A Gene for Arthrogryposis Multiplex Congenita Neuropathic Type Is Linked to D5S394 on Chromosome 5qter

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Summary

Arthrogryposis multiplex congenita (AMC) is a heterogeneous-symptom complex characterized by joint contractures at birth that involve more than one part of the body. We performed a genetic-linkage study of one large Israeli-Arab inbred kindred showing autosomal recessive inheritance of AMC neuropathic type that had been recently investigated by our group. After analysis of ~80% of the genome, D5S1456, which showed no increased homozygosity, showed increased genotype sharing in affected individuals. Linkage analysis in all family members revealed linkage between AMC and D5S1456 on chromosome 5qter (maximum LOD score 5.3 at recombination fraction .001). Analysis of additional markers in this region places the gene causing AMC in this family between D5S1456 and D5S498.

Introduction

Arthrogryposis multiplex congenita (AMC) is a symptom complex characterized by nonprogressive joint contractures that involve more than one part of the body (Hall 1996). This heterogeneous group of disorders may result from different mechanisms that cause decreased movement in utero. Among the genetic forms of AMC, several different syndromes have been reported, and these can be distinguished on the basis of inheritance, location of the basic defect (muscle, CNS, spinal cord, etc.), and other associated findings (Hall 1996). Among the autosomal recessive forms, there have been several reports of multiple affected sibships with AMC neuropathy without other manifestations (McKusick 1994). In some of these families, histological changes involving the spinal cord have been described (Frischknecht et al. 1960; Barzeton et al. 1961), and, in a subset of children with AMC, deletions in the SMN gene have been found (Burglen 1996).

We recently investigated a large Israeli-Arab inbred kindred, originally described by Weissman et al. (1963) and further studied by Lebenthal et al. (1970), with autosomal recessive arthrogryposis of the neuropathic type at the spinal level (Jaber et al. 1995). This family has since been tested for genetic linkage, and, after screening of ~80% of the human genome, the AMC gene was mapped to chromosome 5qter, as reported herein.

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Material and Methods

Samples

Blood samples were obtained from seven sibships, all from a large Israeli-Arab inbred family (Jaber et al. 1995), in each of which at least one individual was affected with AMC (fig. 1). These sibships included a total of 15 affected and 30 unaffected individuals. The study protocol was approved by the Human Subjects Committee of Beilinson Medical Center.

The most common features were flexion contractures of the elbows and knees and marked equinovarus (fig. 2). Because of the diminished range of motion of the affected limbs, the muscles around the contracted joint were markedly hypoplastic. Marked variability among patients was noted in the clinical expression. Although the patients belong to the same sibship, some patients had contractures of both the elbows and the knees and had equinovarus, whereas other close relatives had asymmetric involvement of different joints: either left elbow and right knee only and bilateral equinovarus or only bilateral equinovarus.

Many patients had relatively mild symptoms and have learned how to cope adequately with their handicap, and most of the adult patients are employed, some as carpenters (a common profession in this clan). As described in our previous evaluation of affected individuals, the diagnosis of AMC was based on neurological exami-

