Letters to the Editor

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A Third Locus Predisposing to Multiple Deletions of mtDNA in Autosomal Dominant Progressive External Ophthalmoplegia

To the Editor:

Autosomal dominant progressive external ophthalmoplegia (adPEO) is a mitochondrial disorder characterized clinically by ptosis and progressive muscle weakness—most severely affecting the external eye muscles—with disease onset in early adulthood. Ataxia, dysphagia, sensorineural hypoacusia, neuropathy, tremor, cataract, and/or depression are present in some families (Zeviani et al. 1989, 1990; Servidei et al. 1991; Suomalainen et al. 1992; Melberg et al. 1996). In a Swedish adPEO family, hypogonadism cosegregated with the disease (Melberg et al. 1996). The typical morphological findings are ragged red fibers in the modified Gomori trichrome staining of muscle samples, and accumulation, enlargement, and abnormal shape of the mitochondria, on electron microscopy. Moderate reduction of the activities of respiratory-chain complexes I and IV is detected in biochemical analysis, and mtDNA analysis shows multiple mtDNA deletions in muscle samples (Zeviani et al. 1990; Servidei et al. 1991; Suomalainen et al. 1992, 1997).

We have shown previously that adPEO is a genetically heterogeneous disorder, by assigning two distinct genomic loci; one, in a Finnish family, on 10q24 (MIM 157640; Suomalainen et al. 1995) and the other, in three Italian families, on 3p14-21 (MIM 601226; Kaukonen et al. 1996). However, several adPEO families studied showed exclusion of both of these loci, thus indicating the existence of one or more additional adPEO loci (MIM 601227; Suomalainen et al. 1995; Kaukonen et al. 1996). Here we report a genomewide search and the assignment of a third adPEO locus.

Figure 1 shows the adPEO pedigree used in the genome scan, and figure 2 shows Southern blot–hybridization analyses of muscle mtDNA of patient 306 and a healthy control. The affected status was determined by observation of marked clinical symptoms in the neurological examination and/or by detection of multiple mtDNA deletions in the analysis of the muscle-biopsy specimen. Muscle samples from patients 306 and 408 were examined. The clinical symptoms in this family were milder than those in families with linkage to the 10q and 3p loci (Suomalainen et al 1995; Kaukonen et al. 1996). All the patients had progressive external ophthalmoplegia and ptosis but had no generalized muscle weakness. Age at onset was ~35 years. Several affected family members had sensorineural hypoacusia. Two subjects had goiter associated with hypo- or hyperthyroidism (patients 305 and 306, respectively). Two elderly subjects (patients 310 and 311) suffered from dementia manifesting as impairment of the cognitive functions, with no affective component. An increased serum-lactate level at rest was detected in one patient (patient 408). A typical example of a patient in this family is patient 306, who at age 67 years had ptosis and ophthalmoplegia, bilateral hearing loss, and hyperthyroidism with goiter. Her standard electromyogram was myopathic. Nerve conduction–velocity studies were normal. Multiple mtDNA deletions were detected in an analysis of muscle specimen from the biceps brachialis. Histological analysis of her muscle sample revealed that 3% of the fibers were ragged red and 5% showed partial COX deficiency. No elevation of lactic acid was detected at rest or after standard exercise, and her serum creatine-phosphokinase level was within the normal range. Respiratory-chain analysis showed slightly reduced activities of complexes III and IV (65%–70% of controls' mean), whereas activities of complexes I and II were within the normal range. Informed consent was obtained from all family members, and total DNA was extracted from lymphoblasts or from 10–150 mg of frozen muscle, as described by Zeviani et al. (1988). Southern blot analysis, with *Pvu*II restriction digestion of total DNA, preparation of total human mtDNA as the hybridization probe, and PCR amplifications to detect mtDNA deletions, were conducted as described elsewhere (Zeviani et al 1988; Kaukonen et al. 1996). $\gamma[^{32}P]$-ATP–labeled, PCR-amplified microsatellite markers were separated onto a 5% denaturing polyacrylamide gel and visualized by autoradiography. Fluorescently labeled PCR-amplified microsatellite markers were typed by use of a model 377 Applied Biosystems automatic sequencer (Perkin-Elmer).

