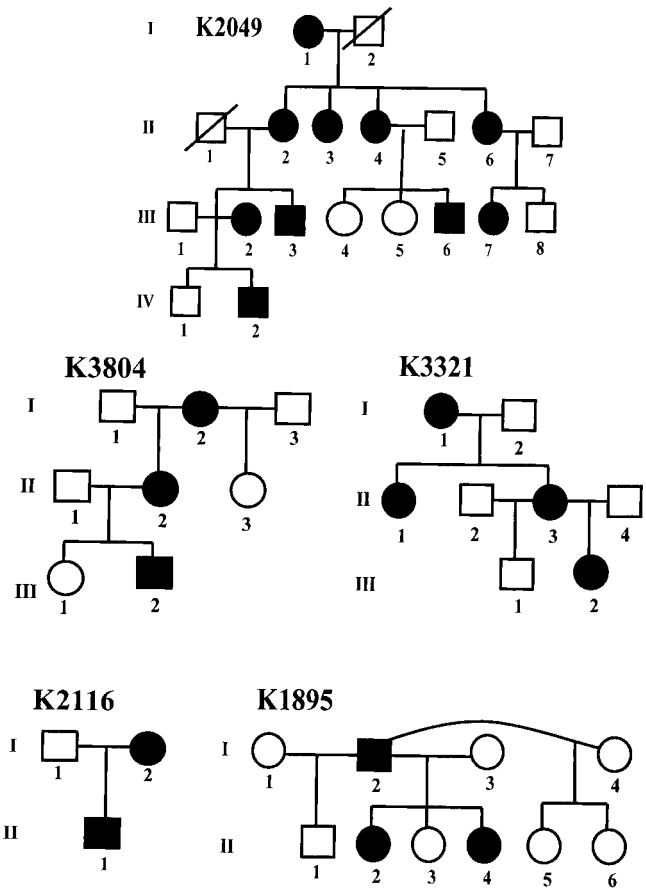

Fig. 1. Pedigrees of the SVAS families with short deletions. K1895 and K2049 have been previously reported [Frangiskakis et al., 1996]. The filled circles/squares represent family members who have deletions of *ELN* and other genes in the WS region.

WS Phenotype

WS, a multi-system disorder that occurs in 1/20,000 births, is distinguishable from other neurodevelopmental disorders by a recognizable pattern of facial features. In the infant and young child these include a broad forehead, bitemporal narrowing, low nasal root, periorbital fullness, stellate/lacy iris pattern, strabismus, bulbous nasal tip, malar flattening, long philtrum, full lips, wide mouth, full cheeks, dental malocclusion with small widely spaced teeth, small jaw, and prominent earlobes. Older children and adults have a more gaunt appearance of the face with a prominent supraorbital ridge, narrow nasal root of normal height, full nasal tip, malar flattening, wide mouth with full lips, small jaw, dental malocclusion, and long neck. WS is associated with an abnormal pattern of growth. The incidence of prenatal growth deficiency is approximately 50–70% [Morris et al., 1988; Pankau et al., 1992]. Feeding problems, prolonged colic, gastroesophageal reflux, and constipation lead to failure to thrive in 80% of infants, and the rate of linear growth is 75% of normal during childhood [Jones and Smith, 1975]. Puberty typically occurs early and is associated with a brief growth spurt [Pankau et al., 1992]; the mean final adult height is below the 3rd centile [Morris et al., 1988; Pankau et al., 1992].

Multiple organ systems are affected in WS. There is an increased incidence of endocrine abnormalities, including hypothyroidism and impaired glucose tolerance [Pober et al., 2002]. Idiopathic hypercalcemia has been most commonly found in the first 18 months and has been documented in 15% of individuals with WS. Hypercalciuria occurs in 30%. Neurologic abnormalities in WS include hypotonia, hyperreflexia, and evidence of cerebellar dysfunction [Morris et al., 1990; Pober and Szekely, 1999]. Reduced cerebral volume but preservation of cerebellar and superior temporal gyrus volumes has been reported in neuroimaging studies [Reiss et al., 2000]. Ophthalmologic findings include esotropia, hyperopia, hypoplasia of the iris stoma, and

reduced stereoacuity [Sadler et al., 1996; Winter et al., 1996]. Dental problems include malformed, small, and/or missing teeth, localized enamel hypoplasia, and increased space between the teeth [Hertzberg et al., 1994]. Malocclusion occurs in 85% of individuals with WS and likely is due to the combined effects of hypotonia, tongue thrust, and connective tissue abnormality. Chronic otitis media and hypersensitivity to sound are common. Gastrointestinal problems in WS include feeding problems, gastroesophageal reflux, constipation in 40%, and colon diverticulosis [Morris et al., 1988, 1990]. Urinary tract abnormalities include renal structural defects in approximately 20%, bladder diverticulae, and nephrocalcinosis [Pober et al., 1993; Pankau et al., 1996].

The connective tissue abnormalities associated with WS include a hoarse/deep voice, hernias, bladder/bowel diverticulae, soft/lax skin, joint laxity or limitation, and cardiovascular disease. The abnormality of elastin results in a diffuse arteriopathy with stenoses occurring throughout the systemic arterial system, especially at origins of brachiocephalic, carotid, and renal arteries. The overall prevalence of cardiovascular disease is 80% and includes SVAS, peripheral pulmonic stenosis (PPS), hypertension, and mitral valve prolapse [Kececioglu et al., 1993]. Musculoskeletal problems may include radioulnar synostosis, kyphosis, lordosis, and scoliosis. Hyperextensibility of joints is common in young children, while joint contractures are more common in older individuals [Kaplan et al., 1989; Morris et al., 1990].

One of the most intriguing aspects of the WS phenotype is the unique cognitive and behavioral profile. Individuals with WS typically have relatively good verbal short-term memory and language, but very poor visuospatial constructive cognition, hallmarks of the Williams Syndrome Cognitive Profile (WSCP). The personality profile includes overfriendliness, empathy, attention deficit disorder, and anxiety.

