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A Variant of Freeman-Sheldon Syndrome Maps to 11p15.5-pter

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Summary

Distal arthrogryposis type 1 (DA1) and Freeman-Sheldon syndrome (FSS) are the two most common known causes of inherited multiple congenital contractures. We recently have characterized a new disorder (DA2B) with a phenotype intermediate between DA1 and FSS. We report the mapping of a gene that causes DA2B to chromosome 11p15.5-pter. Linkage analysis in a single kindred generated a positive LOD score of 5.31 at $\theta = 0$ with the marker D11S922, and recombinants localize the gene to an ~3.5–6.5-cM region between the marker TH and the telomere. Analysis of additional families improves the LOD score to 6.45 at $\theta = 0$ and suggests linkage homogeneity for DA2B.

Introduction

Clubfoot, calcaneovalgus deformities, and dislocated hips are the most common reported types of congenital contractures (Hall et al. 1982). The combined prevalence of all causes of congenital contractures is 0.5%–1.0% (Hall 1985), and a child with more than a single congenital contracture is found in 1/3,000 births (Hall 1992). Despite the relatively moderate degree of pediatric morbidity associated with these contractures, the functional impairment and the associated early-onset osteoarthritis can lead to substantial residual disabilities in teenagers and adults. The occurrence of congenital contractures is usually sporadic, although autosomal dominant inheritance in some kindreds is well documented (Hall et al. 1982; Bamshad et al. 1996*a*). Such families can be utilized to identify genes associated with inherited congenital contractures (Tsipouras et al. 1992; Bamshad et al. 1994; Putnam et al. 1995). This eventually may help us to understand the pathogenesis of sporadic positional limb deformities.

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The distal arthrogryposes (DAs) are inherited primary limb malformation disorders characterized by congenital contractures of two or more different body areas and are without primary neurological and/or muscle disease that affects limb function. Features common to DAs include a consistent pattern of distal joint involvement, limited proximal joint involvement, an autosomal dominant inheritance pattern, reduced penetrance, and variable expressivity. We recently revised and extended Hall et al.'s (1982) classification of the DAs (Bamshad et al. 1996*b*) (table 1). We suggested that Freeman-Sheldon syndrome (FSS; OMIM 193700) should be considered not only a DA but also, in comparison with other DA disorders, most closely related to distal arthrogryposis type 1 (DA1; OMIM 108120). In fact, individuals with DA1 and FSS may have such similar limb phenotypes that they only can be distinguished by differences in their facial morphology. Moreover, an extended family in which different individuals were diagnosed with DA1 or FSS has been reported (Klemp and Hall 1995). For these reasons, in the revised classification of DAs, we categorized FSS as DA2 and suggested that distinct disorders with phenotypes overlapping DA1 and FSS were likely to exist.

Recently, we had the opportunity to evaluate a large, extended kindred in which individuals had been diagnosed with either DA1 or FSS (fig. 1; family M). Twenty-one individuals in five generations of this family are affected. Characteristics of affected individuals (fig. 2) include a triangular face, downslanting palpebral fissures, attached ear lobules, prominent nasolabial folds, a small mouth, small mandible, arched palate, cervical webbing, short stature, severe camptodactyly, ulnar deviation, and positional foot deformities (calcaneovalgus and/or clubfoot). Although the limb phenotype is very similar to that of individuals with DA1 and FSS, the facial features are uncommon findings in patients with DA1. Despite the prominent nasolabial folds, downslanting palpebral fissures, and small mouth of affected individuals in family M, the shape of the mouth and chin is different from that of people with FSS. Moreover, no members of family M had feeding difficulties at birth or underwent surgical revision of the mouth, which are nearly universal features of children with FSS (Carey et al. 1993). Thus, the phenotype of most individuals in family M was intermediate between DA1 and FSS, and,