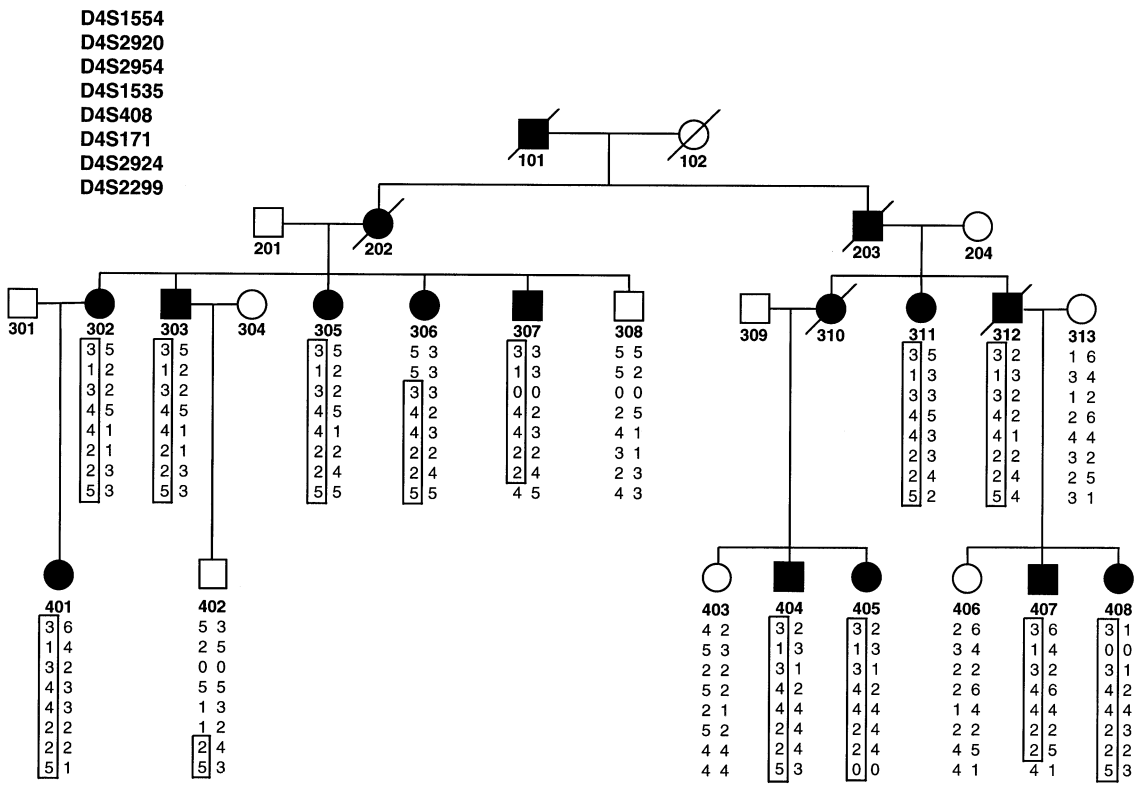

Figure 1 adPEO family with linkage to the markers on chromosome 4q. The individuals with marked clinical symptoms and/or deletions of mtDNA detected by PCR or Southern blot–hybridization analyses are indicated by blackened symbols. The unblackened symbols indicate clinically healthy individuals age >45 years. The markers used in the haplotype construction are shown in the upper-left corner of the figure. The boxes around the haplotypes indicate the shared regions of the affected chromosomes. The recombination events limit the adPEO region, between D4S2924 and D4S2920, to within a distance of 13.5 cM.

