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Reevaluation of the Linkage of an Optic Atrophy Susceptibility Gene to X-chromosomal Markers in Finnish Families with Leber Hereditary Optic Neuroretinopathy (LHON)

To the Editor:

One of the commonest reasons for sudden-onset optic nerve degeneration in young men can be attributed to maternally inherited Leber hereditary optic neuroretinopathy (LHON) (Nikoskelainen et al. 1987). Specific point mutations at either np 11778 (Wallace et al. 1988) or np 3460 (Howell et al. 1991; Huoponen et al. 1991) in mitochondrial DNA (mtDNA) encoding for respiratory enzyme complex I subunits (i.e., ND4 or ND1) can be found in 70% of families. These mutations exist as being either homoplasmic or heteroplasmic, but the correlation between the degree of heteroplasmy and the risk of developing optic atrophy is far from clear (Holt et al. 1989; Vilkki et al. 1990). Neither does heteroplasmy explain the strong male bias seen in LHON families, when the sex ratio of patients with visual impairment is observed.

Our earlier results indicated that susceptibility to optic atrophy in Finnish families with LHON was probably determined by an X-chromosomal gene closely linked to DXS7 with a maximum lod score of 2.48 at recombination fraction (θ) of 0 (Vilkki et al. 1991). Exclusion of such a regulatory gene was previously accomplished in Tasmanian families, albeit with slightly different linkage analysis criteria than were used in Finnish studies (Chen et al. 1989). Likewise, the linkage of a putative optic atrophy liability locus to DXS7 and surrounding markers was later ruled out in British, Italian, and German families (Carvalho et al. 1992; Sweeney et al. 1992). However, using statistical calculations based on segregation analysis in LHON families, Bu and Rotter (1991) ended up with a two-locus model supporting the existence of an X-chromosomal factor as an explanation for the male preponderance in patients with loss of vision.

Contradictory results prompted us to reevaluate the existence of an X-chromosomal visual loss susceptibility gene in Finnish LHON families. Pedigrees included in the previous linkage study were revised through clinical examinations and more extensive genealogical studies. As a result, phenotype status was corrected for some family members. Because we used stricter criteria in age limits, some individuals in the youngest generations were removed from linkage analysis. Furthermore, five new pedigrees (three with the 11778 mutation, one with the 3460 mutation, and one harboring neither of these) and previously untested family members were included in the study. To avoid the effect of possible genetic heterogeneity, families were separated in linkage analysis according to the mtDNA mutation. New markers acquired from the critical region included two hypervariable microsatellites from the MAO A and MAO B loci, both of which are tightly linked to DXS7. Because no recombinations between the MAO and DXS7 loci have been encountered, it is justified to replace the relatively uninformative DXS7 with the allele information gained from the MAO loci.

Ninety-four individuals from the seven families with the ND4 mutation were included in the linkage analysis (fig. 1). Families 1–4 are the same as those in our previous analysis. Linkage analysis was also performed in two families with the ND1 mutation (including family 5 from the previous study) and in two other families having neither the 11778 nor the 3460 mutation (including family 6 from the previous study). All except three individuals with no visual impairment had passed the age of 30 years. Primer sequences and PCR conditions for amplifying MAO A and MAO B microsatellite regions were taken from the original descriptions (Black et al. 1991; Konradi et al. 1992). The linkage data were analyzed with the program package LINKAGE (Lathrop et al. 1984), version 5.04. Two-point lod score (Z) values were calculated with the program MLINK. The criteria for defining phenotype status followed the guidelines used in the previous study; namely, only patients with optic atrophy were classified as affected. Gene frequency of .5 was set for the putative liability gene, but other values between .08 and .9 were also tested. The penetrance value of .01 for optic atrophy was assigned to heterozygous females to cover the assumed effect of X-chromosome inactivation. To minimize the amount of unknown arbitrary parameters in linkage analysis, penetrance values for three liability classes were derived

