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Assignment of a Gene (NEM1) for Autosomal Dominant Nemaline Myopathy to Chromosome 1

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Summary

Nemaline myopathy (NEM) is a neuromuscular disorder characterized by the presence, in skeletal muscle, of nemaline rods composed at least in part of α-actinin. A candidate gene and linkage approach was used to localize the gene (NEM1) for an autosomal dominant form (MIM 161800) in one large kindred with 10 living affected family members. Markers on chromosome 19 that were linked to the central core disease gene, a marker at the complement 3 locus, and a marker on chromosome 1 at the α-actinin locus exclude these three candidate genes. The family was fully informative for APOA2, which is localized to 1q21-q23. NEM1 was assigned to chromosome 1 by close linkage for APOA2, which is localized to 1q21-q23. NEM1 was assigned to chromosome 1 by close linkage to APOA2, with a lod score of 3.8 at a recombination fraction of 0. Recombinants with NGFB (1p13) and AT3 (1q23-25.1) indicate that NEM1 lies between 1p13 and 1q25.1. In total, 47 loci were investigated on chromosomes 1, 2, 4, 5, 7–11, 14, 16, 17, and 19, with no indications of significant linkage other than to markers on chromosome 1.

Introduction

Nemaline myopathy (NEM) was first described by Shy et al. (1963). It is "the most frequently reported, best known and most intensively studied of the congenital myopathies" (Gardner-Medwin and Tizard 1981). It is an autosomal dominant neuromuscular disorder (McKusick 1990) characterized by the presence, in skeletal muscle, of nemaline rods composed at least in part of α-actinin (Jennekens et al. 1983). The disease usually manifests with hypotonia in infancy which is described as nonprogressive, but deterioration can occur, and death may ensue from respiratory muscle involvement. Some cases may be mild or subclinical (Gardner-Medwin and Tizard 1981; Dubowitz 1985). Late-onset variants of the disease occur, including a rapidly progressive adult-onset myopathy (Dubowitz 1985).

McKusick (1990) suggests three candidate genes: (1) complement 3 (C3), since abnormal levels were reported in one family with nemaline myopathy; (2) the gene for central core disease (CCO), since central cores and nemaline rods are periodically seen in the same muscle biopsy (Dubowitz 1985); and (3) the skeletal muscle α-actinin genes, since the nemaline rods are largely composed of α-actinin (Jennekens et al. 1983). The search for linkage was based on a candidate-locus approach, together with highly polymorphic VNTRs (Nakamura et al. 1987) and microsatellite markers (Weber and May 1989) spread throughout the genome.

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Subjects and Methods

Family

The family studied showed autosomal dominant inheritance over five generations (fig. 1). The proband (IV-3) had normal motor development until, in junior school, he developed symmetrical weakness in dorsiflexion of his feet. The weakness progressed over adolescence, to involve all ankle movements and more-proximal limb musculature. His gait was high stepping, and he could not run with his peers. Gradual deterioration continued, but no walking aids were required until after he fractured the neck of his left femur at age 39 years, which necessitated use of a walking stick. As a teenager, he had difficulty eating, experiencing dysphagia and later regurgitation. Mild shoulder-girdle wasting was present, but there was no wasting of the intrinsic hand muscles. The scapulae did not wing, and there was mild pectus excavatum. Upper-limb power was normal, but the lower limbs were grossly wasted, particularly in the lateral compartment of the leg, and there was pes cavus. The reflexes were depressed in the upper limbs and were absent in the lower limbs. Plantar responses were flexor and sensation normal. The motor- and sensory-conduction velocities were normal, excluding a neuropathic process, in particular Charcot-Marie-Tooth (CMT) disease, which may have a similar distribution of weakness in the lower limbs. Electromyography showed myopathic features, with low amplitude, short-duration polyphasic units in the deltoid, tibialis anterior, and vastus lateralis muscle. There were no neurogenic features. A left-deltoid biopsy revealed marked variation in muscle fiber size, from 5 to 120 microns. Nemaline bodies were numerous on staining with Gomori trichrome pH 3.4 (fig. 2, *left*), particularly within type 1 fibers, which were predominant. There were some atrophic fibers which appeared to contain nemaline bodies only. Electron microscopy (EM) confirmed both the nature of the nemaline bodies and the typical lattice appearance with transverse and longitudinal striations (fig. 2, *top*). The proband also had achalasia and chronic upper-intestinal pseudo-obstruction, as demonstrated by barium studies.