Table 1

Extended Classification of the DAs

Syndrome	Label	OMIM Number	Distinguishing Feature(s)[a]
DA type 1A	DA1	108120	...
FSS	DA2	193700	Small, puckered mouth; H-shaped groove in chin
DA type 2B	DA2B	...	Small mouth and chin; triangular face
Gordon syndrome	DA3	114300	Cleft palate; short stature
DA type 4	DA4	...	Scoliosis
DA type 5	DA5	108145	Ophthalmoplegia; ptosis
DA type 6	DA6	...	Sensorineural hearing loss
DA type 7	DA7	158300	Trismus; camptodactyly with dorsiflexion
Multiple pterygium (dominant)	DA8	178110	Pterygia
Congenital contractural arachnodactyly	DA9	121050	Marfanoid habitus; crumpled ear

[a] Positional foot deformities, camptodactyly, and ulnar deviation are features common to all of these disorders.

consequently, we classified this disorder as DA2B. DA2B appears similar to the DA variant described by Moore and Weaver (1989) as a new form of DA, to the disorder described by Kawira and Bender (1985), and to the second case of FSS reported by Freeman and Sheldon (1938).

Individuals in three additional multigenerational families who had been diagnosed previously with "variants" of DA1 or FSS but who had phenotypes similar to DA2B also were evaluated (families L, S, and T; fig. 1). A fourth family, in which one individual had been diagnosed with DA1 and another with FSS, also was studied (family ST; fig. 1).

Subjects, Material, and Methods

Clinical Status of Subjects

All studies were performed with the approval of the Institutional Review Board of the University of Utah and the General Counsel of the Shriners Hospitals for Children. After informed consent was obtained, living members of a single, large kindred of northern-European descent were evaluated by review of their medical history, completion of a questionnaire, and physical examination. An individual was diagnosed as affected on the basis of the presence of two or more of the major clinical manifestations of a DA (Bamshad et al. 1996*b*) and two of the minor features of DA2B. Major diagnostic criteria of the upper limbs included ulnar deviation, camptodactyly, hypoplastic and/or absent flexion creases, and/or overriding fingers at birth. Major diagnostic criteria of the lower limbs included talipes equinovarus, calcaneovalgus deformities, a vertical talus, and/or metatarsus varus. Minor diagnostic criteria included a triangular face, downslanting palpebral fissures, attached ear lobules, a small mouth, a small mandible, an arched palate, cervical webbing, and short stature. Exclusion criteria included primary neurological and muscle abnormalities. Fifteen of the affected individuals in family M were examined by two of the authors (M.B. and J.F.B.), and all individuals in families L and S were examined by M.B. Four affected individuals in family M were deceased, and two individuals were not available for physical examination. Hence, the diagnostic status of these individuals was determined by review of photographs and medical histories. Individuals V-3 and V-4 of family M were diagnosed prenatally via serial ultrasound examinations.

Ascertainment of Genotype

Fifteen milliliters of blood were obtained from all affected individuals and at least their first-degree relatives. Blood was obtained from the umbilical cord when possible. Genomic DNA was prepared from lymphocytes and from cell lines derived from Epstein-Barr virus–transformed lymphocytes as described elsewhere (Bell et al. 1981; Anderson and Gusella 1984). Primers were end-labeled using γ-^{32}P-ATP and polynucleotide kinase. One picomole of end-labeled primer was added to the PCR mix. Genomic DNA sequences were amplified in 1 × buffer (10 mM Tris, pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$) with 25 ng of template genomic DNA, 50 μM dNTPs, 20 pmol of each primer, and 1 unit of *Taq* DNA polymerase in a total reaction volume of 25 μl. Samples were cycled 30 times in a Perkin-Elmer 9600 PCR machine by use of standard profiles. The annealing temperature was decreased 4°C on the sixth cycle. A 20-μl portion of 50% formamide loading dye was added to each reaction after PCR. Samples were denatured for 3 min at 94°C and then electrophoresed through 5% denaturing polyacrylamide gels. Bands were visualized by autoradiography. All experiments with markers defining the DA/FSS haplotype were confirmed by repetition. To evaluate the zygosity of the twins in family M (V-3 and V-4; fig. 1), these samples were genotyped for eight tetranucleotide loci located on separate chromo-