Genotype–Phenotype Relations

SVAS is inherited in an autosomal dominant fashion [Eisenberg et al., 1964; Schmidt et al., 1989]. It had long been noted that SVAS could occur both as a discrete trait within families and also as part of the extensive symptom complex, i.e., WS [Merritt et al., 1963]. The histologic appearance of the arterial wall in familial versus syndromic SVAS (WS) was identical [O'Connor et al., 1985; Conway et al., 1990]. The etiology of the phenotypic overlap was debated, especially since there were some kindreds who had family members with some features of WS such as hoarse voice and hernias in addition to the cardiovascular disease [Grimm and Wesselhoeft, 1980; Morris and Moore, 1991]. The presence of the rare cardiovascular condition, SVAS, as one component of the complex WS phenotype led to the strategy of performing genetic studies in families with clearly inherited autosomal dominant SVAS. The linkage studies of 10 such kindreds identified *ELN*, which encodes elastin, as a candidate gene for the cardiovascular disease [Ewart et al., 1993a; Olson et al., 1993]. The association was further strengthened by the description of a 6;7 translocation that disrupted ELN in exon 27 and cosegregated with the SVAS phenotype in a four-generation family [Curran et al., 1993; Morris et al., 1993a]. Subsequent molecular studies have demonstrated point mutations of *ELN* in exons 1–28 in SVAS kindreds [Li et al., 1997; Metcalfe et al., 2000]. Other reported *ELN* abnormalities resulting in SVAS included deletion of the 3′ end at exon 27 in one family [Ewart et al., 1994] and a 30 kb intragenic deletion of exons 2 through 27 in an additional kindred [Olson et al., 1995]. These data proved that *ELN* mutations caused SVAS [Gosch and Pankau, 1997]. *ELN* was found to be deleted in 99% of WS cases [Ewart et al., 1993b; Lowery et al., 1995; Nickerson et al., 1995]. Therefore, the cardiovascular disorder can be attributed to functional hemizygosity for *ELN* in both autosomal dominant SVAS kindreds and in individuals with WS.

Congenital cutis laxa can be inherited as an autosomal dominant disorder and is characterized by soft, lax skin that is not hyperextensible or fragile. Craniofacial features may include iris hypoplasia, hoarse/deep voice, and long earlobes [Beighton, 1972]; PPS has been seen occasionally. Frameshift mutations in exons 30 and 32 of *ELN* have been found in individuals with cutis laxa [Tassabehji et al., 1998; Zhang et al., 1999]. Tassabehji and colleagues showed that the mutation in exon 32 led to expression of the mutant allele. The resultant dermal elastic fibers were abnormal in architecture, suggesting a dominant-negative effect. It is interesting to note that some individuals with SVAS, as well as individuals with WS, share phenotypic features with cutis laxa. The major difference is that the skin sags and looks prematurely aged at a younger chronologic age in cutis laxa, as compared with SVAS and WS.

Individuals with SVAS who have point mutations in ELN often have extracardiac connective tissue abnormalities including a few of the WS facial features, a hoarse voice, hernias, and soft skin [Morris and Mervis, 1999], but typically lack other features of WS. In the course of evaluating SVAS kindreds for signs of WS, we identified two families who, in addition to connective tissue abnormality, had a history of difficulty in school. They did not have mental retardation, hypercalcemia, or other findings of WS [Frangiskakis et al., 1996]. We demonstrated that affected members of both kindreds had the WSCP. IQs were in the low average range. Both families had small deletions in the WS region; the smallest deletion affected only two genes: *ELN* and *LIMK1*. We concluded that hemizygosity of *LIMK1* played a role in the difficulties these family members had with visuospatial construction.

There have been four other reports of small deletions in the WS region, but only three individuals were formally assessed for the WSCP. Tassabehji et al. [1999] reported four individuals; none of the three tested had the WSCP, but all had higher intelligence than found in our kindreds; two with above-average IQs. Two brothers with a deletion of *ELN* and *LIMK1* had SVAS and hernias but did not have the WSCP. One man with SVAS had a 170 kb deletion including *ELN* and

LIMK1, but not *STX1A* or *RFC2* and could not be tested for the WSCP. One girl with SVAS had an ~850 kb deletion from the centromeric breakpoint through *RFC2*. She was found to have above average intelligence (IQ of 117, with 100 being the 50th centile for the general population) and normal height and facial features and did not fit the WSCP in standardized testing [Karmiloff-Smith et al., 2003].

Botta et al. [1999] reported two unrelated children who had WS facial features, SVAS, and developmental delay. The 2-year old male had a ~850 kb deletion with the centromeric breakpoint between *STX1A* and *ELN* and the telomeric breakpoint of classic WS. The 6-year-old female had moderate mental retardation; her deletion included *ELN* and extended to the telomeric breakpoint of classic WS.

Two additional reports of short deletions in the WS region described individuals for whom IQ data were not available. Korenberg et al. [2000] reported a girl with SVAS, "mild mental retardation," and some WS facial features who had a deletion that did not include *FZD9* (centromeric) or *GTF2I* (telomeric). Del Campo et al. [2002] reported a family with three individuals affected with SVAS and "borderline intellectual functioning," who had a ~700 kb deletion that included *ELN* (centromeric) through *GTF2IRD1* (telomeric).

MATERIALS AND METHODS

Medical Evaluation

Five families were invited to participate in a genetic study regarding SVAS. A dysmorphologist reviewed medical records and examined all participants. Craniofacial features scored included dolichocephaly, broad brow, periorbital fullness, stellate iris, bitemporal narrowing, low nasal root, flat mala, full cheeks, long philtrum, small jaw, malocclusion, full nasal tip, wide mouth, full lips, prominent ear lobes, and facial asymmetry. The presence and extent of SVAS was determined by two-dimensional echocardiography and Doppler blood-flow analyses as described by Ensing et al. [1989]. Individuals were scored as affected if there was narrowing of the ascending aorta demonstrated on echocardiography or if Doppler peak flow velocities were above normal (normal values for adults: aortic 1.0–1.7 m/sec, pulmonary 0.6–0.09 m/sec; for children: aortic 1.2–1.8 m/sec, pulmonary 0.7–1.1 m/sec). Velocities within 0.2 m/sec greater than the normal range were considered weakly positive. Individuals were also scored as positive if SVAS was documented by medical records of cardiac catherization or surgery.

General Measures of Intellectual Ability

The Kaufman Brief Intelligence Test [K-BIT; Kaufman and Kaufman, 1990] was used to measure general intelligence for individuals ages 4 years and older. The K-BIT includes two subtests: vocabulary, which measures crystallized intelligence (language); and matrices, which measures fluid intelligence (nonverbal reasoning). For the general population, the mean standard score for overall IQ and the two subtests is 100, with a standard deviation of 15. The K-BIT is normed for ages 4–90 years. This measure is particularly appropriate for comparisons of general intellectual ability for studies involving kindreds with small deletions in the WS region because it does not assess visuospatial construction, the hallmark weakness of individuals with WS. Thus, if the primary impact of small deletions in this region is on visuospatial construction rather than on intelligence in general, kindred members with and without deletions would be expected to have similar IQs as assessed by the K-BIT.