The marker set used for the genomewide gene search was chosen by use of marker-location information obtained from Généthon (Dib et al. 1996), the Cooperative Human Linkage Center, and the Genetic Location Database (LDB), with an average intermarker spacing of 15 cM. An autosomal dominant model was used in linkage calculations, and the frequency of the disease allele was estimated to be .00001. To avoid the potential danger of considering young, clinically unaffected, and non-muscle-biopsied patients as healthy, we performed the primary calculations as an affected-only analysis, using even-allele frequencies for each marker allele. Individuals with marked clinical symptoms and/or multiple mtDNA deletions in their muscle were considered to be affected, and all other family members were considered to have an "unknown" affected status. We performed chromosome 4q calculations also by considering the clinically healthy family members age >45 years to be healthy, with .8 penetrance to allow for exceptionally late appearance of the disease.

Data-simulation analyses were performed with the SLINK and MSIM options of the LINKAGE package (Ott 1989; Weeks et al. 1990). We calculated the average expected LOD score from 2,000 replicates with a five-allele marker, using even-allele frequencies, to be 3.34 (SD = 0.62) at recombination fraction (θ) of .0 with the affected-only model, and to be 4.23 (SD = 0.92) when information from clinically healthy family members age >45 years was included. The pairwise and multipoint LOD-score values were calculated with the FASTLINK option (Cottingham et al. 1993; Schaffer et al. 1994) of the MLINK and LINKMAP programs of LINKAGE (Lathrop et al. 1984).

The two known adPEO loci on chromosomes 10q24 and 3p14-21 were first analyzed by genotyping the markers linked to these loci, as described elsewhere (Suomalainen et al. 1995; Kaukonen et al. 1996). These loci were unequivocally excluded from carrying the disease gene in this family, on the basis of haplotype construction and multipoint linkage analyses done across the critical regions. Many recombination events were detected in the disease chromosomes, and the multipoint LOD scores were <-2 across the entire regions of interest (data not shown).

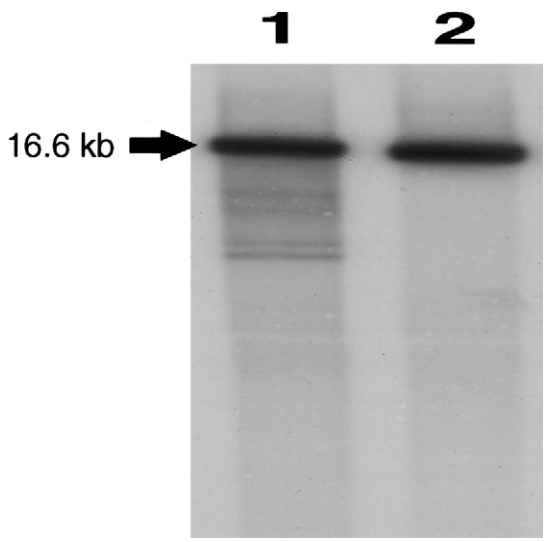

Figure 2 Southern blot–hybridization analysis of total muscle DNA, with full-length mtDNA as a probe. Muscle DNA of patient 306, showing normal-size mtDNA of 16.6 kb (*arrowhead*), and additional bands of lower molecular weight, representing mtDNA populations with multiple large deletions (lane 1) and a muscle sample from a control individual with no mitochondrial disease, with only normal-size mtDNA molecules (lane 2) are shown.

After analysis of 315 markers, primary evidence of linkage was obtained with marker D4S408, which provided two-point LOD scores of 1.89, with the affected-only model, and 2.34, with the inclusion of clinically normal family members age >45 years as healthy, with .8 penetrance in the linkage calculations (table 1). Haplotypes across this chromosomal region were constructed with informative markers D4S1554, D4S2920, D4S2954, D4S1535, D4S408, D4S171, D4S2924, and D4S2299. Recombination events detected in subjects 306 and 402 (fig. 1) limit the third adPEO locus to <13.5 cM, between markers D4S2920 and D4S2924, on 4q34-35. The intermarker distances and cytogenetic localization of this adPEO locus were established by use of the mapping information of the LDB.

