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Autosomal Dominant Distal Myopathy: Linkage to Chromosome 14

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Summary

We have studied a family segregating a form of autosomal dominant distal myopathy (MIM 160500) and containing nine living affected individuals. The myopathy in this family is closest in clinical phenotype to that first described by Gowers in 1902. A search for linkage was conducted using microsatellite, VNTR, and RFLP markers. In total, 92 markers on all 22 autosomes were run. Positive linkage was obtained with 14 of 15 markers tested on chromosome 14, with little indication of linkage elsewhere in the genome. Maximum two-point LOD scores of 2.60 at recombination fraction .00 were obtained for the markers MYH7 and D14S64—the family structure precludes a two-point LOD score ≥3. Recombinations with D14S72 and D14S49 indicate that this distal myopathy locus, *MPD1,* should lie between these markers. A multipoint analysis assuming 100% penetrance and using the markers D14S72, D14S50, MYH7, D14S64, D14S54, and D14S49 gave a LOD score of exactly 3 at MYH7. Analysis at a penetrance of 80% gave a LOD score of 2.8 at this marker. This probable localization of a gene for distal myopathy, *MPD1,* on chromosome 14 should allow other investigators studying distal myopathy families to test this region for linkage in other types of the disease, to confirm linkage or to demonstrate the likely genetic heterogeneity.

Introduction

The distal myopathies are a heterogeneous group of disorders showing both autosomal dominant and autosomal recessive inheritance, with diverse phenotypic features and pathological changes (Mastaglia 1991). Gowers (1902) described a patient with weakness and wasting of the hand, forearm, and anterior tibial muscles, which had developed by the age of 10 years; and by the age of 18 years there was also severe wasting of the sternomastoids, wasting of the tongue, and weakness of the facial muscles. This is accepted as the first description of the hereditary distal myopathies (MIM 160500; McKusick 1992), although it has been suggested that Gowers's patient may have had myotonic dystrophy, which shows a similar pattern of muscle atrophy and weakness (see Walton and Gardner-Medwin 1981). The better-known of the distal myopathies are the dominantly transmitted forms, occurring particularly in Scandinavia, which may have a late onset and either a relatively benign course (Welander 1951) or a more rapid progression (Edström et al. 1980). Recessive forms have also been described, particularly in Japan, where a distinctive myopathy characterized histologically by the presence of rimmed vacuoles in muscle fibers occurs (Nonaka et al. 1981).

To date, genetic linkage has not been demonstrated in any of the forms of distal myopathy, nor have any candidate genes been identified (McKusick 1992). We report here positive linkage to the long arm of chromosome 14 in a family with dominantly inherited distal myopathy with phenotypic features closely resembling those described in Gowers's original report (Gowers 1902). These results have previously been reported in brief (Meredith et al. 1994).

Subjects and Methods

Family

The condition was dominantly transmitted with high penetrance in three generations of the family, which is of English/Welsh origin (fig. 1). Nine affected and three unaffected members from three generations were examined.

Clinical Features

Age at onset was 4–25 years, with selective weakness of the toe and ankle extensors and the neck flexors, followed, after several years, by progressive weakness of the finger extensors. The finger flexors and intrinsic hand muscles were relatively unaffected, but certain proximal muscle groups such as the hip abductors and external rotators and shoulder abductors were mildly affected. Although progression was gradual, there was eventually a moderate degree of incapacity, as in II:2, the oldest living affected family member, who was initially investigated in 1969 and who

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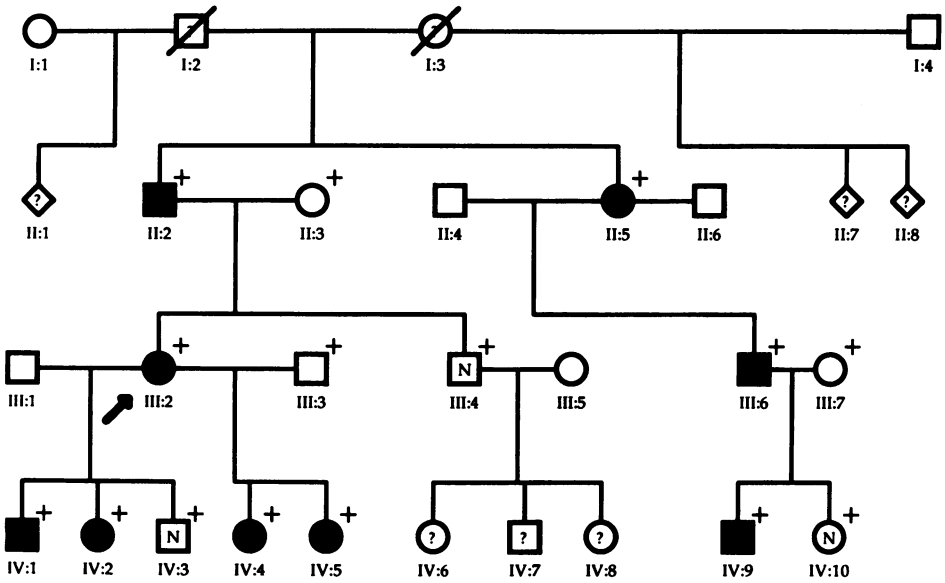