The Mental Scale of the Bayley Scales of Infant Development, 2nd edition [Bayley, 1993] was used to measure general intelligence for the only participant who was less than 4 years old (age 15 months). The BSID-II is a full scale measure of intellectual ability and assesses both verbal and nonverbal (including visuospatial construction) abilities. For the general population, the mean standard score is 100, with a standard deviation of 15. The BSID-II is normed for ages 1–42 months.

Receptive Vocabulary Ability

The Peabody Picture Vocabulary Test-Revised [PPVT-R; Dunn and Dunn, 1981] was used to measure receptive vocabulary for participants in kindreds 3321 and 2116. Due to participants' time constraints, this test was not administered to members of kindred 3804. For the general population, the mean standard score is 100, with a standard deviation of 15.

WSCP

The WSCP was measured according to the criteria in Mervis et al. [2000]. The Differential Ability Scales [DAS; Elliott, 1990a] was administered to all participants except the 15-month-old. The DAS is designed to provide specific information about an individual's strengths and weaknesses across a wide range of intellectual activities. Core subtests measure verbal, nonverbal reasoning, and spatial (primarily visuospatial construction) abilities. One of the diagnostic subtests measures verbal short-term memory. The 4- and 5-year-old were given the Preschool level of the DAS; all other participants completed the School-Age level. Standard scores on the DAS subtests are expressed as T scores; for the general population, the mean is 50 with a standard deviation of 10.

An individual is considered to fit the WSCP if he or she meets all four criteria. These criteria are:

1. T score for Recall of Digits, naming/definitions, or similarities > 1st centile;
2. Pattern Construction T score < 20th centile;
3. Pattern Construction T score < mean T score;
4. Pattern Construction T score < Recall of Digits T score.

The youngest participant was too young to complete the DAS. This toddler's fit to the WSCP was measured based on the first edition of the Bayley [1969]. Mervis and Bertrand [1997] and Mervis et al. [1999a] found that on this assessment, toddlers with WS passed a larger

proportion of language items than nonlanguage items in the critical range of items (the 10 items scored before the first item was failed through the last item that was passed). In contrast, all but one of the toddlers with Down syndrome passed a larger portion of nonlanguage items than language items. Most of the nonlanguage items on the Bayley measure visuospatial construction. A toddler is considered to have a profile consistent with the WSCP if he or she passes a larger proportion of language items than nonlanguage items. Definitive assessment of the WSCP requires the child to be old enough for the upper preschool or school age version of the DAS.

Molecular Cytogenetics

Chromosome preparations were made according to standard cytogenetics protocols. Cultures of lymphocytes and lymphoblastoid lines were harvested using colcemid, KCL hypotonic, and 3:1 methanol/acetic acid fixative. The metaphase preparations were spread on microscope slides following usual cytogenetic practices.

To define the size of WS region deletions, fluorescent in situ hybridization (FISH) experiments used both commercial probes for the Williams–Beuren syndrome region (Cytocell, Banbury, UK; Vysis, Downer's Grove, IL) and/or homebrew probes for several loci/subregions in and around the WS classical deletion region.

Probes consisted of cosmids, BACs, and PACs provided by the University of Utah laboratory of Mark Keating (cosA, cosF, BCL, STX, cos30, ELN, LIMK, and cos11) and by Lucy Osborne (209 is cos209c11, 220 is 220e11, 1186 is RP5-1186P10) at the University of Toronto, Toronto, Canada. Prepared DNA was labeled by nick translation (Vysis' Nick Translation Kit) using digoxigenin for indirect-labeled probes or with green or red fluorophores for the direct-labeled probes (Molecular Probes, Eugene OR; or Vysis).

Hybridizations were carried out using 100 ng of labeled probe DNA, cDenhyb2 (Insitus, Albuquerque, NM) as the hybridization solution following the manufacturer's recommended procedures and 0.5 mg of Cot-1 DNA in the probe mixture. The FISH slides were denatured at 70°C in 70% formamide, 2× SSC, pH 7 for 2–3 min. The hybridization reactions ran overnight; slides were subsequently washed at 45°C for 15 min in 50% formamide, 2× SSC, pH 7, then for 8 min in 2× SSC at 37°C.

Detection of indirect-labeled probes was performed with fluorescently-labeled anti-digoxigenin antibodies (Roche Applied Science, Indianapolis, IN) following the manufacturer's recommendations.

Observation and photodocumentation of results were performed on a Zeiss Axioscop equipped for epifluorescence photography with a 100 W mercury lamp and suitable filter sets from Chroma Technology (Brattleboro, VT). Images were captured on Fuji film NPZ exposed at ASA 3200.

Molecular Genetics

DNA was isolated from either whole blood or lymphoblastoid cell lines using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN).

All SNPs were genotyped using the Assays-on-Demand* SNP Genotyping system (Applied Biosystems, Inc., Foster City, CA) and 50 ng of genomic DNA as recommended by the manufacturer. Assays were run on an Applied Biosystems 7700 Genetic Analysis system and genotypes were determined using system software.

RESULTS

Clinical/Medical Characteristics

Craniofacial features and medical problems in the five kindreds are detailed in Table I. Although all families were ascertained due to SVAS, there were some individuals with deletions (14%) who did not have detectable cardiovascular disease. (A parallel finding has been reported by Li et al. [1997] for SVAS families with ELN point mutations.) In addition to the cardiovascular disease documented in 86% of affected individuals, other connective tissue abnormalities were hoarse voice in 50%, mitral valve prolapse in 10%, hernia in 32%, spine abnormality (kyphosis, lordosis, or scoliosis) in 18%, and joint laxity or contractures in 23%. In addition, 14% had strabismus. Other lower-frequency WS features were each found in a single different person in the study; these were small widely spaced teeth, an extra sacral crease, limitation of supination at the elbow, a pelvic kidney, and diverticulosis. All family members had normal growth parameters. In each kindred, calcium studies had been done in the youngest affected member during infancy, and all were normal. One unexpected finding was a history of a seizure disorder in 27% of affected individuals. Seizures have been reported in WS, but are not especially common. In kindred 2049, siblings III-2 and III-3 were treated for seizures in childhood and the disorder persists in III-2. Seizures were treated in IV-2 for 2 years as a toddler, then resolved. In kindred 2116, II-1 had onset of petit mal seizures in childhood; his unaffected father had an unspecified seizure disorder. In kindred 3321, both I-1 and II-1 had late onset of a seizure disorder (after age 40).