The same set of 4q markers was used in pairwise and multipoint linkage calculations. The best two-point LOD scores obtained were 2.62, with marker D4S1535 (affected-only model), and 3.51, when clinically healthy family members age >45 years were considered healthy, with .8 penetrance. The affected-only multipoint calculations across the critical region gave a maximum LOD score of 3.8; 4.7 was obtained when data on the healthy family members were included in the analyses (fig. 3).

Our sample contained four informative Italian families with adPEO (each family alone was informative enough to provide the maximum expected LOD score of >2 at $\theta = .01$, with 2,000 replicates) not previously assigned to known adPEO loci. To study the possible linkage to the 4q locus in these families, haplotypes were constructed across the entire 4q adPEO region. Many recombination events were observed across the region in the disease chromosomes, and the multipoint calculations across the region remained <-2 (data not shown), thus clearly excluding the chromosome 4 locus as the cause of the disease in these families.

adPEO appears to be a genetically heterogeneous disorder, with at least four different nuclear loci causing very similar phenotypes. This heterogeneity could be explained by causative genes that encode different components of related metabolic pathways or by different subunits of an enzyme complex. In databases, we have not found evidence of functionally related proteins previously mapped within the three chromosomal adPEO loci (GeneMap '98). To date, two other autosomally inherited diseases associated with mtDNA deletions have been mapped to distinct nuclear regions. Wolfram syndrome is an autosomal recessive neurodegenerative disorder sometimes associated with single or multiple mtDNA deletions, and it has been shown to be linked to chromosome 4p (Polymeropoulos et al. 1994; Bar-

Table 1

Pairwise LOD Scores of Chromosome 4q Markers

Marker and Analysis[a]	Maximum LOD score at θ =				
	.00	.01	.05	.10	.15
D4S1554:					
1	1.61	1.57	1.38	1.15	.92
2	2.28	2.22	1.99	1.70	1.41
D4S2920:					
1	$-\infty$	.44	.93	.96	.85
2	$-\infty$	1.32	1.74	1.69	1.51
D4S2954:					
1	1.29	1.25	1.10	.92	.75
2	1.73	1.68	1.51	1.29	1.08
D4S1535:					
1	2.62	2.56	2.30	1.98	1.64
2	3.51	3.43	3.12	2.72	2.30
D4S408:					
1	1.89	1.85	1.65	1.41	1.18
2	2.34	2.29	2.10	1.85	1.60
D4S171:					
1	1.75	1.72	1.59	1.42	1.23
2	1.86	1.83	1.72	1.55	1.36
D4S2924:					
1	1.97	1.91	1.71	1.46	1.22
2	1.68	1.65	1.53	1.37	1.19
D4S2299:					
1	$-\infty$	−1.80	−.56	−.16	−.02
2	$-\infty$	−.98	.20	.52	.59

[a] "1" denotes affected-only analyses, and "2" denotes analyses done with inclusion of clinically healthy individuals age >45 years, with .8 penetrance. Pedigree of the family is shown in figure 1.

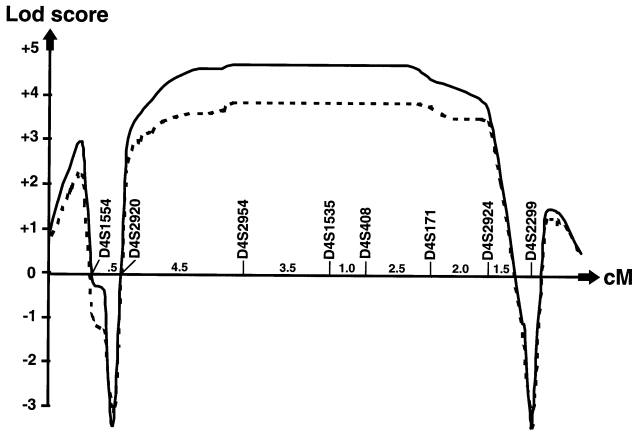

Figure 3 Multipoint LOD-score calculation across the 4q adPEO region. The dotted line represents the analyses done with information from the affected individuals, giving a maximum LOD score of 3.8. The solid line indicates the multipoint calculations done when data from clinically healthy individuals age >45 years were added, giving a maximum LOD score of 4.7. The markers used in the calculations are shown above the X-axis, and the intermarker distances are shown between the markers.

rientos et al. 1996*a*, 1996*b*). The defective gene (WFS1) was recently identified and it appears to function in the survival of islet β-cells and neurons (Inoue et al. 1998). A recessively inherited mitochondrial neurogastrointestinal encephalomyopathy with multiple mtDNA deletions was recently shown to be caused by mutations in the thymidine-phosphorylase gene on chromosome 22q13.32-qter (Hirano et al. 1998; Nishino et al. 1999).