The family history extended over five generations. No firm diagnosis had been made, the disorder having been labeled as either an atypical limb-girdle dystrophy or a variant of facioscapulohumeral dystrophy. The pattern of weakness was extremely consistent in the three generations studied. Mild proximal arm wasting and weakness were present in some family members; however, weakness of ankle movements with foot drop was prominent in all affected family members from the onset. There was no wasting of intrinsic hand muscles, and sensation was completely normal.

The proband's deceased uncle (III-10) had more extensive weakness when he was examined by a neurologist in 1978; symptoms included mild bilateral facial weakness, mild weakness of sternocleiodomastoid and trapezius, mild winging of the scapulae and gross wasting and weakness of the quadriceps and of the anterior tibial group of muscles. Electromyography showed end-stage muscular disease, with no evidence of neurogenic disorder. Muscle biopsy was performed in 1978, and review showed severe atrophic changes only. Gomori trichrome stain was not available. EM showed Z-band streaming. The conclusion at that time was that the uncle had a variant of facioscapulohumeral dystrophy. The proband's father (III-4) had also had a muscle biopsy, which showed only nonspecific myopathic changes. Serum creatine kinase levels were not elevated in the index case and were not examined in other family members.

The disease status of young family members V-3 and V-6 has been designated "unknown," as clinical signs of disease were absent in both, but the family considered V-6 to have "the family walk." Electromyography was not felt to be appropriate, since there were no definite clinically weak muscles to direct testing toward and since, at the stage of minimal clinical involvement, electromyography results may also be within normal limits.

Blood Samples

Blood samples were taken, and immortalized cell lines were established for all living family members. Venous blood samples of up to 40 ml from adults were split 50:50 into lithium heparin and EDTA tubes for, respectively, immortalization with Epstein-Barr virus (Neitzel 1986) and immediate DNA isolation by either conventional phenol-choloroform or salt-precipitation techniques (Miller et al. 1988).

RFLP Analysis

DNA isolated either from the EDTA sample or from cell lines was digested with the restriction endonuclease (New England Biolabs and TOYOBO) appropriate for the RFLP under investigation, according to the instructions of the manufacturer. Fragments were