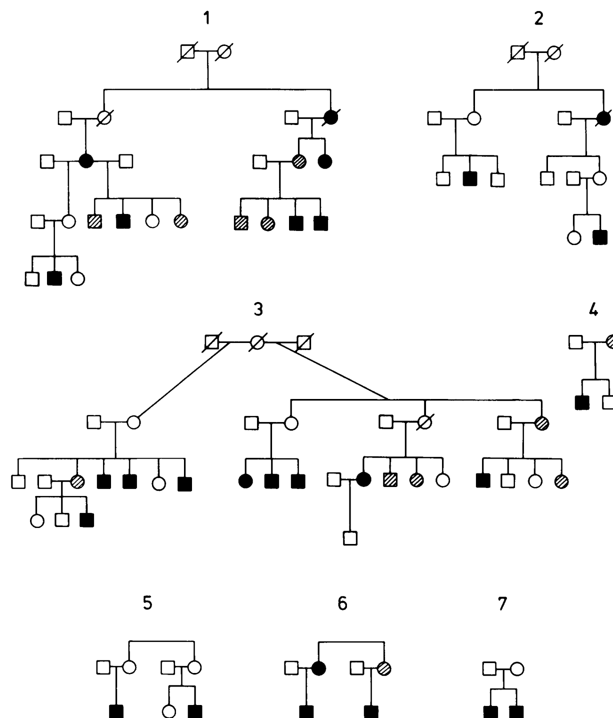

Figure 1 ND4 families included in the linkage analysis. Blackened symbols indicate patients with optic atrophy, and striped symbols indicate individuals with microangiopathy.

empirically from the clinical data. For males in age groups 20–30 years, 30–40 years, and 40–60 years, the penetrances were 78%, 90%, and 100%, respectively. Since the age at onset for females with the ND4 mutation in our series was usually between 30–50 years, penetrance values of 50%, 60%, and 70% were used in the above-mentioned age groups.

In order to avoid the effect of possible heterogeneity in linkage analysis, families were tested separately according to their mtDNA mutation. Statistically significant Z's were not obtainable from the two ND1 families and two non-ND1, non-ND4 families because of small sample size. However, a sufficient number of recombinations between the MAO locus and the putative visual loss susceptibility gene were present to exclude tight linkage (data not shown). The results from pairwise linkage analysis between the liability to develop optic atrophy and the 15 X-chromosomal markers in families with the ND4 mutation are presented in table 1. Every meiosis except one in family 4 was informative for the MAO B microsatellite marker. Because MAO A and MAO B loci are located only about 200 kb from each other (Chen et al. 1991), information on family 4 was taken from the MAO A locus, and the combined data were used in linkage analysis. The previously untested three ND4 pedigrees and new family members were analyzed only in respect to the MAO, DXS84, and DXS255 loci. Allele information for the other markers was taken from the previous study comprising families 1–4. Recombinations were encountered between the possible susceptibility locus and every marker except the particularly uninformative DXS85. Thus, no evidence of tight linkage to any X-chromosomal marker was seen. Alterations in the liability-locus gene frequency had no marked effect on Z values (data not shown).

The results of our present study clearly demonstrate

Table 1

Two-point *Z* Values for Linkage between Liability to Develop Optic Atrophy and 15 X-chromosomal Marker Loci

			Z at θ =					
Locus	Location	Probe	0	.05	.1	.2	.3	.4
DXS143	p22.3	dic56	$-\infty$	−1.90	−1.10	−.30	−.10	−.01
DXS85	p22.2	782	−.01	−.01	−.00	−.00	−.00	−.00
DXS43	p22.2	D2	$-\infty$	−3.01	−1.84	−.72	−.21	−.01
DXS41	p22.1	99-6	.29	.42	.45	.41	.28	.13
DXS84	p21.1	754	$-\infty$	−.86	−.46	−.15	−.00	.00
MAO	p11.3		$-\infty$	−1.60	−.57	.01	.20	.14
DXS255	p11.23	M27β	$-\infty$	−1.73	−.76	−.14	−.07	−.12
DXS14	p11.21	58.1	.32	.26	.21	.12	.04	.00
DXS1	q11.2-q12	p8	$-\infty$	−.66	−.37	−.12	−.02	.01
DXYS1	q21.31	DP34	$-\infty$	−1.71	−1.10	−.53	−.23	−.10
DXYS2	q21.3	7b	$-\infty$	−.72	−.41	−.14	−.03	.01
DXYS12	q21.33	St25	$-\infty$	−1.40	−.84	−.34	−.12	−.02
F9	q26.2-q27	P1	$-\infty$	−.75	−.46	−.20	−.10	−.01
F8C	q28	F8A	$-\infty$	−1.99	−1.30	−.59	−.24	−.06
DXS15	q28	DX13	$-\infty$	−4.05	−2.65	−1.26	−.56	−.18