The facial features of WS found in these kindreds are recorded in Table I and are shown in Figures 2–6. A long philtrum was the most common WS feature seen in these families, with a frequency of 41%. Young children had more facial features than adults, but no child had more than six. In classic WS, the number of WS facial features is $\geq$9. In the review of childhood photos of the participants of 4/5 kindreds, many had some WS features such as full cheeks and periorbital fullness as a child, but these were not evident when examined as adults. In the remaining kindred, 2116, the mother had only one WS facial feature (periorbital fullness); her son never had any significant WS facial features. In kindreds 2049, 1895, 3804, and 3321, one family member had been previously diagnosed with WS due to the presence of SVAS, plus some WS facial features in infancy, only to have the diagnosis questioned later, when the face did not clearly resemble WS. In kindred 2116, the son was initially diagnosed with WS, but the diagnosis was questioned due to the absence

TABLE I. Clinical Features of Individuals With Deletions in All Five Kindreds

Kindred	K2049										K1895			K3804			K2116		K3321			
Craniofacial features	I-1	II-2	II-3	II-4	II-6	III-2	III-3	III-6	III-7	IV-2	I-2	II-2	II-4	I-2	II-2	III-2	I-1	II-1	I-1	II-1	II-3	III-2
Dolichocephaly	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Broad brow	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Periorbital fullness	+	+	−	−	−	−	−	−	−	+	−	+	−	−	−	+	+	−	−	−	−	−
Stellate iris	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bitemporal narrowing	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−
Low nasal root	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Flat mala	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−
Full checks	−	−	−	−	+	−	−	−	−	+	−	+	+	+	+	+	−	−	−	−	−	+
Long philtrum	+	+	−	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	+
Small jaw	−	−	−	−	+	−	−	−	−	+	−	+	−	+	−	+	−	−	−	−	−	−
Malocclusion	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−
Full nasal tip	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Wide mouth	+	−	+	−	+	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−
Full lips	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Prominent ear lobes	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Facial asymmetry	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Other																						
Strabismus	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−
Hoarse voice	+	+	+	−	+	−	+	−	−	+	+	+	−	−	−	+	−	−	+	+	−	−
SVAS	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
MVP	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−
Hernia	−	−	U	−	−	−	−	−	−	−	I	−	−	−	−	I	U	−	−	I	I	I
Diverticulosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Seizure	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	+	+	+	−	−
Spine abnorm.	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+
Joint abnorm.	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	+	−	−	−	−

SVAS, supravalvar aortic stenosis; MVP, mitral valve prolapse; U, umbilical hernia; I, inguinal hernia.

Fig. 2. Kindred 2049: Shown are eight of the affected family members. **Top** row (L–R): I-1, II-2, II-3, II-4. **Bottom** row (L–R): III-7, III-3, III-2, IV-2.

of WS facial features. In addition to WS facial features seen in infants, in kindreds 3804 and 2049, women over 40 years had some WS features. (There were no affected males over 40 years in the study.) The change in facial features over time is illustrated in Figure 7.

Standardized Assessments

Performance of kindred members on the standardized assessments is presented in Table II. K-BIT IQ was compared for affected and unaffected members of kindreds 3804 and 3321. (No unaffected members of kindred 2116 were available for cognitive testing.) As expected, mean IQ was similar for unaffected (97.0) and affected (92.33) members ($t = -0.73$, $P = 0.48$). Comparisons of T scores for the DAS Recall of Digits subtest also indicated similar levels of performance for affected ($T = 46.5$) and unaffected (39.6) members ($t = 1.39$, $P = 0.20$). PPVT-R standard scores were compared for unaffected and affected members of K3804; mean scores were almost identical for affected (88.75) and unaffected (88.67) members. Thus, unaffected and affected kindred members performed at about the same levels for IQ (on a measure that does not include visuospatial construction), receptive vocabulary, and verbal short-term memory.

Fig. 3. Kindred 1895: **Top** row (L–R): II-2 (affected), I-2 (affected). **Bottom** row (L–R): II-3 (unaffected), II-4 (affected).

In contrast, a comparison of affected and unaffected members of kindreds 3804 and 3321 on T scores for the DAS Pattern Construction subtest indicated that performance was significantly worse for affected ($T = 31.83$) than unaffected ($T = 43.0$) individuals ($t = -3.60$, $P = 0.006$). Furthermore, a comparison across all three kindreds of the difference between a participant's Pattern Construction and Recall of Digits T scores indicated a significant difference between affected (-14.75) and unaffected ($+3.40$) members ($t = -5.79$, $P < 0.001$). All of the affected members earned a higher score on the Recall of Digits subtest than on the Pattern Construction subtest; in contrast, all but one of the unaffected members earned a higher score on the Pattern Construction subtest. There was no overlap between the difference scores of the affected and unaffected groups. According to the DAS Handbook [Elliott, 1990b], a T score difference of 9 points indicates a significant difference for an individual in his or her Pattern Construction and verbal short-term memory abilities. By this criterion, all but one of the affected participants had significantly stronger verbal short-term memory ability than Pattern Construction ability. None of the unaffected participants demonstrated a significant difference in these two abilities.

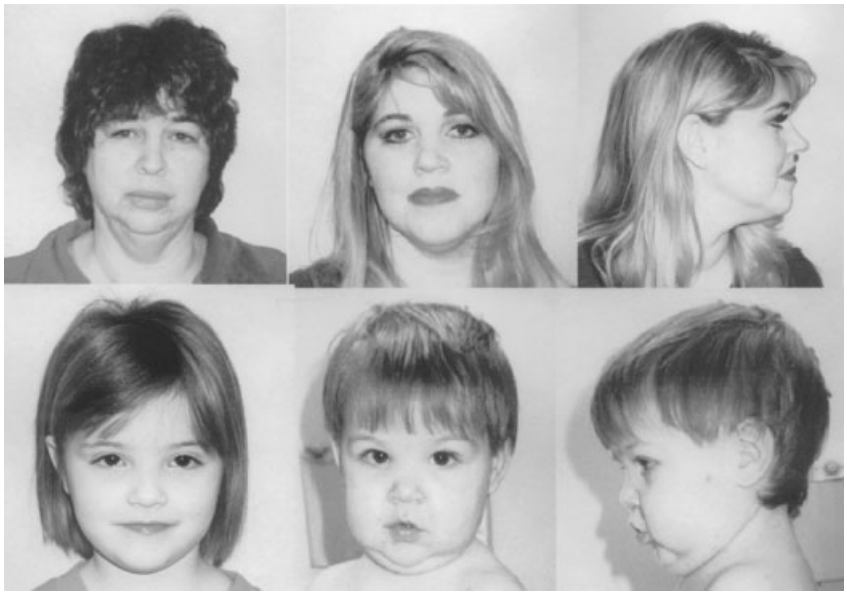

Fig. 4. Kindred 3804: **Top** row (L–R), I-2 (affected), II-2 (affected), II-2 profile. **Bottom** row: III-1 (unaffected), III-2 (affected), III-2 profile.