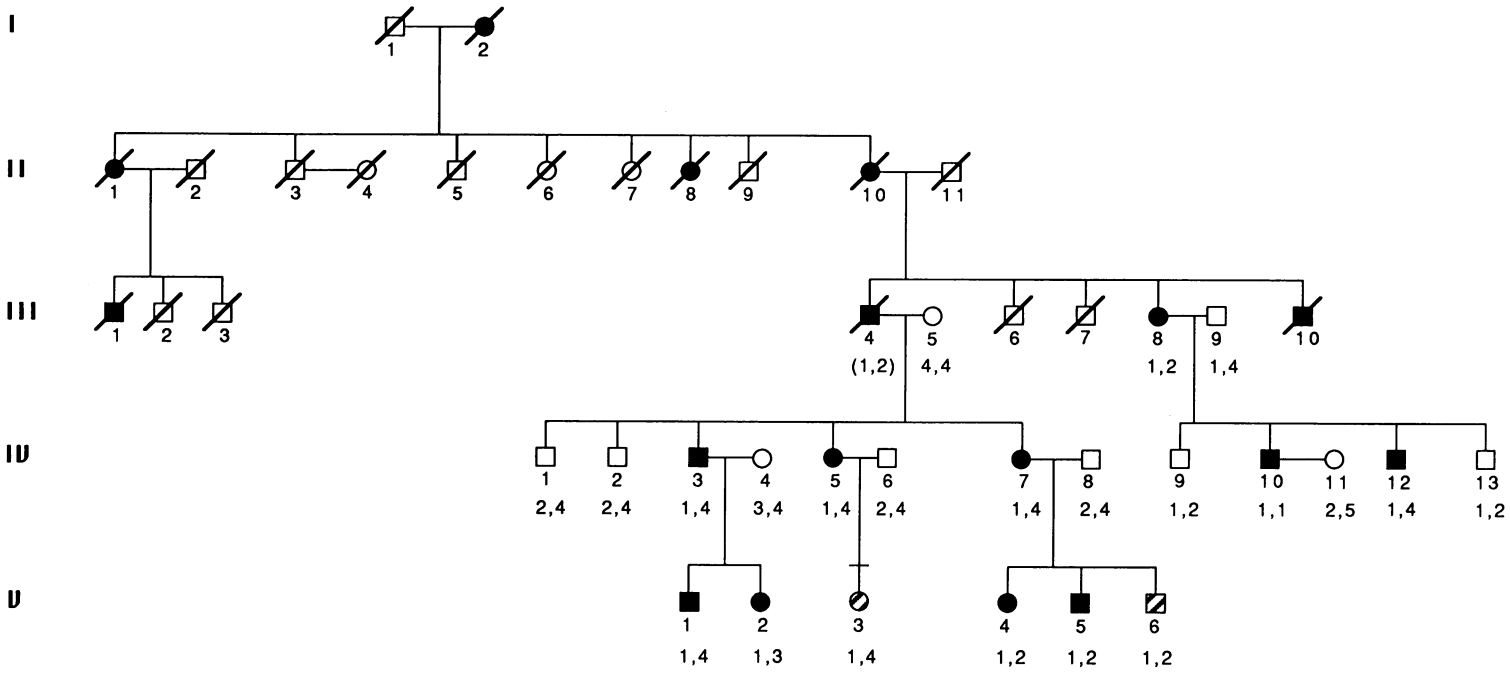

Figure 1 Pedigree of family studied. APOA2 (Mfd3) genotypes are shown. The genotype for III-4 was inferred.