Figure 1 Pedigree of the family segregating distal myopathy. The proband (III:2) is indicated by the arrow. Each individual marked with a cross was examined clinically, and an immortalized cell line was established for him or her. Blackened symbols denote affected individuals; an "N" in the center of the symbol denotes that the individual is clinically unaffected, and a question mark (?) in the center of the symbol denotes that the individual's status is unknown.

was reexamined in 1992 when he was still walking but had difficulty maintaining an erect posture when standing. In all affected individuals the deep-tendon reflexes were preserved, and the plantar responses were flexor. There was no myotonia, sensory impairment, or other neurological abnormality. Serum creatine kinase levels were elevated (216–531 U/liter [normal <180 U/liter]) in three affected individuals.

Electrodiagnostic Studies

Electromyography and nerve conduction studies were performed in the proband (III:2; fig. 1) and in II:2 and IV:2. Muscle sampling with a concentric needle electrode showed striking myopathic motor-unit potential changes (i.e., low amplitude and brief duration units, with many polyphasic units) and a full, low-amplitude (<0.5 mV) interference pattern particularly in affected distal limb muscles and, to a lesser extent, in some proximal muscles. Occasional spontaneous fibrillation potentials and positive waves were present in some affected muscles in III:2. Myotonia was not found. Results of motor- and sensory-nerve conduction studies in the upper and lower limbs were normal.

Histological Changes

An open biopsy taken from the left vastus lateralis muscle in III:2 showed occasional necrotic and regenerating fibers, excessive variation in fiber size, increased numbers of fibers with internal nuclei, occasional angulated atrophic fibers, and nuclear clumps (fig. 2). There were no rimmed vacuoles or sarcoplasmic inclusions. Histochemistry showed only nonspecific changes with moth-eaten fibers and increased enzyme activity in angulated fibers in the NADH-TR preparations (fig. 2). There was no pathological fiber-type grouping. There were no sarcoplasmic inclusion bodies as found in the family described by Edström et al. (1980). Results of dystrophin and desmin immunohistochemistry were normal. A biopsy of the left tibialis anterior muscle from II:2, taken in 1969, showed evidence of end-stage disease with extreme muscle-fiber atrophy, many central nuclei and nuclear clumps, and condensation of endomysial connective tissue.

Blood Samples

Blood samples were taken, and immortalized cell lines were established for the 15 family members who volunteered to participate in the study (fig. 1). Venous blood samples (≤40 ml) from adults were split 50:50 into lithium-heparin and EDTA tubes for, respectively, lymphocyte isolation and immortalization with Epstein-Barr virus (Neitzel 1986) and immediate DNA isolation by either conventional phenol-chloroform or salt-precipitation (Miller et al. 1988) techniques.

Linkage Analysis

Ninety-two polymorphic markers spread over all autosomes were tested. These were D1S80, D1S185, D1S176, CRP, D1S104, AT3, and ACTN2; APOB, D2S44, HOX4,