WSCP

To determine if participants' pattern of intellectual strengths and weaknesses fit the WSCP, individuals' performance on the six core DAS subtests and the Recall of Digits diagnostic subtest was compared to the four criteria in Mervis et al. [2000]. An individual was considered to fit the WSCP if he or she met all four criteria. Individuals who met three or fewer criteria were considered not to fit the WSCP. The fit of each participant to each of the four criteria as well as his or her overall fit to the WSCP are indicated in Table III. As expected, all kindred members fit WSCP criteria 1, since none of the kindred members has mental retardation. However, each of the unaffected members did not meet one or more of the remaining WSCP criteria; none of the unaffected members fit the WSCP. In contrast, all of the affected members met all four criteria and therefore fit the WSCP.

Fig. 5. Kindred 2116 affected individuals: I-2, II-1.

Genotype

While high resolution chromosome studies were normal, FISH analysis of the WS region showed deletions in all five kindreds (Fig. 8). The probes used to define the sizes of the deletions, from centromere to telomere end, were A, F, BCL, STX, Cos30, ELN, LIMK,

Fig. 6. Kindred 3321 affected individuals: **Top** row: I-1, II-1. **Bottom** row: II-3, III-2.

Fig. 7. Facial features over time in a boy with SVAS (K2049, IV-2), ages, **top** row (L–R), 2 months, 9 months, 2 years. **Bottom** row (L–R), 4 years, 4 years profile, 10 years. Note that in the two youngest pictures, the face is most similar to Williams syndrome (WS).

209, 220, 1186, and 11. The FISH results for the five kindreds are shown in Table IV. As shown in Figure 9, the small deletions in these five families nearly span the classic WS deletion region, which includes ~1.6 Mb from *FKBP6* through *GTF2I*. Kindred 2049 has the smallest deletion of 83.6 kb that includes the telomeric portion of *ELN* and all of *LIMK1* [Frangiskakis et al., 1996]. Kindred 1895 is not deleted for markers from *STX1A* or *CYLN2*, but markers from *ELN*, *LIMK1*, and *WBSCR5* are deleted, for a deletion size of ~350 kb. Kindred 3804 has a deletion of ~600 kb that includes *STX1A* through *LIMK1*, while 2116 has a larger deletion of ~850 kb which includes *BCL7B* through *WBSCR5*. Kindred 3321 has the only small deletion (~750 kb) extending significantly telomeric from *ELN* and *LIMK1*, encompassing *GTF2IRD1* but not *GTF2I*. SNP analysis was informative for more precisely defining the telomeric breakpoint region in kindreds 3804 and 2116 and the centromeric breakpoint in K2116 (Fig. 9).

DISCUSSION

WS usually occurs sporadically, though familial cases including male to male transmission have been reported [Morris et al., 1993b; Sadler et al., 1993]. The commonly deleted region spans ~1.6 Mb, and 95% of WS individuals studied have the same deletion breakpoints [Meng et al., 1998; Morris et al., 1999a,b; Osborne, 1999; Bayes et al., 2003]. Maternal and paternal origins of deletions occur with equal frequency [Ewart et al., 1993b; Urban et al., 1996] and there is no proven parent of origin effect [Wu et al., 1998]. The sporadic deletion is the result of unequal crossing over between grandparental chromosomes [Dutly and Schinzel, 1996; Perez Jurado et al.,

TABLE II. Deletion Status, Fit to WSCP, and Performance on Standardized Assessments in Kindreds 3804, 2116, and 3321

Kindred	Patient	CA	DEL?	WSCP?	KBIT IQ	DAS Pattern Construction	DAS Recall of Digits	PPVT-R
3804	I-2	45	Yes	Yes	94	T = 28	T = 55	—
	II-2	25	Yes	Yes	100	T = 34	T = 48	—
	II-3	19	No	No	107	T = 42	T = 39	—
	III-1	5	No	No	109	T = 50	T = 43	—
	III-2	15 mo	Yes	Yes	100 BSID II	Cannot stack blocks		—
2116	I-1	32	Yes	Yes	97	T = 41	T = 58	94
	II-1	13	Yes	Yes	109	T = 37	T = 50	112
3321	I-1	53	Yes	Yes	101	T = 31	T = 41	106
	I-2	53	No	No	95	T = 38	T = 32	86
	II-1	31	Yes	Yes	94	T = 38	T = 58	88
	II-3	25	Yes	Yes	91	T = 36	T = 46	86
	II-4	26	No	No	81	T = 39	T = 37	84
	II-1	6	No	No	93	T = 46	T = 47	106
	III-2	4	Yes	Yes	74	T = 24	T = 31	75

TABLE III. Fit to WSCP: Overall* and Individual Criteria in Kindreds 3804, 2116, and 3321

Kindred	Patient	Del?	Overall	WSCP 1	WSCP 2	WSCP 3	WSCP 4
3804	I-2	+	+	+	+	+	+
	II-2	+	+	+	+	+	+
	II-3	−	−	+	−	+	+
	III-1	−	−	+	−	−	−
	III-2	+	+[a]				
2116	I-2	+	+	+	+	+	+
	II-1	+	+	+	+	+	+
3321	I-1	+	+	+	+	+	+
	I-2	−	−	+	+	+	−
	II-1	+	+	+	+	+	+
	II-2	+	+	+	+	+	+
	II-3	−	−	+	+	−	−
	III-1	−	−	+	−	+	+
	III-2	+	+	+	+	+	+

*Requires fit ("+") to all four of the individual WSCP criteria.
[a]Based on comparison of proportions of verbal and nonverbal items passed on the Bayley [1969].