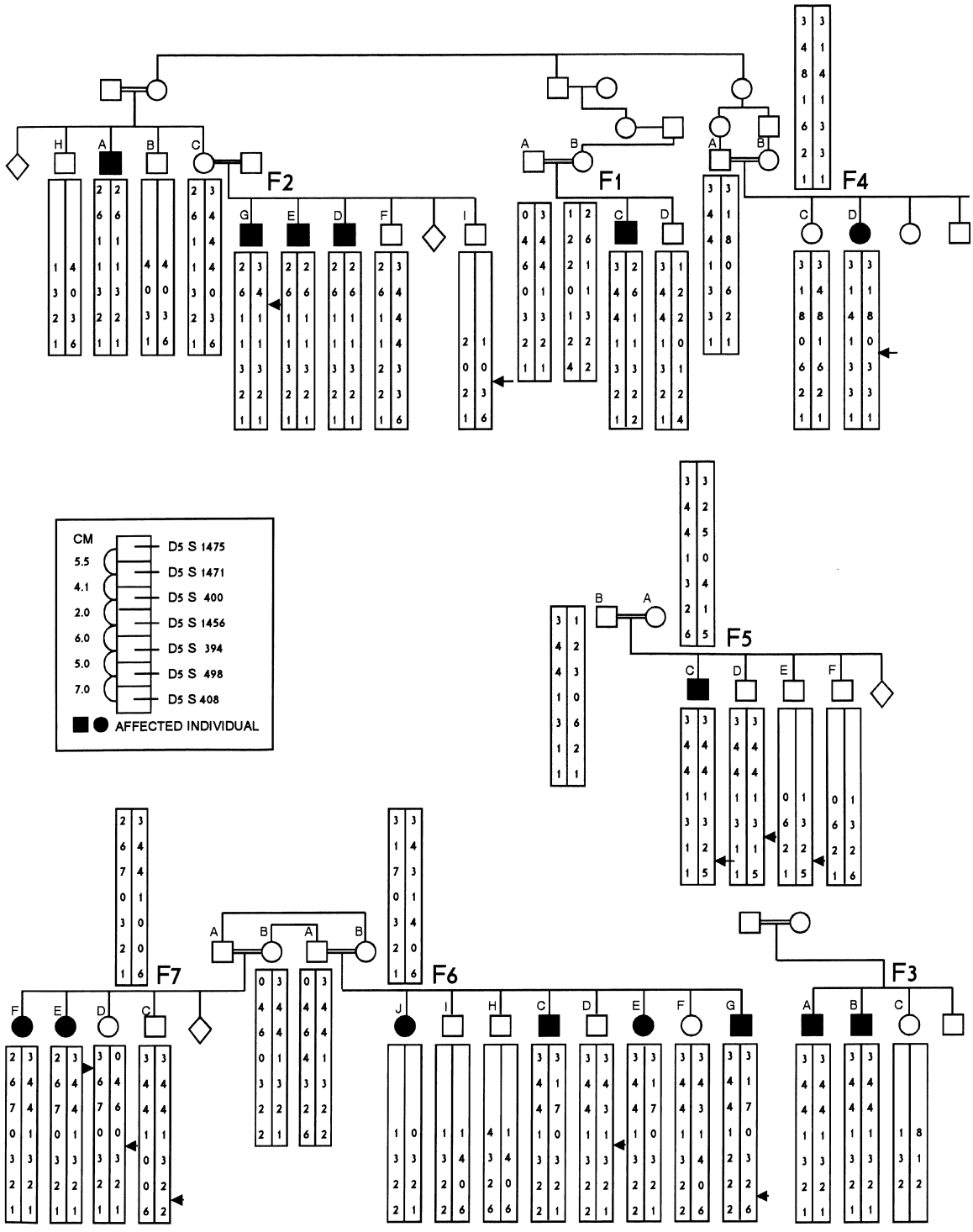

Figure 1 Haplotype of seven markers studied in Israeli-Arab sibships with AMC neuropathic type. The order and distance between the markers are depicted.

nation, electromyogram, somatosensory evoked-potential test, Hofman-reflex response, and nerve conduction test, which were performed in two patients. Markedly decreased nerve conduction with very low amplitude was found in affected motor nerves, as evidence of demyelinizing axonal damage. We concluded that this represents a neuropathic type of AMC (Jaber et al. 1995). Females were less affected than males (male:female ratio

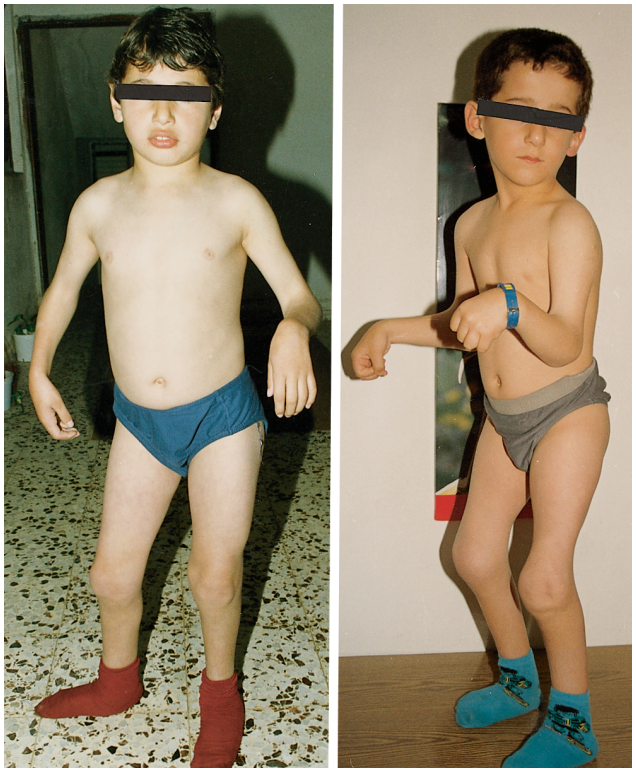

Figure 2 Typical clinical findings in affected members of family with AMC neuropathic type.