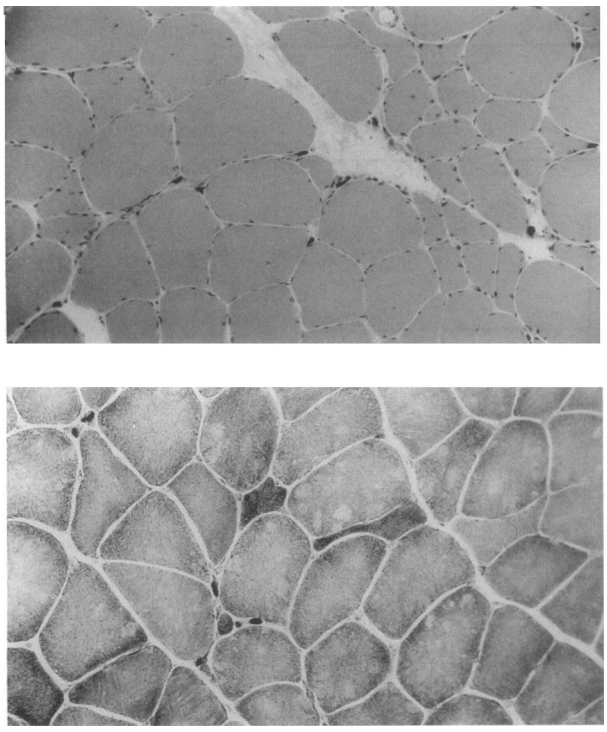

Figure 2 *Top,* Vastus lateralis biopsy from III:2, showing excessive variation in myofibril size and nuclear clumps: hemotoxylin and eosin (× 400). *Bottom,* Vastus lateralis biopsy from III:2, showing darkly stained angulated atrophic type II fibers and moth-eaten type I fibers: NADH-TR (× 400).

and D2S102; D3S1307, D3S1304, D3S1285, D3S1287, D3S1279, D3S1282, D3S1262, and D3S1311; HD, D4S174, and D4S171; D5S268, D5S112, D5S39, APC, and D5S210; DMDL; D7S472, D7S435, D7S474, GCK, D7S440, and CF; D8S166, D8S84, D8S198, and D8S200; D9S104, D9S15, and D9S53; D10S28; D11S875, D11S873, CD3D, and D11S836; D12S43 and D12S60; RB1; D14S72, D14S50, MYH7, D14S64, D14S54, D14S49, D14S52, D14S76, D14S53, D14S74, D14S48, D14S81, D14S45, D14S51, and D14S31; D15S87; D16S291, D16S292, D16S287, D16S295, D16S298, D16S300, D16S308, D16S265, D16S186, D16S301, D16S260, D16S266, and D16S305; D17S30, D17S122, GX-Alu, HOXB, and D17S26; D18S40 and D18S51; D19S75, APOC2, and DM; D20S66; D21S210 and D21S213; and D22S258.

VNTR Analysis

Probes for genotyping the VNTR markers D2S44, D10S28, D14S13, and D17S26 were purchased from Promega. For each family member, 4 μg of DNA isolated as above was digested overnight with *Hae*III (Amersham). Fragments separated in a 0.8% agarose gel electrophoresis were alkali-transferred to Hybond N^+ nylon membranes (Amersham) and hybridized with inserts labeled by random priming (Amersham). Cronex 4 film (Du Pont) was exposed for 7–10 d at −80°C with single intensifying screens.

Microsatellite and PCR Polymorphisms

A Cyclone Plus DNA Synthesiser (Milligen/Biosearch) was used to synthesize primers for microsatellite (Weber and May 1989) and PCR polymorphisms. Primer sequences were obtained from the Genome Data Base. Microsatellite PCR conditions for a reaction volume of 20 μl were as follows: 4 μl of 5 × buffer (335 mM Tris-HCl [pH 8.8] at 25°C, 83 mM $(NH_4)_2SO_4$, 100 μM dNTP, 0.2 μl of 25 mM $MgCl_2$, 50 ng of each primer, 0.1 μl of ^{32}P-dCTP (3,000 Ci/mmol; Amersham), 50 ng of target DNA, and 0.5 unit of *Tth* polymerase (Biotech International). The reactions were overlaid with mineral oil, and the cycling conditions were as follows: 94°C for 5 min to denature, 58°C for 6 min to anneal and elongate for one cycle, followed by 35 cycles of 94°C for 1 min and 58°C for 6 min. Where necessary, annealing temperatures were raised to improve primer specificity. Products were then refrigerated at 4°C until gel electrophoresis. Aliquots of the reaction product were mixed with an equal volume of formamide-loading buffer and, depending on expected allele size, were electrophoresed on standard 6% or 4% acrylamide denaturing sequencing gels (Sambrook et al. 1989, chap. 13). PCR reaction conditions for four nonmicrosatellite polymorphisms (APOB, AT3, HD, and D17S30) were similar, except that no radioactive nucleotide was included in the reaction mix and aliquots of the reaction products were electrophoresed in 2% agarose.

Linkage Analysis

Two-point linkage analysis was carried out using the method of maximum likelihood and LOD scores (Morton 1955), by the computer program LIPED (Ott 1974). Analysis of the combined data from all the markers was performed using the EXCLUDE program (Edwards 1987; Sarfarazi et al. 1989). The chromosomal positions of loci were estimated from the NIH/CEPH Collaborative Mapping Group (1992) linkage maps, the second-generation linkage map of the human genome (Weissenbach et al. 1992), and data from the Cooperative Human Linkage Center. Multipoint linkage analysis was performed using the CRIMAP version 2.4 package, as described elsewhere (Mulley et al. 1993), at 100% and 80% penetrance.