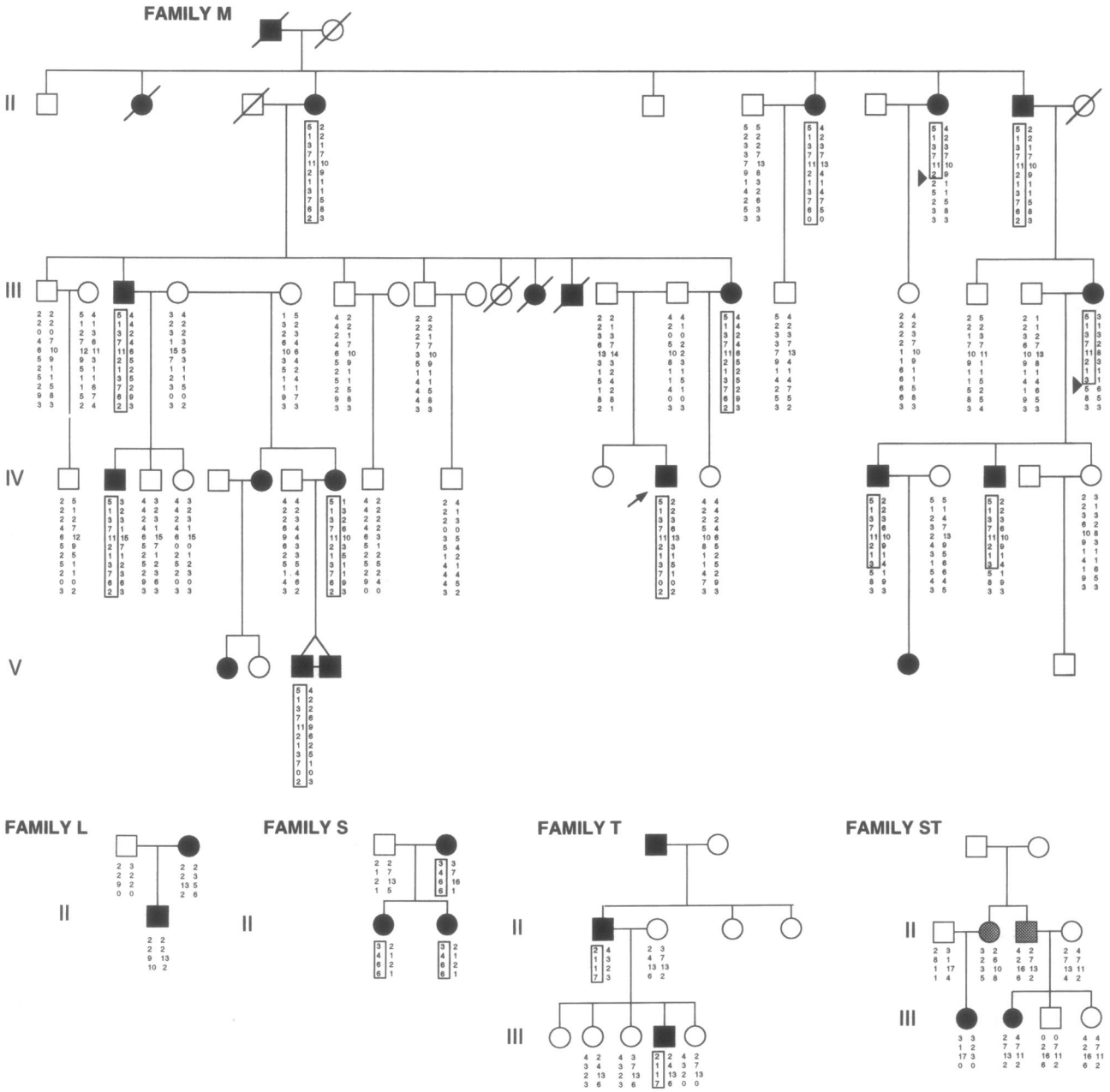

Figure 1 Pedigrees of four families (M, L, S, and T) with DA2B and of a fifth family (ST) without DA2B but in which one individual has DA1 and another has FSS. Affected individuals are denoted by a blackened symbol, unaffected individuals are denoted by an unblackened symbol, and individuals whose status is not known are denoted by a gray symbol. The haplotype segregating with DA2B is boxed. Recombination events are indicated by arrowheads. Genotypes for the microsatellite markers D11S4893, DRD4, D11S1363, D11S4177, D11S922, D11S4046, TH, D11S1318, D11S988, D11S1923, and D11S1323 in family M and D11S1363, D11S4177, D11S922, and D11S988 in families L, S, T, and ST are shown.

somes. Primer sequences and information are available from the Genome Data Base (Baltimore).

Linkage Analysis

Two-point analysis was performed using MLINK of the LINKAGE package (Lathrop et al. 1984). Disease penetrance was set at .95, without a sex difference. Normal and disease allele frequencies were set at .9999 and .0001, respectively. Genotypes for all individuals typed in the reported families are available on request.