2:1), and it was concluded that there is incomplete penetrance of the disease in females. In addition, it seems that the clinical picture in females is milder than that in males (Jaber et al. 1995).

DNA Analysis

DNA was isolated from the blood samples by standard methods (Sambrook et al. 1989).

Microsatellite Marker Analysis

Standard amplification conditions for PCR were followed for the entire human-genome linkage screening with Cooperative Human Linkage Center (CHLC) markers and specifically for markers D5S820, D5S1475, D5S1456, and D5S408 (Genetic Research). Généthon's markers D5S1471, D5S400, D5S394, and D5S498 were typed by use of previously published markers and conditions (Dib et al. 1996).

Linkage Analysis

For the initial screening of linkage to the disease gene, we genotyped 30 members of the AMC family (15 affected individuals and 15 unaffected siblings), as well as 15 unrelated individuals. For each marker, we compared the degree of homozygosity and genotype sharing in each of these three groups. Genotyping of all family members and linkage analysis were performed for each marker that showed increased homozygosity or genotype sharing in the 15 affected individuals. Genetic-linkage analysis was done by use of the MLINK, ILINK, and LINKMAP subroutines of the computer program LINKAGE, version 5.10 (Lathrop et al. 1984). Homozygosity mapping was performed in this inbred family in the way suggested by Lander and Botstein (1987).

An autosomal recessive mode of inheritance was assumed for the linkage analysis. On the basis of our updated study of the family, the penetrance was assumed to be 90% in males and 70% in females. The gene frequency was estimated at .05, on the basis of the assumption of complete ascertainment with 40 affected individuals among 16,000 people living in the village. The family lives in a village with a high frequency of consanguineous marriages, and, on the basis of our previous study of this population, the calculated range of the inbreeding coefficient was .035–.1624 (Jaber et al. 1992). Since genotypes of all individuals were known or could be deduced, marker-allele frequencies did not affect the LOD scores in our study. Two-point LOD scores were calculated by use of MLINK, and multipoint calculations were done by use of LINKMAP of the LINKAGE package. The large pedigree was broken into seven small unrelated nuclear families. In families with inbreeding loops, the loops were broken by counting twice an individual who has both parents and children included in the pedigree. The order of the markers used, starting from the centromere, was D5S1475-D5S1471-D5S400-D5S1456-D5S394-D5S498-D5S408 (Dib et al. 1996) (for distances, see fig. 1).

Results

The initial screening of the affected individuals by molecular homozygosity testing showed 47% heterozygosity with the marker D5S1456, which was identical to the heterozygote rate in the unaffected siblings. However, further analysis of the results showed an increased genotype sharing of allele 1 among affected individuals (23/30), compared with that in the unaffected siblings (16/30) and in unrelated individuals (10/30). Because of the increased genotype sharing at D5S1456, we further analyzed all family members in these sibships, by conventional linkage testing. The results provided evidence of linkage with D5S1456; the maximum LOD score was 5.30 at recombination fraction .001.

Six additional markers, around this marker, were then tested. Figure 1 depicts the haplotype of each of the individuals in the seven sibships studied with the following markers: D5S1475, D5S1471, D5S400, D5S1456, D5S394, D5S498, and D5S408. The results

Table 1

Two-Point LOD Scores for Chromosome 5q Markers

Marker	LOD Score at Recombination Fraction of												Maximum Recombination Fraction	Maximum LOD Score
	.001	.01	.02	.03	.04	.05	.06	.08	.10	.15	.20	.30		
D5S1475	−1.20	.11	.55	.79	.95	1.04	1.11	1.18	1.20	1.12	.95	.54	.10	1.20
D5S1471	−1.94	−.52	.02	.33	.54	.68	.79	.92	.99	.99	.89	.54	.13	1.02
D5S400	4.21	4.21	4.16	4.10	4.03	3.95	3.86	3.66	3.45	2.90	2.34	1.25	.003	4.21
D5S1456	5.30	5.23	5.13	5.03	4.92	4.80	4.67	4.41	4.14	3.45	2.74	1.40	.001	5.30
D5S394	4.16	4.15	4.10	4.05	3.98	3.91	3.83	3.66	3.47	2.96	2.42	1.33	.000	4.16
D5S498	4.20	4.10	4.00	3.89	3.78	3.67	3.56	3.35	3.13	2.60	3.08	1.12	.001	4.20
D5S408	−2.21	−.74	−.13	.26	.54	.74	.90	1.11	1.23	1.29	1.17	.68	.14	1.30

of the two-point linkage analysis with these markers are given in table 1.

