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A Gene for Distal Arthrogryposis Type I Maps to the Pericentromeric Region of Chromosome 9

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Summary

Club foot is one of the most common human congenital malformations. Distal arthrogryposis type I (DA-1) is a frequent cause of dominantly inherited club foot. Performing a genomewide search using short tandem repeat (STR) polymorphisms, we have mapped a DA-1 gene to the pericentromeric region of chromosome 9 in a large kindred. Linkage analysis has generated a positive lod score of 5.90 at $\theta = 0$, with the marker GS-4. Multiple recombinants bracketing the region have been identified. Analysis of an additional family demonstrated no linkage to the same locus, indicating likely locus heterogeneity. Of the autosomal congenital contracture disorders causing positional foot deformities, this is the first to be mapped.

Introduction

Distal arthrogryposis type I (DA-1; OMIM 108120) is a highly penetrant autosomal dominant disorder characterized by multiple flexion contractures of the distal extremities (Hall et al. 1982; Hall 1985, 1989, 1992). The prevalence at birth of any type of congenital contracture is 0.5%–1.0%, and >150 conditions have been described in which multiple congenital contractures may be a feature (Hall 1985). The most common reported congenital contractures are dislocated hips (0.5%) and club feet (0.2%) (Hall et al. 1982). A child with multiple congenital contractures is identified in 1 of every 3,000 births (Hall 1992), and the prevalence of DA-1 is ~1 in 10,000–50,000 (J. G. Hall, personal communication). DA-1 is a common known cause of inherited club foot deformity.

All affected individuals demonstrate flexion contractures at birth. The most frequently affected joints are the hands (98%) and the feet (88%) (Hall et al. 1982). The hands are typically held in a characteristic position consisting of a tight fist with medial overlapping of the fingers and an adducted thumb. Approximately 40% have an equinovarus deformity of one or both feet. Neurologic exams are normal, and intelligence is unaffected. There is marked intra- and interfamilial variability in the severity of expression. As affected children begin to exercise, their hands can become quite functional. Affected adults may demonstrate residual camptodactyly and/or ulnar deviation of the fingers at the metacarpal phalangeal joint. Twenty percent of affected adults have straight and fully functional fingers. Hence, absent distal interphalangeal creases may be the only residual finding in the upper extremities. Overlapping of the toes may be the only residual manifestation in the feet. The pathogenic mechanisms responsible for DA-1 have yet to be defined. Misplaced, hypoplastic, or absent tendons have been documented in some patients (Hall et al. 1982).

To initiate the effort to clone and characterize the genes for the multiple congenital contracture syndromes, genetic linkage analysis was performed on a large kindred with DA-1. It is demonstrated that DA-1 maps to a 65-cM pericentromeric region of chromosome 9. Genetic heterogeneity is suggested by a smaller family in which linkage is excluded.

Material and Methods

Clinical Status

All studies were performed with the approval of the institutional review board of the University of Utah and the general counsel of the Shriner's Hospitals for Crippled Children. After informed consent was obtained, two unrelated kindreds of northern European descent were evaluated by history and physical examination (family F) or review of medical records and photographs (family T). An individual was diagnosed as affected on the basis of a positive family history and the presence of one or more of the clinical manifestations of DA-1. Major diagnostic criteria of the upper extremity included ulnar deviation, camptodactyly, absent flexion creases, and overriding fingers. Major diagnostic features of the lower extremity included metatarsus varus, talipes equinovarus, a vertical talus, and a calcaneovalgus deformity.

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In each family (fig. 1) DA-1 segregated as an autosomal dominant trait. Eighteen individuals in the two families were affected. To date, family F is the largest kindred with DA-1 reported. One or more major diagnostic features were present at birth in all affected individuals in family F. Intrafamilial phenotypic variation ranged from ulnar deviation (II-4) to bilateral hip dislocation, bilateral equinovarus, and camptodactyly (IV-17). Individuals II-1 and III-4 of family T exhibited camptodactyly of the hands and feet, ulnar deviation, and a small mouth, but not positional deformities of the feet. This family was originally diagnosed with Freeman-Sheldon syndrome (FSS) and reclassified as DA-1, as none of the family members exhibited the characteristic facial features of FSS, such as hypertelorism, a long philtrum, or a small nose with small nostrils. In both families, DA-1 is distinguished from DA-IIE by the lack of trismus in most individuals, normal height and intelligence, and camptodactyly without hyperextension of the metacarpophalangeal joints. All participants were notified regularly via a newsletter of progress on the project.