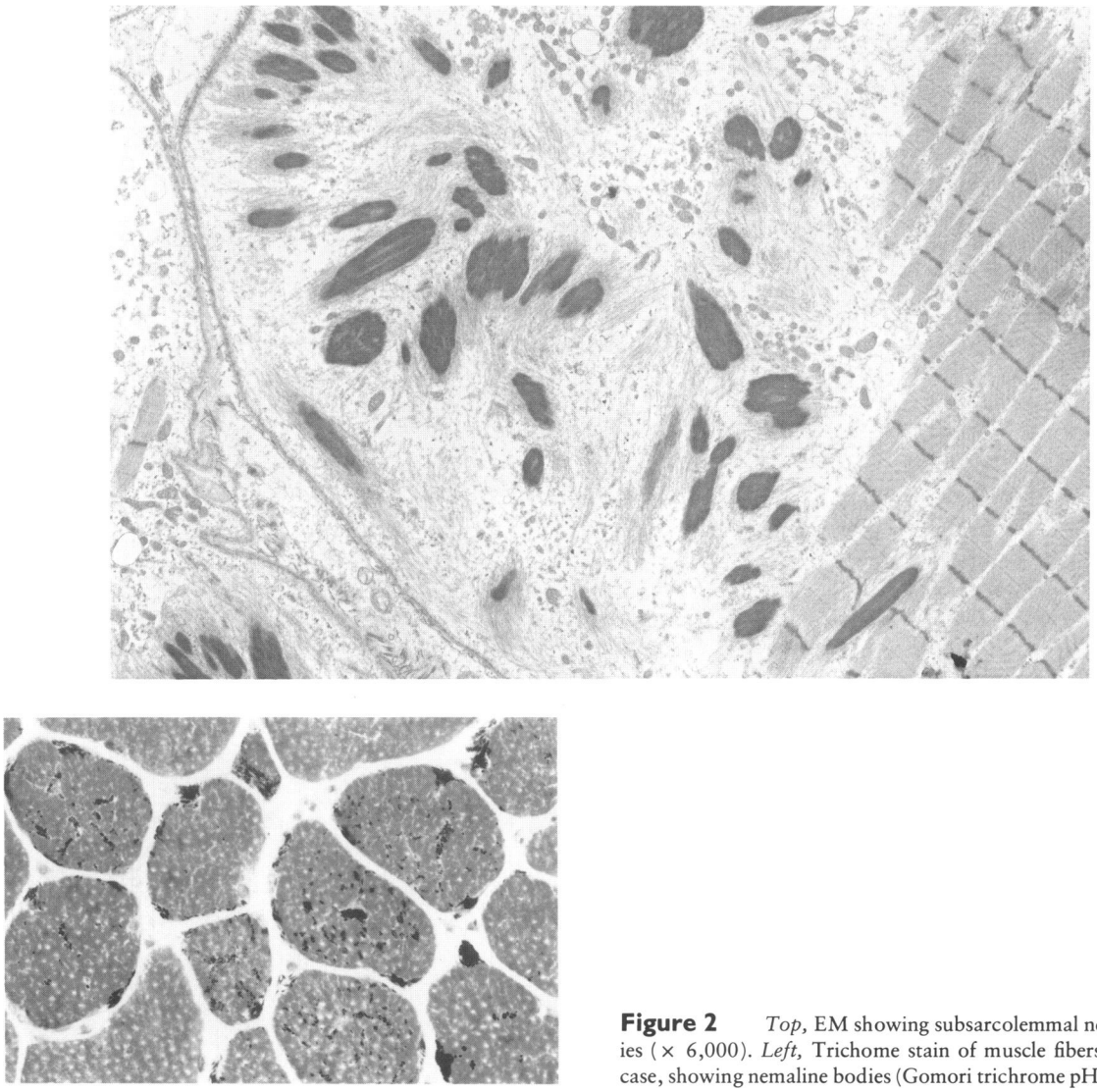

Figure 2 *Top,* EM showing subsarcolemmal nemaline bodies (× 6,000). *Left,* Trichome stain of muscle fibers from index case, showing nemaline bodies (Gomori trichrome pH 3.4 × 455).

separated in 0.8%–1.5% agarose-gel electrophoresis, were alkali transferred to nylon membranes (Genescreen Plus from NEN; Hybond N^+ from Amersham) and hybridized with nick-translated or random prime–labeled (Amersham) whole plasmids or isolated inserts, depending on the probe (Laing et al. 1990). Cronex 4 film (DuPont) was exposed for 1–10 d at −70°C with single intensifying screens. Probes for genotyping the markers NGFB, D1S2, D1S61, D5S21, D5S39, D11S84, D19S7, and D19S9 were obtained from the NIH Repository of Human DNA Probes and Libraries (ATCC); D1S47 was from Collaborative Research International; and D2S44, D4S139, D10S28, D14S13, and D17S26 were from Promega. Other clones were obtained from their originators: α-spectrin clone 3021E1 from B. Forget, α-spectrin clone pHalphaSp5 from P. Curtis, D4S10 from J. Gusella, TCRB and TCRA from T. Mack, C3 from G. Fey, RYR from D. H. McLennan, and APOC2 from R. Williamson.

Microsatellite and PCR Polymorphisms

Primers for microsatellite and PCR polymorphisms (Weber and May 1989) were synthesized on a Gene Assembler Plus (Pharmacia) and were gel purified. Microsatellite PCR conditions for a reaction volume of 20 μl were as follows: 4 μl 5 × buffer (335 mM Tris-HCl [pH 8.8] at 25°C; 83 mM $(NH_4)_2$ SO_4, 1 mM

dNTP, 1 mg gelatin/ml, and 2.25% Triton X-100), 1.2 μl 25 mM $MgCl_2$, 50 ng each primer, 0.2 μl ^{32}P-dCTP (3,000 Ci/mmol; Amersham) 10 ng target DNA, and 1 unit *Taq* polymerase (Biotech International). The reactions were overlaid with mineral oil, and temperature 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 39 cycles of 94°C for 1 min; and 58°C for 6 min, with the final elongation time extended to 15 min. Aliquots of the reaction product were mixed with an equal volume of formamide loading buffer and were electrophoresed on standard 6% acrylamide denaturing sequencing gels (Sambrook et al. 1989, chap. 13). PCR reaction conditions for nonmicrosatellite polymorphisms were similar, except that (*a*) no radioactive nucleotide was included in the reaction mix and (*b*) aliquots of the reaction product were electrophoresed in 3% agarose.