Results

In family M, a total of 21 individuals fulfilled the minimal diagnostic criteria for the DAs. All tested obli-

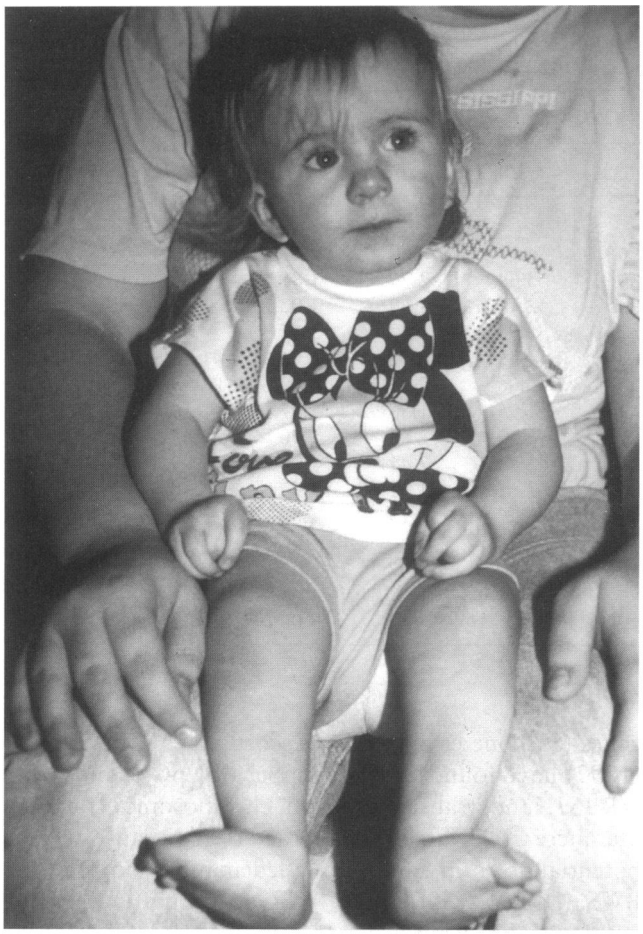

Figure 2 Photograph of individual V-5 of family M. Note the bilateral overlapping fingers, camptodactyly, and calcaneovalgus deformities.

gate carriers were affected. Each affected individual had ulnar deviation, camptodactyly, and hypoplastic flexion creases. Limb abnormalities were always bilateral but were not necessarily symmetrical. Each affected individual had positional deformities of the feet. Calcaneovalgus deformities associated with a vertical talus were found in 20 individuals, and a clubfoot was found in 1 individual. One individual (IV-2; fig. 1) was diagnosed with FSS in the newborn period. Three or more of the minor diagnostic criteria were found in all obligate carriers. Cervical webbing was observed in two individuals. No individuals had a cleft palate, an ophthalmoplegia, hearing loss, pterygia, or an abnormal neurological examination. There was no evidence for a parent-of-origin effect or for anticipation, in family M.