Ascertainment of Genotype

Twelve milliliters of blood were obtained from all affected individuals and at least their first-degree relatives. Genomic DNA was prepared from lymphocytes and 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 $MgCl_2$) using 25 ng of template genomic DNA, 50 μM dNTPs, 20 pmol of each primer, and 1 U of *Taq* DNA polymerase in a total reaction volume of 25 μl. Samples were cycled 30 times in a Perkin-Elmer 9600 PCR machine using standard profiles. The annealing temperature was decreased 4°C on the sixth cycle. Twenty microliters of 50%-formamide loading dye was added to each reaction following PCR. Samples were denatured for 3 min at 94°C and then electrophoresed through 5% denaturing polyacrylamide gels. Bands were visualized by autoradiography.

Linkage Analyses

Two-point and map-order analysis were performed using MLINK and CILINK of the LINKAGE package (Lathrop et al. 1984). Penetrance was set at .95. Allele frequencies were set at .0001 and .9999. Genetic heterogeneity was tested using the HOMOG (Ott 1991) program.

Results

Many chromosomal abnormalities have been identified in infants with arthrogryposis, yet none is found consistently in DA-1 (Reed et al. 1985). A high-resolution karyotype of the proband in family F was normal (data not shown). Because the positioning of the hands in DA-1 newborns is very similar to that of an individual with trisomy 18, we initially assessed linkage between chromosome 18 markers and DA-1. No significant linkage was identified.

As the fundamental defect is unknown and no other candidate regions existed, we conducted a systematic genome search with short tandem repeat (STR) markers. Highly polymorphic STR markers were chosen on the basis of their PIC and location sufficient to exclude linkage within ~30 cM (NIH/CEPH Collaborative Mapping Group 1992; Weissenbach 1992). A lod score of 4.02 at $\theta = .0$ (table 1) between a chromosome 9 marker, D9S43, and the DA-1 locus prompted further analyses of markers from chromosome 9.

Sixteen additional polymorphic markers spanning ~65 cM were tested in family F. Two-point lod scores demonstrated linkage between DA-1 and other polymorphic markers within this region (table 1). The maximum lod score of 5.90 was achieved with marker GS-4 (θ=.0), indicating odds >749,000:1 that a DA-1 locus is in this region. This marker was completely informative at each meiosis.

By using the Cooperative Human Linkage Center (CHLC) framework map of chromosome 9 (9frame.v2; available via anonymous ftp to ftp.chlc.org [Buetow 1994]) as a starting point and mapping additional markers using CILINK, a pericentromeric regional map was constructed (fig. 2). Eight CEPH families were genotyped for markers UT2100, GS-4, UT5132 , UT6023, and UT5494; four CEPH families were genotyped for UT726 and UT5024. A locus was added if its placement was supported, by a lod score of 3, over alternative locations in the map. Markers GS-4, D9S15, and GS-2 map to a 135-kb region on the same cosmid contig (Fujita et al. 1992). Markers UT726 and UT5494 could be placed unequivocally on the map, while only regions indicated by confidence intervals for D9S52, D9S43, UT5132, UT6023, and UT5024 were identified.

To determine whether DA-1 in one additional family (fig. 1, family T) also mapped to the pericentromeric region of chromosome 9, we performed linkage analysis using markers UT2100, D9S50, D9S15, GS-4, UT726, and UT5132. Two-point lod scores (table 1) suggested that DA-1 in family T is not linked to the chromosome 9 locus (lod = −1.76 at $\theta = .0$ for GS-4). A test for locus heterogeneity yielded a χ^2 value of 2.9 ($P<.09$). In light of the small size of family T, this marginally significant result indicates that DA-1 is likely a genetically heterogeneous disorder. We designate the disease locus in the pericentromeric region of chromosome 9 as *DA-1A*.

Analysis of haplotypes that segregated with DA-1 in family F identified recombination events between *DA-1A* and polymorphic markers in five affected individuals (fig. 1). One recombination event was identified between