The microsatellite and PCR polymorphism primers were synthesized according to the sequences published by Boerwinkle et al. (1989) (APOB), Weber and May (1989) (APOA2, APOC2, CRP, D8S84, and D19S47), Wu et al. (1989) (AT3), Wallis et al. (1990) (D9S15), and Weber et al. (1990) (CD3D). The primer sequences for the unpublished microsatellite marker GXAlu for NF1 were provided by G. Xu. The microsatellite for the chromosome 1 α-actinin gene (ACTN2) originated in the Boston laboratory (author's unpublished data). The 10 $(CA)_n$ repeat markers for chromosome 16 originated from the Adelaide Laboratory (authors' unpublished data).

Skeletal muscle α-actinin genes

The skeletal muscle α-actinin genes were localized to chromosomes 1 and 11 by using somatic cell hybrids (A. H. Beggs, unpublished data). The chromosome 1 α-actinin gene was localized to 1q42-qter by in situ hybridization (A. H. Beggs and J. H. M. Knoll, unpublished data).

Linkage analysis

Two-point LOD scores were calculated using the LIPED computer program (Ott 1974). The linkage data for all the probes were then used as input for

Table 1

Lod Scores for Linkage between NEM1 and Loci on Chromosomes 1 and 19

Locus (location[a])	Lod Score at θ of							
	0	.001	.01	.05	.10	.20	.30	.40
Chromosome 1:								
D1S47 (pter-p34.1)	−99	−5.67	−2.73	−.79	−.10	.34	.36	.20
D1S2 (pter-p31)	−99	−4.70	−2.72	−1.37	−.83	−.35	−.13	−.03
D1S57 (pter-p31)	.16	.16	.16	.13	.11	.06	.03	.01
NGFβ (p13)	−99	−2.27	−.31	.87	1.19	1.20	.89	.43
MUC1 (q21-q23)	1.45	1.45	1.42	1.29	1.13	.80	.47	.18
SPTA1 (q21)	.12	.12	.11	.09	.08	.04	.02	.01
CRP (q21-q23)	2.11	2.11	2.07	1.89	1.67	1.19	.71	.26
APOA2 (q21-q23)	3.80	3.80	3.74	3.48	3.14	2.42	1.62	.74
D1S104 (q)	1.11	1.10	1.08	.96	.82	.51	.23	.05
D1S61 (q)	1.11	1.10	1.08	.96	.82	.53	.26	.06
AT3 (q23-q25.1)	−99	−2.13	−1.15	−.52	−.29	−.11	−.03	.01
ACTN2 (q42-qter)	−99	−7.01	−4.03	−2.01	−1.20	−.51	−.19	−.04
Chromosome 19:								
C3 (p13.3-p13.2)	−99	−5.01	−3.01	−1.61	−1.01	−.45	−.18	−.04
D19S7 (cen-q12)	−99	−2.02	−1.04	−.40	−.18	−.04	−.01	−.00
D19S9 (q12-q13)	−99	−9.61	−5.65	−2.93	−1.82	−.80	−.31	−.07
D19S75 (q13.1)	−99	−14.26	−8.30	−4.25	−2.62	−1.21	−.56	−.21
RYR (q13.1)	−99	−3.99	−2.17	−.87	−.39	−.05	−.02	−.01
D19S47 (q12-q13.1) ...	−99	−9.47	−5.49	−2.80	−1.74	−.82	−.41	−.18
APOC2 (q13.2)	−99	−8.90	−4.93	−2.27	−1.25	−.42	−.12	−.02
APOC2[b] (q13.2)	−99	−11.27	−6.30	−2.97	−1.67	−.61	−.19	−.04
KLK1 (q13.3-q13.4) ...	−99	−11.45	−6.52	−3.16	−1.84	−.71	−.24	−.05

[a] Sources: Human Gene Mapping 10.5 (1990); Dracopoli et al. (1991); and A. H. Beggs, et al. (unpublished data).