Since DA1 maps to chromosome 9p21-q21 (Bamshad et al. 1994), we initially assessed linkage between chromosome 9 markers and DA2B. Linkage to markers spanning intervals of ~10 cM was excluded. Next, we performed a genomewide search using short tandem repeats (STRs) with high PIC values and spaced at ~30-cM intervals. After testing only 30 markers, we detected linkage between DA2B and the D11S922 locus (Z_{max} = 5.31), indicating odds >200,000:1 that a DA2B locus is in this region. Fifteen additional markers spanning ~20 cM around D11S922 were tested, and the experiments were repeated to confirm results. Positive LOD scores were identified with D11S4893, DRD4, D11S1363, D11S4177, D11S4046, TH, D11S1318, D11S988, HBB, D11S1923, and D11S1323 (table 2).

On the basis of the marker-order estimates of the regional linkage map of chromosome 11 (Hudson et al. 1995; Fain et al. 1996; http://www-genome.wi.mit.edu) and the physical map of the chromosome 11 region distal to *HRAS1* (Russell et al. 1996), we constructed a map of the region surrounding the DA2B locus (fig. 3). *HRAS1* is located ~500 kb from the telomere of chromosome 11 (Russell et al. 1996), and the nearest anchored polymorphic STR locus is D11S1318, which is 3–6 Mb proximal to *HRAS1* (Hudson et al. 1995). Analysis of haplotypes that segregated with DA2B alleles identified recombination events between the DA2B locus and polymorphic markers in two individuals in family M (fig. 1). Recombination events were found between TH and DA2B in II-9 and between D11S988 and DA2B in III-20 (fig. 1). D11S2071 is the most telomeric marker on chromosome 11p (Russell et al. 1996), and it did not identify any additional recombinants in family M. When only recombinant events in affected individuals are considered, the DA2B critical region is localized to an ~3.5–6.5-cM interval between D11S1318 and the telomere.

Three additional families with "variants" of DA1 also were studied. Each affected individual in these three families exhibited at least two of the major and two of the minor criteria of DA2B (e.g., family L; fig. 4). Members of a fourth family (family ST; fig. 1), in which one individual had been diagnosed with DA1 and another had been diagnosed with FSS, did not meet the diagnostic criteria for DA2B but, nevertheless, were tested because of the similarities of their diagnoses. Subsequently, we performed linkage analysis using markers D11S1318, D11S922, and D11S4177. Linkage analysis of the three informative families generated a combined LOD score of 6.45. Although the use of these families alone precludes achieving an adequate significance level, analysis of their haplotypes suggests that DA2B may be a genetically homogeneous disorder. The affected individuals in family ST did not share a haplotype by common descent.

Discussion

We have mapped DA2B to chromosome 11p15.5-pter, and recombinants bracket the DA2B locus to a