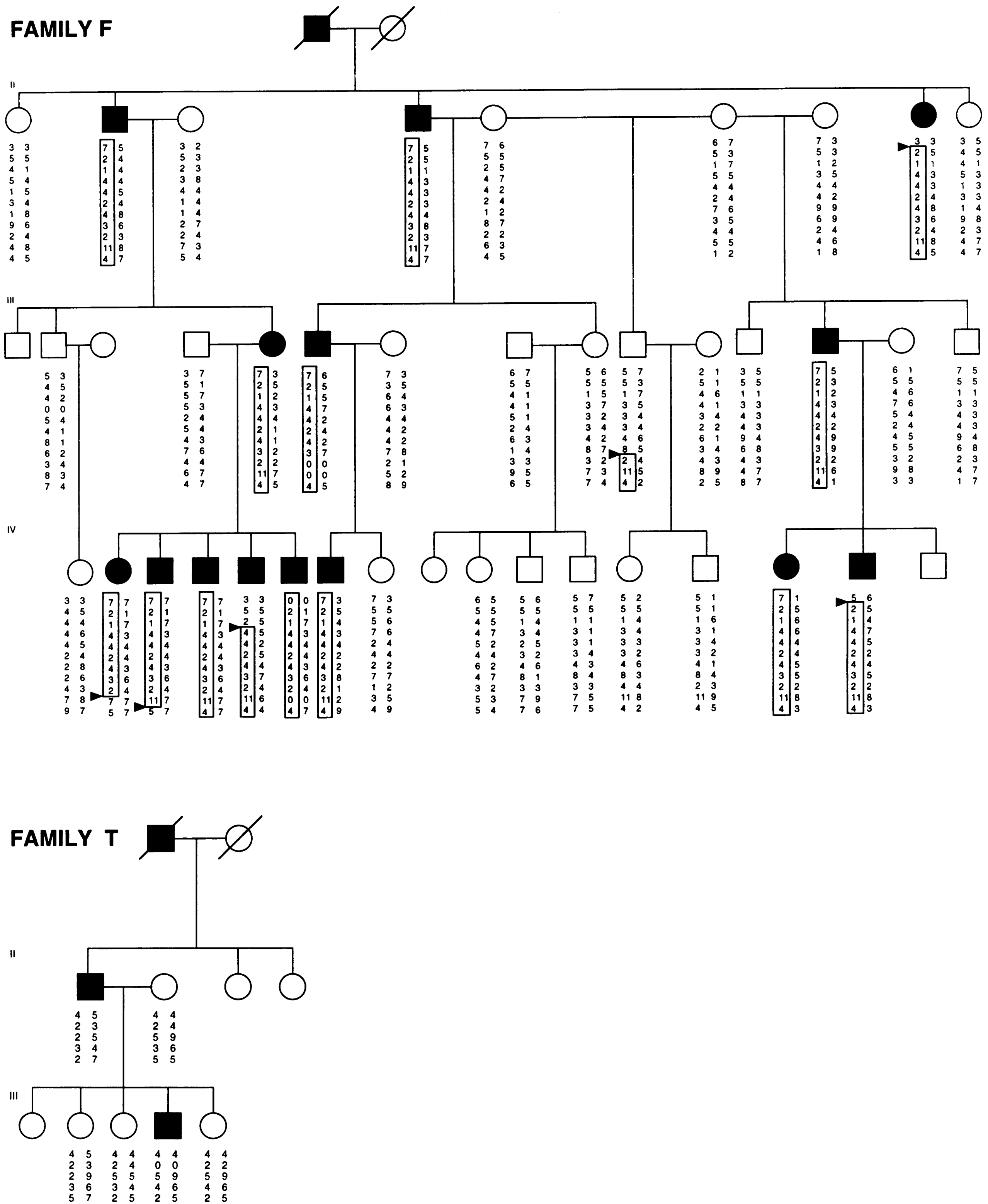

Figure 1 Pedigrees of two families with DA-1. Affected individuals are denoted by a blackened symbol, unaffected by an unblackened symbol. The haplotype segregating with DA-1 is boxed. Recombination events are indicated by arrowheads. Genotypes for microsatellite markers D9S156, D9S126, D9S169, UT5132, D9S50, D9S15, UT726, GS-4, UT5024, UT6023, and UT5494 in family F and D9S50, D9S15, GS-4, UT726, and UT5132 in family T are shown. A manuscript describing the unpublished markers UT5132 (D9S248), UT726 (D9S234), UT5024 (D9S245), UT6023 (D9S769), and UT5494 (D9S768) is in preparation.