Results

Linkage

In total, 92 marker loci spread over all 22 autosomes were typed in the distal myopathy pedigree. Two markers on chromosome 8 gave positive two-point LOD scores >1.00. D8S84 gave a LOD score of 1.11 at recombination

Table 1

Results of Two-Point Linkage Analyses, for Linkage between MPD1 and Markers on Chromosome 14

Locus	Location[a]	LOD Score at θ = 0	.001	.01	.05	.10	.20	.30	.40
D14S72	q11	−3.13	−1.56	−.59	.03	.23	.31	.26	.15
D14S50	q11	2.31	2.31	2.27	2.12	1.93	1.51	1.06	.57
MYH7	q11	2.60	2.59	2.55	2.36	2.12	1.61	1.08	.53
D14S64	q11-q32	2.60	2.59	2.55	2.36	2.12	1.61	1.08	.53
D14S54	q11-q32	.41	.41	.40	.35	.29	.17	.09	.02
D14S49	q11-q32	−2.49	−1.25	−.30	.30	.47	.49	.36	.19
D14S52	q11-q32	−99	−3.41	−1.45	−.22	.17	.32	.23	.10
D14S76	q11-q32	−99	−6.39	−3.42	−1.46	−.72	−.17	.01	.05
D14S53	q11-q32	−99	−3.31	−1.38	−.14	.27	.49	.44	.27
D14S74	q11-q32	−99	−.99	−.02	.55	.69	.66	.52	.30
D14S48	q11-q32	−99	−.47	.49	1.00	1.06	.87	.56	.26
D14S81	q11-q32	.20	.20	.19	.15	.10	.04	.01	.00
D14S45	q32.1-qter	−99	−2.20	−1.21	−.57	−.34	−.15	−.07	−.02
D14S51	q32.1-qter	−99	−3.77	−1.80	−.53	−.10	.15	.15	.08
D14S13	q32.1-qter	−99	−1.61	−.63	−.03	.15	.19	.13	.04

[a] From NIH CEPH Collaborative Mapping Group (1992) and Weissenbach et al. (1992).

fraction (θ) .00, and D8S200 gave a LOD score of 1.09 at θ = .00. These two markers were, however, only informative in one branch of the family, and other markers on chromosome 8—i.e., D8S166 and D8S198—which gave information for the other branch of the family, showed multiple recombinants. Thus, significantly negative LOD scores were obtained for these markers, and chromosome 8 is unlikely to be the site of the disease locus. More strongly positive two-point LOD scores were obtained with markers on chromosome 14. D14S50, MYH7, and D14S64 showed no recombinants (table 1) and two-point LOD scores of 2.31, 2.60, and 2.60, respectively. A multipoint LOD score of 3.00 was obtained for MYH7, and 2.99 for D14S64, when the information for D14S72, D14S50, MYH7, D14S64, D14S54, and D14S49 was used (fig. 3), under the assumption of 100% penetrance. A multipoint LOD score of 2.80 was obtained for MYH7 at a penetrance of 80%. Recombinants with D14S49 and D14S72 indicate that the disease locus, *MPD1*, should lie between these markers.

EXCLUDE

The EXCLUDE program (Edwards 1987) was used to estimate the likelihood that the distal myopathy gene in this family lies on chromosome 14 and to estimate the proportion of the genome excluded as the possible site for the disease gene. Running the EXCLUDE program for the entire data set gave a probability of 99.9% that the gene responsible for autosomal dominant distal myopathy in this family, *MPD1*, lies on chromosome 14. The same probability of 99.9% for the disease gene lying on chromo-