Table 2

Pairwise LOD Scores between Chromosome 11 Markers and DA2B Families M, T, and S

	LOD Score at θ =						
Family and Marker	.00	.01	.05	.10	.20	.30	.40
M:							
D11S4893	5.08	4.99	4.64	4.19	3.22	2.16	1.02
DRD4	4.43	4.35	4.04	3.64	2.79	1.85	.86
D11S1363	2.70	2.65	2.45	2.18	1.62	1.02	.43
D11S4177	3.55	3.49	3.25	2.94	2.29	1.57	.81
D11S922	5.31	5.22	4.86	4.39	3.38	2.26	1.06
D11S4046	4.92	4.84	4.50	4.06	3.11	2.08	.96
TH	2.33	2.29	2.15	1.95	1.53	1.07	.56
D11S1318	−1.36	3.39	3.72	3.55	2.84	1.90	.85
D11S988	−5.46	1.50	2.56	2.73	2.41	1.74	.89
D11S1923	−6.46	.78	1.89	2.11	1.91	1.37	.68
D11S1323	−4.01	−.11	.90	1.20	1.19	.87	.42
T:							
D11S922	.84	.82	.75	.66	.47	.27	.08
S:							
D11S922	.30	.29	.26	.21	.13	.06	.02

critical region 3.5–6.5 cM telomeric of TH. A complete physical map from the telomere to *HRAS1* recently has been reported, and the length of this contig is ~500 kb (Russell et al. 1996). The closest marker anchored on a chromosome 11 physical map is D11S1318, which is estimated to be 3–6 cM proximal to *HRAS1* (Hudson et al. 1995). This region is syntenic with a region on mouse chromosome 7. Although there are a variety of murine limb mutants with phenotypes characterized by positional foot deformities, none map to the syntenic regions of murine chromosome 7.

The subtelomeric portions of human chromosomes are reported to contain the highest concentration of genes (Saccone et al. 1992). Accordingly, Weitzel et al. (1992) have found a large number of CpG islands near *HRAS1,* and transcripts from chromosome 11p15.5-pter increasingly are being characterized because of their potential roles in Beckwith-Wiedemann syndrome (or BWS; OMIM 130650), WAGR syndrome (Wilms tumor, aniridia, genital hypoplasia, and mental retardation), and tumorigenesis (Stubbs et al. 1994). Nevertheless, no outstanding candidate genes for DA2B have been reported in this region. *MYF3,* the gene for myogenic factor 3, is the human homologue of murine *MyoD-1* and is involved in the specification of the muscle-cell lineage. It has been mapped to human chromosome 11p15.4 by use of a panel of somatic-cell hybrids (Gessler et al. 1990), and this placement is further supported by a *Not*I map of 11p15 (Higgins et al. 1994). Therefore, *MyoD-1* is excluded by the recombinants in family M.

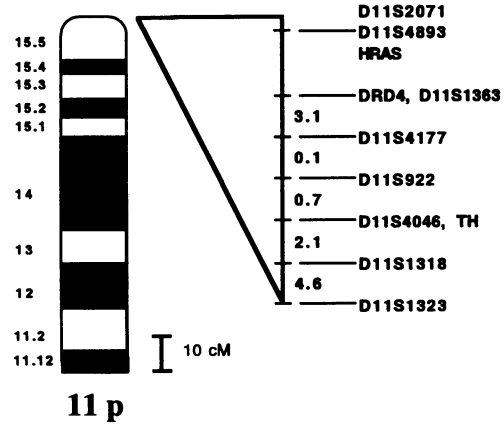

Figure 3 Regional map of chromosome 11p. The markers ordered on the map are supported by 1,000:1 odds. Distance between the markers is in centimorgans.

Haplotype analysis of family M suggests that penetrance of DA2B is complete. All obligate gene carriers had limb malformations that could be detected clinically. Most individuals had bilateral but asymmetrical involvement of the upper and lower limbs. The limitations of extension producing the camptodactyly and ulnar deviation appeared to be more extreme than that found in DA1, leading to more functional restrictions and disabilities. Twenty individuals had calcaneovalgus deformities, and one individual had a clubfoot. Calcaneovalgus anomalies clearly were shown to be present by use of fetal ultrasound examination of at least three affected individuals at ~18–20 wk of estimated gestational age. The distribution and severity of contractures