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Table 1

Pairwise Lod Scores between Chromosome 9 Markers and DA-1 in Two Unrelated Families

Family and Locus	θ						
	.00	.01	.05	.10	.20	.30	.40
Family F:							
D9S156	−19.1	−3.70	−.97	0.4	.66	.64	.31
D9S126	.60	3.81	4.13	3.95	3.19	2.18	.99
D9S169	−2.21	1.03	1.53	1.58	1.37	1.01	.55
UT2100	4.04	3.98	3.72	3.38	2.65	1.82	.87
D9S52	2.86	2.82	2.64	2.40	1.87	1.27	.60
D9S43	4.02	3.96	3.70	3.36	2.64	1.81	.86
UT726	3.83	3.77	3.50	3.15	2.40	1.57	.73
D9S165	3.16	3.11	2.90	2.63	2.05	1.38	.63
D9S50	3.79	3.72	3.46	3.12	2.37	1.53	.61
D9S15	5.29	5.21	4.85	4.39	3.38	2.25	1.01
UT5132	5.29	5.21	4.86	4.39	3.39	2.26	1.03
GS4	5.90	5.80	5.41	4.90	3.79	2.55	1.17
GS2	3.20	3.14	2.89	2.57	1.88	1.12	.37
UT683	5.31	5.23	4.87	4.41	3.40	2.28	1.04
UT6023	−3.20	1.52	2.58	2.74	2.39	1.67	.75
UT5024	−.75	−.06	.45	.59	.57	.40	.15
UT5494	−4.47	−2.59	−.60	.19	.70	.67	.36
Family T:							
D9S50	−1.76	−1.55	−1.05	−.70	−.32	−.13	−.03
D9S15	−.74	−.67	−.47	−.32	−.15	−.06	−.01
GS4	−1.76	−1.55	−1.05	−.70	−.32	−.13	−.03
UT726	−1.76	−1.55	−1.05	−.70	−.32	−.13	−.03
UT5132	−1.76	−1.55	−1.05	−.70	−.32	−.13	−.03

D9S169 and the *DA-1A* locus (IV-5), and one recombination event was found between the *DA-1A* locus and UT5024 (III-10; unaffected). This pattern localizes the *DA-1A* locus between D9S169 and UT5024. Individual III-10 may not be a recombinant if DA-1 is incompletely penetrant. The recombinant event in IV-2 would extend the localization from D9S169 to UT6023. This pattern is consistent with the marker-order estimates of the regional map.

Discussion

We have mapped *DA-1A* to a 65-cM pericentromeric region of chromosome 9, between markers UT2100 and UT6023, and we demonstrated that at least one other DA-1 locus may exist elsewhere in the genome. Haplotype analysis confirms that DA-1 is completely penetrant.

Our results do not permit a precise physical localization of *DA-1A*. Of the flanking markers studied, D9S52 has been assigned to 9p21-9qter, D9S165 to 9p21-9q21, and D9S15 to 9q21 (Atwood et al. 1994). The genetic distance of 65 cM translates to a large cytogenetic distance spanning half of the short arm, the centromere, the heterochromatic area of the long arm, and at least two additional bands. This is consistent with the pericentromeric recombination suppression suggested by Hulten's chiasma data, which predict only 2 cM between 9p21 and 9q21 in males (Povey et al. 1992). Thus, we did not assign *DA-1A* to either side of the centromere.

Candidate genes in the pericentromeric region include *COL15A*, which encodes the alpha-1 polypeptide of type XV collagen (Huebner et al. 1992; Meyers et al. 1992), and *CNTFR*, which encodes the receptor for ciliary neurotrophic factor. COL15A has recently been localized distal to UT5024, between D9S176 and D9S180 (A. Bale, personal communication) and thus is excluded by recombinants in III-10 and IV-2 in family F. Joint contractures have been reported in patients with cartilage-hair hypoplasia (McKusick et al. 1965; van der Burgt et al. 1991), an autosomal recessive chondrodystrophy characterized by short stature, short limbs, joint laxity, and sparse, fine hair (CHH; OMIM 250250). The CHH gene was recently mapped to 9p13-9q11, an interval that overlaps the location of *DA-1A* (Sulisalo et al. 1994).

Congenital contractures are features of >150 disorders, including at least 6 conditions categorized as types of distal arthrogryposis (DA) (see table 2). Patterns of intrafamilial variation in DA kindreds suggest that the features distinguishing DA types may not be present in all affected family members. Gordon syndrome (OMIM 114300) is separated from DA-1 by the presence of a cleft lip/palate, yet this feature can skip generations, making individuals indistin-