[b] Microsatellite polymorphism Mfd5 (Weber and May 1989).

Table 2

Combined Linkage Data Used to Obtain NEM1 Exclusion Map

Chromosomal Position[a] (locus)	θ	Lod Score	r^b	s^c	Location[d]
1.05 (D1S47)	.35	.39	1.9	7.3	pter-p34.1
1.23 (D1S57)	.00	.16	. . .	. . .	pter-p31
1.34 (D1S2)	.10	−.83	1.9	3.7	pter-p31
1.50 (NGFB)	.25	1.25	1.6	10.6	p13
1.57 (MUC1)	.00	1.45	.0	4.8	q21-q23
1.58 (SPTA1)	.00	.12	. . .	. . .	q21
1.59 (CRP)	.00	2.11	.0	7.0	q21-q23
1.61 (APOA2)	.00	3.80	.0	13.0	q21-q23
1.62 (D1S104)	.00	1.11	.0	3.7	q
1.65 (D1S61)	.00	1.11	.0	3.7	q
1.67 (AT3)	.43	.01	.9	2.1	q23-q25.1
1.95 (ACTN2)	.10	−1.20	3.5	7.0	q42-qter
2.07 (APOB)	.10	−1.17	2.6	5.3	p24-p23
2.23 (D2S44)	.10	−3.46	7.8	15.6	p
4.04 (D4S10)	.10	−2.19	4.9	9.9	pter16.3
4.95 (D4S139)	.10	−1.56	3.5	7.0	q
5.21 (D5S21)	.10	−2.86	6.5	12.9	p13-p11
5.41 (D5S39)	.10	−.26	.6	1.2	q12-q14
7.92 (TCRVB)	.10	−.44	1.0	2.0	q35
8.45 (D8S84)	.25	.07	1.7	5.2	q12-q13
9.50 (D9S15)	.31	.25	2.1	7.1	q13-q21.1
10.09 (D10S28)	.10	−2.52	5.7	11.3	pter-p13
11.75 (D11S84)	.10	−.44	1.0	2.0	q22
11.86 (CD3D)	.00	.19	. . .	. . .	q23
14.24 (TCRA)	.10	−1.28	2.9	5.8	q11.2
14.94 (D14S13)	.10	−.76	1.7	3.4	q32
16.04 (D16S94)	.10	−.85	1.9	3.8	p13.3
16.14 (D16S292)	.10	−2.16	4.9	9.7	p13.13-p13.12
16.14 (D16S287)	.10	−1.49	3.4	6.7	p13.12-p13.11
16.21 (D16S294)	.10	−.39	.9	1.8	p12.3-p12.2
16.31 (D16S298)	.10	−1.21	2.7	5.4	p12.1-p11.2
16.36 (D16S300)	.10	−2.22	5.0	10.0	p11.2-cen
16.57 (D16S304)	.10	−.93	2.1	4.2	q12.2
16.81 (D16S301)	.10	−1.43	3.2	6.5	q22.1
16.93 (D16S303)	.10	−1.67	3.8	7.7	q24.2-q24.3
16.93 (D16S305)	.10	−1.93	4.3	8.7	q24.3
17.37 (NF1)	.10	−.54	1.2	2.4	q11.2
17.85 (D17S26)	.10	−1.18	2.7	5.3	q
19.10 (C3)	.10	−1.01	2.4	4.8	p13.3-p13.2
19.50 (D19S7)	.10	−.18	.4	.9	cen-q12
19.64 (D19S9)	.10	−1.82	3.8	7.5	q12-q13.2
19.65 (D19S75)	.10	−2.62	5.9	11.8	q13.1
19.68 (RYR)	.10	−.39	.6	1.2	q13.1
19.72 (D19S47)	.10	−1.74	3.9	7.8	q12-q13.1
19.77 (APOC2)	.10	−1.25	2.8	5.6	q13.2
19.77 (APOC2)	.10	−1.67	3.8	7.5	q13.2
19.80 (KLK1)	.10	−1.84	4.1	8.2	q13.3-q13.4

[a] Location of marker on chromosome is expressed as % of pter-qter.