1996; Osborne et al., 1997]. This region of chromosome 7 is predisposed to the meiotic error due to the presence of a large number of repetitive sequences. The repetitive sequences flanking the commonly deleted region are estimated to be 300–400 kb in size [Francke, 1999; Osborne, 1999] and include both functional and nonfunctional copies of duplicated genes *GTF2I*, *NCF1*, *STAG3*, *POM121*, *FKBP6*, and *WBSCR20* and clusters of *PMS2*-like genes. Osborne et al. [2001] have demonstrated that inversions also occur in this region; in 4 of 12 individuals with WS, a parent had an inversion. Bayes et al. [2003] demonstrated that when inversions are present, the breakpoints are outside the WS deletion region.

The possible relation of the genes in the region to specific aspects of the WS phenotype is unknown for the majority of the genes, though the importance of the ELN deletion to the phenotype is well established. The families reported here all have connective tissue abnormalities as part of the phenotype. These features, such as the hernias, are likely the result of *ELN* haploinsufficiency. None of the affected family members had classic WS face ($\geq$9 of scored facial features), however, all but one showed some facial features at some point in their lifespan. Interestingly, the greatest number of WS features was noted in young infants, and an increase also was noted in women >40 years. (No males >40 years were available for evaluation.) These particular WS features, especially the periorbital fullness and full cheeks, may also be related to differences in elastic fibers. After birth, dermal elastin protein production is highest in the first year of life, with little subsequent synthesis. With aging, elastic fibers degenerate. As compared to SVAS families, the differences noted in the WS face after infancy could be due to the craniofacial effects of another gene deleted in the region or could be the result of persistent hypotonia in WS. Thus, *ELN* abnormality is responsible for some WS facial features, but is not sufficient for the classic WS facial phenotype.

Relative to unaffected members of their kindreds, individuals from kindreds 3804 and 3321 with small deletions in the WS region showed a specific deficit in visuospatial construction, performing significantly worse on the DAS Pattern Construction subtest than unaffected members. However, affected individuals did not show a global deficit in intelligence or specific difficulties with either receptive vocabulary or verbal

TABLE IV. FISH Results for Kindred With Deletions in the WS Region of 7q11.23

	Probes										
Kindreds	A	F	BCL	STX	30	ELN	LIMK	209	220	1186	11
K2049	+	+	+	+	+	−	−	+	+	+	+
K1895	+	+	+	+	−	−	−	+	+	ND	+
K3804	+	+	+	−	−	−	−	+	+	+	+
K2116	+	−	−	−	−	−	−	+	+	+	+
K3321	+	+	+	+	+	−	−	−	−	+	+
Classic WS	+	−/dim	−	−	−	−	−	−	−	−	+

The probes used to define the size of the deletion are, from centromeric end to telomeric end: A, F, BCL, STX, 30, ELN, LIMK, 209, 220, 1186, and 11. Results of metaphase FISH experiments in five kindreds with deletions in the WS region as compared to classic, sporadic WS. +, two FISH probe signals present; −, only one FISH probe signal present, consistent with a deletion; −/dim, one strong probe signal present plus either one absent or one diminished probe signal. ND, not done. Probes F and 1186, respectively represent the centromeric and telomeric extremes of the 'classic' Williams syndrome (WS) region deletion region. Probes A and 11 flank the classic deletion region. In individuals with classic WS, there are two possible results relative to probe F. On the deleted chromosome 7, the probe F signal is either absent or obviously diminished compared to the normal signal.