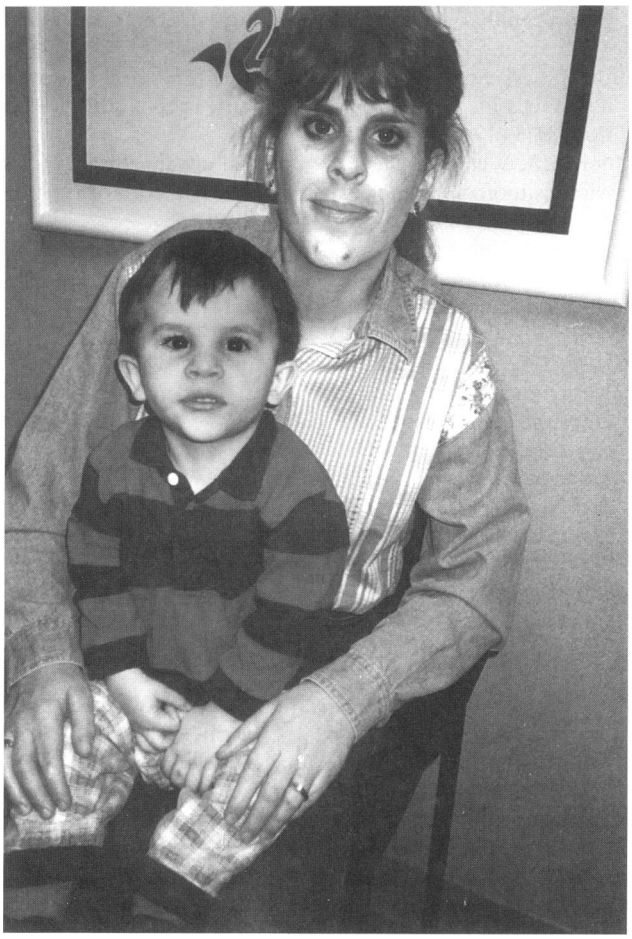

Figure 4 Photograph of individuals I-2 and II-1 of family L. Note the triangular faces, the small mouths, deep nasolabial folds, and downslanting palpebral fissures.

differed substantially among the affected MZ twins (V-3 and V-4), suggesting that epistatic forces influence the development of the phenotype associated with DA2B. The proband of family M (IV-12) was ascertained through his membership in the Freeman-Sheldon Parents Support Group. Despite having positional limb deformities identical to those of children with FSS, he did not meet the proposed diagnostic criteria for FSS (Carey et al. 1993; Bamshad et al. 1996*b*). His oral commissure was of normal length, his lips were not puckered, and he had no feeding difficulties as a newborn. He lacked a prominent philtrum and did not have an H-shaped dimple on his chin. As a newborn, he apparently had a relatively smaller mouth and prominent nasolabial folds, which led to the diagnosis of FSS.

Subsequently, we reviewed the photographs and medical histories of the ~80 families participating in our project, in order to identify a gene for FSS. Several of these families had features atypical of FSS but appeared to meet the diagnostic criteria for DA2B. DNA was available from one of these families (family T; fig. 1). DNA was also available from two families with variants of DA1 and features consistent with DA2B (families L and S; fig. 1). These families appear to map to the DA2B locus. However, it is apparent that the DA2B locus is excluded in some families in which FSS and DA1 are segregating.

Mapping of the genes causing DAs is a prerequisite to defining further the relationships among these conditions and to creating a classification based on the biological mechanisms causing congenital contractures. This strategy already is fostering better clinical management of patients with DA disorders, since the natural histories of DA1, DA2, and DA2B appear to be substantially different. The mapping of the DA2B locus is a further step toward understanding the molecular basis of inherited positional limb anomalies. To date, *FBN2*, the gene for fibrillin-2, is the only cloned gene that causes a multiple congenital contracture disorder. Although the etiologic mechanisms causing positional limb deformities are heterogeneous, once *DA2B* is cloned, we will be able to test whether a subset of sporadic positional limb deformities are caused by mutations in this gene.

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