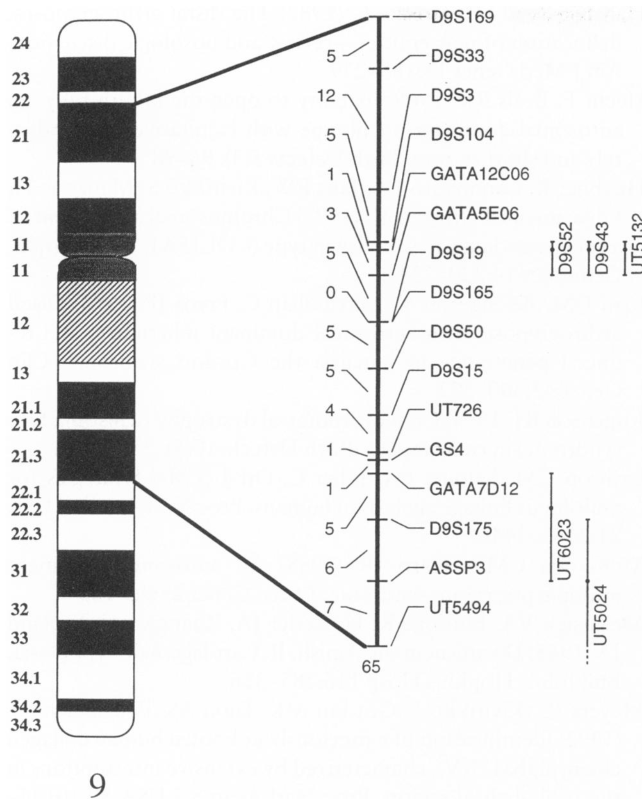

Figure 2 Regional map of chromosome 9. Markers ordered on the map are supported by 1,000:1 odds. Confidence intervals are provided for markers not placed on the map. The most likely placement is denoted by a dot in the confidence interval. Distance between markers is reported in centimorgans.

guishable from DA-1 (Gordon et al. 1969; Halal and Fraser 1979; Ioan et al. 1993).

FSS (OMIM 193700) is characterized by camptodactyly with ulnar deviation of the hands, talipes equinovarus, and specific facial features, most notably a small mouth with trismus (Freeman and Sheldon 1938; Vanek et al. 1986). Individual II-4 of family F has trismus, and additional family members have complained of limited ability to open their mouth. Hall et al. (1982) reclassified two families reported by Jorgenson (1974) as FSS to DA-1, and recently DA-1 and FSS were found segregating in a single kindred (J. G. Hall, personal communication). Allelic or locus heterogeneity could explain the similarities between Gordon syndrome, FSS, and DA-1. This is currently being investigated. Other disorders often confused with DA-1 include the trismus-pseudocamptodactyly syndrome (Hecht and Beals 1969; OMIM 158300) and autosomal dominant multiple pterygium syndrome (McKeown and Harris 1988; OMIM 265000).

Identification of the *DA-1A* gene will permit a more rigorous definition of the relationships among these conditions. Classification of the DAs on the basis of biological mechanisms would improve diagnostic capabilities and enable clinicians to provide more efficient management and achieve greater accuracy in predicting long-term sequelae. If closely linked markers can be identified, an accurate diagnostic screening test could be developed.

Cloning and characterization of *DA-1A* and analysis of disease mutations will be an important step toward understanding the molecular basis of congenital contractures and limb development. A number of genes have recently been identified that regulate the limb morphogenesis cascade. One important class of such genes is the homeobox-containing genes (Hox genes), especially the Hox-4 cluster (Tabin 1992). It is likely that additional levels of regulatory genes exist downstream that signal precise tendon and muscle positioning. *DA-1A* could be a regulatory element of this type.

The club-foot deformity is one of the most common human congenital limb malformations. Numerous studies have concluded that idiopathic club foot is caused by a major gene with a polygenic background (Wang et al. 1988), and a recent analysis suggests that the majority of

Table 2

Clinical Conditions Associated with Distal Contractures

Disorder	Features[a]
Distal arthrogryposis type I	PFD, CAMP, UD
Gordon syndrome (DA-2A)	PFD, CAMP, UD, short stature, cleft palate
Distal arthrogryposis type 2B	PFD, CAMP, UD, short stature, ptosis
Distal arthrogryposis type 2C	PFD, CAMP, UD, cleft lip/palate
Distal arthrogryposis type 2D	PFD, CAMP, UD, scoliosis
Distal arthrogryposis type 2E	PFD, CAMP, UD, trismus
Freeman-Sheldon syndrome	PFD, CAMP, UD, small mouth
Trismus-pseudocamptodactyly	PFD, CAMP with dorsiflexion, trismus
Multiple pterygium (dominant)	PFD, CAMP, UD, short stature, scoliosis

SOURCE.—Hall et al. (1982).

[a] PFD = positional foot deformity; CAMP = camptodactyly; UD = ulnar deviation.

idiopathic club-foot cases may be caused by a single gene (Dietz et al. 1993). Although the etiologic mechanisms of club foot are heterogeneous, it is reasonable to test whether mutations in *DA-1A* can be identified in sporadic affected individuals.

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