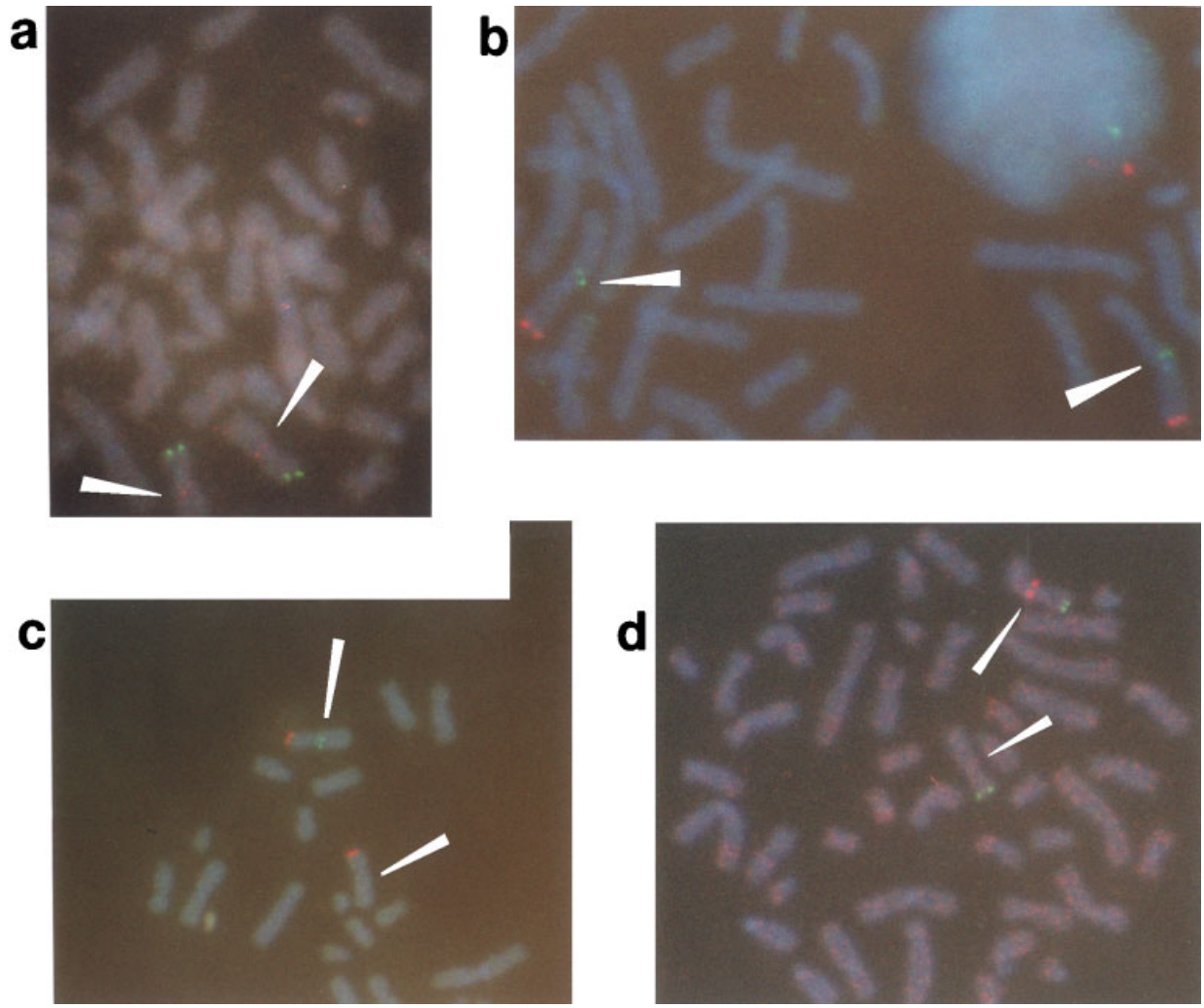

Fig. 8. **A**–**D**: FISH results demonstrating a deletion extending centromeric to the *ELN* locus (K3804) and a deletion extending telomeric to the *ELN* locus (K3321). The normal, no-deletion result (A, B) shows two locator probes (telomeres of chromosome 7) and two signals (arrows) for the probe for the proximal (more centromeric) locus *STX1A* or for the distal probe 209. The deleted result (C, D) shows the locator probes and the arrows indicate either one STX or 209 signal on one chromosome 7 and no signal on the homologue. A, normal, STX probe in K3321; B, normal 209 probe in K3804; C, one deleted, STX probe in K3804; D, one deleted 209 probe in K3321.

short-term memory. When intelligence was tested using a measure (K-BIT) that assessed verbal ability and nonverbal reasoning ability but did not include visuospatial construction, affected and unaffected kindred members obtained similar IQs. Performance on measures of receptive vocabulary (PPVT-R) and verbal short-term memory (DAS Recall of Digits) also was similar for affected and unaffected members. In kindred 2116, we were unable to compare affected individuals to unaffected because no unaffected members were available for testing. Testing did confirm that for both affected individuals in this kindred, performance on DAS Pattern Construction was significantly lower than performance on DAS verbal short-term memory, however. The same pattern of intellectual similarities and dissimilarities between affected and unaffected members shown in kindreds 3804 and 3321 was previously reported for kindreds 1895 and 2049 [Frangiskakis et al., 1996]. In contrast, even the highest functioning individuals who have the classic WS deletion obtain significantly lower IQs than unaffected relatives on the K-BIT (Mervis, unpublished data).

Results presented in this article indicate that all affected members of kindreds 3804, 3321, and 2116, but none of the unaffected relatives tested, fit the WSCP as defined by Mervis et al. [2000]. Data presented in Frangiskakis et al. [1996] demonstrated that all affected members of kindred 1895 and most affected members of kindred 2049 also fit the WSCP. No unaffected members fit the WSCP. Thus, affected individuals show the classic WS pattern of intellectual strengths and weaknesses, even though their performance on the cognitive assessments are significantly and substantially higher than those reported for individuals with classic WS deletions. Mean K-BIT IQ for affected members of kindreds 3804, 3321, and 2116 was 27 points higher than the mean

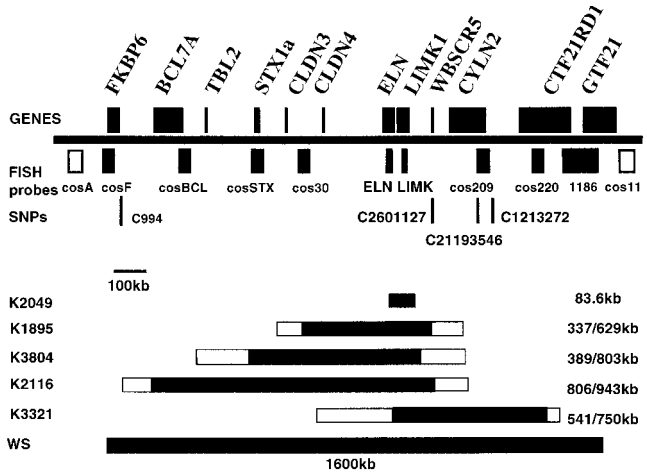

Fig. 9. Deletions present in short deletion families. The WS region is shown with several genes above the line, the classic deletion is represented as the solid bar at the bottom of the diagram. FISH probes used are represented below the line. Of 20 SNPs genotyped across the region, 3 were informative in refining the telomeric deletion end point, and 1 was informative in refining the centromeric end point. The nomenclature for the SNPs is the assay ID no. For each pedigree the minimal deletion is shown as a filled bar. The open bars represent the location of the deletion breakpoint. Approximate minimum and maximum size of deletion is presented to the right of each kindred diagram. All five pedigrees show deletion of all or part of ELN and LIMK1. The location and size of the genes are represented approximately to scale. Scale bar = 100 kb.

reported by Mervis et al. [2003] for a sample of 250 individuals with WS classic deletions. Similarly, mean T score for DAS Pattern Construction was 8 points higher and mean T score for DAS Recall of Digits was 10 points higher [Mervis et al., 2003]. Mean PPVT-R standard score was 26 points higher for affected kindred members than for 127 individuals with classic WS deletions [Mervis et al., 1999a].

Because all five of these kindreds have affected members with the WSCP, and because all five share a *LIMK1* deletion, these data suggest that *LIMK1* is important in normal visuospatial constructive cognition. It is important to note, however, that visuospatial construction ability is a heritable trait as shown by twin and adoption studies, and varies in the general population [Mervis et al., 1999b]. In WS, the variability is found at the lower end of the distribution, and the mean level of visuospatial constructive ability is lower than expected based upon overall cognitive ability. Those individuals with WS with the highest overall intelligence tend to perform best on the tests of visuospatial construction, since they are able to use verbal compensatory strategies including talking their way through the construction task, that take advantage of their relatively good verbal and nonverbal reasoning abilities and good verbal working memory. It is likely that visuospatial construction follows a quantitative trait loci model, in which multiple genes contribute individually to the normal distribution [Mervis et al., 1999b]. Based upon our clinical studies, *LIMK1* is likely to be one of those genes. Expression studies provide additional supportive evidence. *LIMK1* encodes a protein kinase that is strongly expressed in the developing brain, especially the cerebral cortex [Frangiskakis et al., 1996]. The protein interacts with the transmembrane receptor neuregulin [Wang et al., 1998] and also phosphorylates cofilin, a regulator of the actin cytoskeleton important in cell movement and axonal growth of neurons [Arber et al., 1998; Yang et al., 1998].