The clinical symptoms of the patients in the family with linkage to 4q seem to be less severe than those in families with linkage to 10q and 3p. The muscular symptoms are limited to facial muscles: all of the patients presented with ophthalmoplegia and ptosis but with no exercise intolerance or generalized muscle weakness. Most patients had sensorineural hypoacusia, and some had goiter or dementia. It remains uncertain whether the latter symptoms are a result of the adPEO-gene defect, because of the relatively high prevalence of these disorders in the general population.

To date, ~65 expressed sequence tags representing different genes have been localized to the 4q adPEO region, and eight of these represent known genes (GeneMap '98). The adenine nucleotide translocator is a key metabolic enzyme of the mitochondria, transporting ADP and ATP across the inner mitochondrial membrane. The gene for the heart- and muscle-specific isoform (ANT1) has been localized to 4q35 (Fan et al. 1992). The ANT1 knockout mice showed ragged red muscle fibers and proliferation of mitochondria, lactic acidosis, severe exercise intolerance, and cardiomyopathy (Graham et al. 1997). Our patients lacked the cardiac symptoms, but otherwise the symptoms of the patients resembled those of ANT1 knock out mice, making ANT1 a good candidate gene for adPEO. Whether ANT1 is involved in the pathogenesis of adPEO is being analyzed. In addition, the gene for dominantly inherited facioscapulohumeral muscular dystrophy has been localized to the 4q adPEO region (Wijmenga et al. 1990). adPEO and this dystrophy share sensorineural hearing loss as a symptom of the disease, but our patients had neither generalized muscle weakness nor retinal changes. The eventual characterization of the first adPEO gene will not only reveal one of the pathogenic mechanisms causing the genetically heterogeneous disease but will also enhance the search for the remaining adPEO genes and will improve our fundamental understanding of mtDNA stability and maintenance in the cell.

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JYRKI KAUKONEN,[1] MASSIMO ZEVIANI,[2] GIACOMO PIETRO COMI,[3] MARIA-GRAZIA PISCAGLIA,[4] LEENA PELTONEN,[1,*] AND ANU SUOMALAINEN[1]

[1]*National Public Health Institute, Department of Human Molecular Genetics, Helsinki;* [2]*National Neurological Institute "Carlo Besta," Division of Biochemistry and Genetics, and* [3]*Centro Dino Ferrari, Istituto di Clinica Neurologica, Università degli Studi di Milano, Instituto di Ricovero e Cura a Carattere Scientifico, Ospedale Maggiore Policlinico, Milan; and* [4]*Divisione di Neurologia, Ospedale degli Infermi, Rimini, Italy*

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, http://www.chlc.org/ (for markers)
GeneMap '98, http://www.ncbi.nlm.nih.gov/genemap/ (for markers)
Genetic Location Database (LDB), http://cedar.genetics.soton.ac.uk/public_html/index.html (for markers)
Online Mendelian Inheritance in Man (OMIM), http://www

.ncbi.nlm.nih.gov/Omim/ (for adPEO loci in a Finnish family [MIM 157640], in three Italian families [MIM 601226], and from other sources [MIM 601227])

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Address for correspondence and reprints: Dr. Jyrki Kaukonen, National Public Health Institute, Department of Human Molecular Genetics, Mannerheimintie 166, 00300 Helsinki, Finland. E-mail: Jyrki.Kaukonen@ktl.fi

∗ Present affiliation: Department of Human Genetics, University of California School of Medicine, Los Angeles.

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