[b] Equivalent number of recombinants. An ellipsis denotes that too little information was available and that the data were not included in EXCLUDE maps.

[c] Equivalent number of informative meioses. An ellipsis denotes that too little information was available and that the data were not included in EXCLUDE maps.

[d] Sources are as in table 1, plus Adelaide Laboratory (unpublished data) and (J. L. Weber, personal communication: Marshfield markers release 3).

the computer program EXCLUDE (Edwards 1987; Sarfarazi et al. 1989). The chromosomal positions of loci on chromosome 1 were estimated for input to the EXCLUDE program, on the basis of the CEPH consortium map of chromosome 1 (Dracopoli et al. 1991). For other loci the chromosomal positions were estimated from the band localization of the markers on the chromosomes (Sarfarazi et al. 1989). Family members V-3 and V-6, who were of unknown disease status, were not included in the linkage analysis.

Results

Candidate Genes

Recombinants were obtained with polymorphisms for C3, the ryanodine receptor gene, and markers flanking the CCO gene on chromosome 19 and the α-actinin gene on chromosome 1 (table 1). The chromosome 19 data entered into the EXCLUDE program (table 2) gave a zero likelihood that the autosomal dominant NEM (NEM1) locus lies on chromosome 19.

Chromosome 1

Two-point lod scores for chromosome 1 loci versus the disease in the family indicate linkage to APOA2 at 1q21-q23 (table 1). The family was fully informative for APOA2, showing no recombinants (fig. 1) and a lod score of 3.8 at a recombination fraction (θ) of 0. Recombinants with NGFB and AT3 indicate that the disease locus lies between 1p13 and 1q25.1. The two family members of uncertain disease status (V-3 and V-6) both have the allele associated with disease phenotype at APOA2 and the allele associated with normal phenotype at NGFB.

EXCLUDE

Using the entire data set (table 2). The EXCLUDE program gives a probability of 99.98% that the gene responsible for autosomal dominant NEM in this family lies on chromosome 1. Running the EXCLUDE program for the data set minus the data for chromosome 1 demonstrates that a sizable portion of the genome has been excluded as the site of the disease gene in this family.

Discussion

The large kindred segregating NEM1 afforded the opportunity to perform a linkage analysis avoiding the potential difficulties associated with genetic heterogeneity. The pedigree investigated has a late-onset variant of NEM in that the proband showed normal milestones until he began junior school. It may be that the late-onset variant and the more typical NEM are allelic, with different mutations resulting in variable phenotypes, as with Duchenne and Becker dystrophy (Monaco et al. 1988).

Both the linkage to APOA2 and recombinants with NGFB and AT3 indicate that candidate genes must lie within the region 1p13-1q25.1. The NEM1 gene is therefore none of the three candidate genes for NEM that have been suggested by McKusick (1990)—i.e., C3 (Davies et al. 1983) on chromosome 19, CCO (Haan et al. 1990; Kausch et al. 1990) on chromosome 19, and the known skeletal muscle α-actinin genes on chromosomes 1 and 11 (A. H. Beggs, et al., unpublished data). It is possible, however, that as yet unidentified α-actinin genes localized within the appropriate region of chromosome 1 might be involved.

It is of interest that a gene for one form of CMT neuropathy (i.e., CMT1b) has been linked to a similar region of chromosome 1 (Bird et al. 1980; Vance 1991). The affected members of the NEM1 kindred investigated showed positive indications of NEM and did not show clinical features of CMT1b, in that there was no slowing of nerve-conduction velocity, no sensory abnormality, and no neuropathic signs on either electromyography or muscle biopsy. It would seem unlikely, though possible, that the diseases are allelic.

It will be essential to investigate other NEM families to confirm the localization and to investigate possible genetic heterogeneity. Examination of further chromosome 1 loci will be necessary to more accurately delineate the physical region of chromosome 1 containing the NEM1 gene.

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