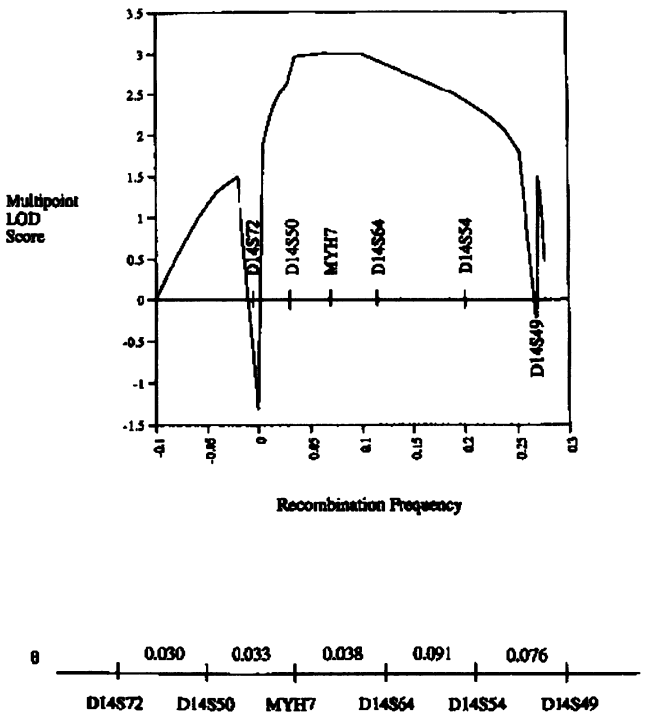

Figure 3 Multipoint analysis for the markers D14S72, D14S50, MYH7, D14S64, D14S54, and D14S49, assuming 100% penetrance. The graph shows multipoint LOD scores for chromosome 14; the LOD score for a penetrance of 80% was 2.8 at MYH7. Shown beneath the graph is the background map used to localize the disease genes. θ is sex-averaged and is calculated by using Haldane's mapping function.

some 14 was obtained from EXCLUDE in an affecteds-only analysis. Running the EXCLUDE program for the data set minus the data for chromosome 14 showed that a considerable proportion, ≥50%, of the genome had been excluded as the possible site of the disease gene in this family.

Discussion

The pedigree investigated displays a form of distal myopathy that is quite distinct from the better-known distal myopathies described in Scandinavia and Japan. Welander (1951) described a benign Scandinavian form with weakness first occurring in the fingers and wrists and later progressing to involve the anterior tibial and calf muscles. There was also early involvement of the thumb, wrist, and finger muscles in the severe Scandinavian form described by Edström et al. (1980), with weakness progressing to other muscles and leading to major disability within 15 years of onset of the disease. In comparison, the distal myopathy described here is very similar to that reported by Gowers (1902), with early involvement of the distal lower limb muscles and the sternomastoids and with relative sparing of the hand and forearm muscles.

The distal myopathy in this family shows obvious dominant inheritance. A considerable portion of the genome has been scanned with the 92 markers tested. The best evidence of linkage is seen with chromosome 14. The EXCLUDE program (Edwards 1987) gives a 99.9% likelihood of linkage to chromosome 14, both in an affecteds-only analysis and when data for all the family members are used, while multipoint analysis gives a LOD score of 3.00 at MYH7, under the assumption of 100% penetrance, and gives a LOD score of 2.8 at 80% penetrance. There is little evidence of linkage elsewhere. It is unlikely that a larger LOD score will be obtained from study of this family, since the Australian branch of the family has lost contact with any remaining relatives in England/Wales, and, despite attempts to identify such relatives, none have been found. It is also possible that there are no other affected relatives.

Both the linkage to MYH7 and D14S64 and the recombinations with D14S49 and D14S72 indicate that the mutated gene, *MPD1,* should lie in the region of these markers. Both the distal myopathy in this family and the other distal myopathies affect restricted groups of muscles in defined locations of the body; other muscle diseases affect other restricted muscle groups. Exactly what type of genes that, when mutated, could cause muscle diseases restricted to certain muscle groups is uncertain. They could perhaps be genes coding for muscle structural proteins, though this might seem unlikely because such proteins should be expressed in all muscle groups. The mutated genes might, on the other hand, be genes involved in delineating body segments during development. Recently a repeated homeodomain has been implicated in another restricted form of muscle disease, facioscapulohumeral muscular dystrophy (Wijmenga et al. 1992), although the exact relationship between the homeodomain and the disease has been brought into question by the identification of recombinations between the homeodomain marker and the disease (Weiffenbach et al. 1993).

Two muscle genes that are known to lie within the *MPD1* linkage region on chromosome 14 are the alpha and beta cardiac myosin genes MYH6 and MYH7 (Matsuoka et al. 1989). Although beta cardiac myosin is expressed in skeletal muscle (Jandreski et al. 1987), it is unlikely that mutations in that gene are the basis of the distal myopathy in the family under investigation, since mutations in the beta cardiac myosin gene lead to hypertrophic cardiomyopathy, which is not present in this family. Markesbery et al. (1974) reported autopsy findings of cardiomyopathy in their late-onset hereditary distal myopathy patients, but the pattern of muscle weakness in their patients was different from that in the family described here. Nevertheless, the cardiac myosin genes must be considered as candidate genes for *MPD1* and should be investigated for mutations in affected family members.

Despite the phenotypic differences between the Gowers and other forms of distal myopathy, it is possible that some are allelic, with different mutations producing the different phenotypes. The suggested linkage to chromosome 14 should now allow other investigators researching distal myopathy families to confirm linkage or demonstrate the heterogeneity that might be expected from the variable clinical phenotypes seen in the condition.

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