Across the 5 kindreds, only 2 of 21 affected individuals did not have the WSCP. The three individuals studied by Tassabehji et al. [1999] also did not have the WSCP. It is possible that this simply represents variable expression. Alternatively, it is likely that these individuals successfully utilized verbal compensation strategies to solve the Pattern Construction problems [Mervis et al., 1999b]. This is especially likely for the high-IQ child studied by Karmiloff-Smith et al. [2003].

Although the deletions in the five families that we have studied nearly span the WS deletion region, none is deleted for the most telomeric gene (*GTF2I*) or the most centromeric gene (*FKBP6*). None of the family members has mental retardation; mean IQ is almost two standard deviations above the mean for classic WS, and all affected members have similar IQ's to nonaffected family members when visuospatial skills are excluded. This pattern suggests that genes in the breakpoint regions of the classic deletion are likely to be involved in the mental retardation in WS.

The previous case reports that are most relevant to this possibility are those by Botta et al. [1999] and Karmiloff-Smith et al. [2003]. The child reported by Botta et al. [1999] as having moderate mental retardation (IQ of 48) was deleted for *GTF2I*. In contrast, the girl with an IQ of 117 reported by Karmiloff-Smith et al. [2003] did not have a deletion of *GTF2I*, but did have a deletion of *FKBP6*, suggesting that *FKBP6* deletion does not cause mental retardation. Thus, evidence from deletion studies, including the current report, supports a role for *GTF2I* in mental retardation in WS. *GTF2I* is expressed in both fetal and adult tissues, most notably in the brain. This gene encodes two proteins: BAP-135, which is a target for Bruton's tyrosine kinase, and TFII-I. TFII-I is a transcription factor that shuttles between the cell nucleus and the cytoplasm, activating other genes [Perez Jurado et al., 1998]. This protein has recently been found to have a role in transcriptional repression as well [Hakimi et al., 2003].

Lumping and Splitting

Abnormality of the *ELN* gene is the etiology of three different, well-described phenotypes and accounts for some overlap in clinical features of these conditions [Morris and Mervis, 2000]. Autosomal dominant cutis laxa, associated with mutations in *ELN* exons 30 and 32, has clinical findings primarily in the skin. The phenotype of lax, loose skin occurs as a result of a dominant-negative effect. Autosomal dominant SVAS is clinically important as an arteriopathy, though other connective tissues are affected as manifested by the common signs and symptoms of hernia, soft skin, and hoarse voice. WS is a contiguous gene syndrome that includes SVAS and other connective tissue abnormalities related to hemizygosity of ELN, but also is well known for additional important phenotypic features including the WSCP,

infantile hypercalcemia, mild mental retardation, and a unique personality. Within these three clinical diagnoses, there is a considerable variability. Some individuals with SVAS have trivial cardiovascular findings, whereas others die in infancy with bilateral outflow tract obstruction. Some individuals with WS have normal intelligence, but most have mental retardation ranging from severe to mild.

So where do the families described here fit? Clinically, are they best described as phenotypic variants of SVAS or of WS? We have shown that the families we have studied with short deletions do have significant weakness in visuospatial construction relative to unaffected kindred members. Furthermore, affected members fit the WSCP. However, none of the affected members has mental retardation, and mean IQ as well as mean standard scores for receptive vocabulary, verbal short-term, memory, and visuospatial construction are considerably higher than for WS. In further contrast to WS, mean K-BIT IQ of affected kindred members is similar to that of unaffected members; the same pattern holds for receptive vocabulary and for verbal short-term memory. Some affected family members have WS facial features, but the gestalt changes over time, and none has the classic WS face.

Clinically, the families we report with the short deletions in the WS region are distinguishable from other SVAS families with point deletions only by the difficulty with visuospatial construction. When the first two families were evaluated over 10 years ago, we were struck by the similarities to WS. However, having had the opportunity to follow the families over time, it is our opinion that these families are best thought of as variants of the SVAS phenotype, rather than "partial" WS. One of the reasons for our position concerns the natural history of SVAS versus WS. If one accepts the argument that an important reason for making a diagnosis is to provide anticipatory guidance, then SVAS is more appropriate, because these individuals have a natural history and prognosis more similar to other SVAS kindreds than to classic WS.

The problem of nomenclature in genotype–phenotype conflicts has been discussed by Biesecker [1998], who suggested a diagnostic coding system that takes both genotype and phenotype into account. For example, an individual with isolated SVAS could have a diagnosis "SVAS (MIM:18550), *ELN* 1821 del C." A person with cutis laxa could have the diagnosis designated as "Autosomal Dominant Cutis Laxa (MIM:123700), *ELN* 2039 del C". A person with classic WS would be coded "WS (MIM:194050) del 7q11.23, *FKBP6* through *GTF2I*." The kindreds described here also could be classified using this method. For example, an affected individual from kindred 3804 could have the diagnosis termed "SVAS (MIM:18550) del 7q11.23, *STX1A* through *RFC2*." This scheme may be useful as investigators continue to explore genotype/phenotype relationships in WS.

CONCLUSION

Extensive morphologic, cardiac, psychological, cytogenetic, and molecular genetic evaluations of five families with varying partial deletions of the WS region on chromosome 7 have provided evidence for genotype–phenotype associations. *ELN* mutation, previously shown to cause the characteristic arteriopathy of both SVAS and WS, is also associated with some of the WS facial features, especially periorbital fullness, full cheeks, and long philtrum. In SVAS families, these features are most likely to be detected in infancy and after the fourth decade. However, *ELN* hemizygosity alone does not account for classic WS craniofacial characteristics. Presence of visuospatial construction deficits in three additional SVAS families with small deletions including *LIMK1* supports the role of that gene in the development of normal visuospatial constructive cognition. Hemizygosity of *LIMK1* in WS contributes to the impaired visuospatial constructive cognition, resulting in the WSCP. *GTF2I* deletion is likely associated with the mental retardation found in 75% of individuals with WS. Since individuals with WS who have normal intelligence have lower IQ's than their families, it is likely that *GTF2I* is negatively impacting their intelligence as well. All five families reported here have normal intelligence. The five deletions nearly span the WS region; only *FKBP6* (centromeric) and *GTF2I* (telomeric) are not included in any of the deletions. While either of these genes may be related to the mental retardation in WS based upon our current study, our results combined with previously reported findings strongly implicate *GTF2I*.

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