Recombination events were analyzed and showed that in none of the affected individuals in which these events had occurred between the disease locus and D5S498 and/or D5S408 had they involved the two centromeric markers. Reciprocally, in those affected individuals with recombination events between AMC and D5S1471 and/or D5S400 and/or D5S1456, in no case did these events involve the more telomeric markers (D5S498 and D5S408). On the basis of the homozygosity analysis in affected individuals by use of the marker D5S394 (mapped between D5S1456 and D5S498), it appears that, since the founder individual, there has not been any recombination event with the AMC gene. There were two recombination events, in the interval of the disease locus, between D5S1456 and D5S498 (in individuals F5-D and F4-D). However, the lack of homozygosity for the same allele is evidence of recombination events in previous generations. Complete homozygosity was found in all the affected individuals, across all the sibships, with marker D5S394 genotype 3. On the basis of the frequency (18/30) of allele 3 in the parental generation, the chance that all affected individuals are homozygous for allele 3 at this locus is 6.14×10^{-7} (analogous to a LOD score of 6.21 in favor of association). Several unaffected siblings (F1-A, F7-D, F7-B, F6-A, F6-D, and F5-D) were also homozygous for genotype 3; some of these (F1-A, F7-B, and F6-A) are clearly heterozygotes, with allele 3 on a different haplotype, whereas others appear to represent either incomplete penetrance (F5-D) or recombination between this marker and the AMC gene (F6-D and F7-D).

Discussion

Our study mapped the gene causing AMC neuropathic type in the Israeli-Arab family to chromosome 5qter, between D5S1456 and D5S498, and demonstrates increased homozygosity with D5S394.

Only a few familial cases suggesting an autosomal recessive inheritance of AMC have been reported (Ek 1958; Frischknecht et al. 1960; Bargeton et al. 1961; Swinyard 1963; Laitinen and Hirvensalo 1966; Pena et al. 1968; Gustavson and Jorulf 1976). Whether the gene causing AMC in the family that we studied is the same as the one that is responsible for other cases of familial arthrogryposis is yet to be clarified. In some familial cases this can be tested by linkage study, although in small families the answer will await identification of the gene itself.

Since AMC is a heterogeneous group of disorders, various mechanisms may play a role in the development of AMC in the family that we studied. In a few patients with AMC, morphological changes in the spinal cord and Betz cells have been reported (Ek 1958; Frischknecht et al. 1960; Bargeton et al. 1961). These cases indicate that, in some AMC patients, the involved gene may play a role in the development or organization of the spinal-cord cells. Indeed, Burglen et al. (1996) have demonstrated that a subset of children with AMC have deletions of SMN. Genes such as those for MSH homeobox homologue 2 (HOX 8), dopamine receptor, beta synuclein, and others that have been mapped to this general region will have to be considered after the location of the AMC gene in this family has been further narrowed.

Of interest are our findings of haplotype sharing, at the critical region, by all affected individuals in this consanguineous family. Complete homozygosity was found in all affected individuals with marker D5S394 genotype 3, whereas the frequency of allele 3 in the parental generation was 18/30. Analysis of the homozygosity at D5S394 therefore provides significant statistical evidence in favor of linkage. Analysis of genotype sharing, by affected individuals, at markers D5S1456 (23/30 individuals) and D5S498 (27/30 individuals) helps in the effort to estimate the critical interval, but it is not accurate for calculation of the recombination distances. A single recombination at D5S1456 in a generation preceding those available for study caused a relatively high proportion of heterozygotes (47%, a proportion similar to that in unaffected siblings), although this marker is only 5.5 cM away from D5S394 (which showed com-

plete homozygosity). This emphasizes the importance of genotype-sharing analysis over heterozygosity/homozygosity comparison, whenever homozygosity molecular testing is used for screening for linkage.

The results of this genetic-linkage study place the AMC disease locus between marker D5S1456, on the centromeric side, and marker D5S498, on the telomeric side. Further and fine mapping of the AMC gene in this family will allow carrier testing, which is so important in this markedly inbred kindred. In addition, it will provide the means for identification of the AMC gene.

Acknowledgments

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