that the earlier close linkage to DXS7 is implausible. The altered Z is due to revised pedigrees, the use of liability classes, and separation of the families according to the associated mtDNA mutation. Furthermore, close linkage of the putative liability locus to any other of the 14 X-chromosomal markers used is, for the most part, excluded under the circumstances employed in this study. However, statistically significant values covering the whole X chromosome would require more family material, more closely spaced informative markers, and LINKMAP analysis. Our present results are in concordance with those of other groups (Chen et al. 1989; Carvalho et al. 1992; Sweeney et al. 1992). Testing our pedigrees with parameters, derived from studies by Bu and Rotter (1991), with low visual loss susceptibility gene frequency (.08) and quite high penetrance in heterozygous females (.11) had no major effect on the results (data not shown).

In the course of this study, while searching for a gene whose existence is hypothetical, the pitfalls of linkage analysis itself became well noticed. In addition to the X-chromosomal inheritance of a possible visual loss susceptibility gene, one has to make arbitrary assumptions about the frequency and penetrance of that gene as well as about the expression of the disease in heterozygous women. For example, in the absence of liability classes, when the linkage between the putative regulatory gene and the MAO locus was observed, penetrance values of 1.0, .9, and .71 in homozygotes and hemizygotes yielded Z values of $-\infty$, -0.11, and 1.22, respectively, at θ of 0. Cautiousness in maximizing the Z over several unknown parameters when mapping a gene conferring susceptibility to optic atrophy in LHON has already been stressed (Chen and Denton 1991). The reasons for the male bias in LHON still remain unknown. They are likely to be more complicated than previously expected and more laborious to approach, e.g., with linkage analysis. In addition to a possible nongenetic or hormonal basis, one can decipher the male preponderance through multifactorial genetic models, including the participation of several X-chromosomal genes or a combination of a certain autosomal and an X-chromosomal factor in provoking loss of vision.

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Compound Heterozygosity for the ΔF508 and F508C Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Mutations in a Patient with Congenital Bilateral Aplasia of the Vas Deferens

To the Editor:

In the November 1992 issue of the *Journal* Macek et al. (1992) describe a peculiar pattern of heteroduplex formation in a case of compound heterozygosity for the ΔF508 and F508C mutations in the CFTR gene (cystic fibrosis transmembrane conductance regulator gene). The individual carrying these mutations was the clinically normal father of a cystic fibrosis (CF) patient. We would like to report a similar molecular finding, observed in a completely different clinical setting.

There is increasing evidence that congenital bilateral aplasia of the vas deferens (CBAVD) is a primarily genital form of CF. Simple or compound heterozygosity for mutations in the CFTR gene has been found in up to 64% of individuals affected by this condition (Dumur et al. 1990; Anguiano et al. 1992). These patients can now be effectively treated with the technique of microsurgical sperm aspiration from the epididymis and consecutive in vitro fertilization (Silber et al. 1990). On the basis of the high prevalence of mutations in the CFTR gene, we have recently established a pretherapeutic genetic screening program for couples where the male partner is affected by CBAVD. DNA from both partners is screened for ΔF508 (by heteroduplex analysis) and other mutations in CFTR exons 7, 10, 11, and 21 (by direct sequencing after PCR amplification).

In a patient with surgically proved CBAVD we observed the same slightly shifted heteroduplex bands for ΔF508 as were described by Macek et al. Apart from a pneumonia in early infancy, the patient had never had any CF-typical symptoms. Sweat electrolytes were within the normal range. Direct sequencing showed heterozygosity for the ΔF508 and F508C mutations and the amino acid polymorphism M470V (Kerem et al. 1990). DNA from the patient's parents, both of whom were free of CF symptoms, was then analyzed with the same techniques. Results are summarized in table 1. The father was found to be heterozygous for the ΔF508 mutation. In the mother, heterozygosity for the F508C mutation and the M470V polymorphism was detected. In the patient and his mother, F508C and M470V were present on the same chromosome (*cis* configuration), as can be deduced from the segregation pattern (table 1).

Table 1

CFTR Gene Haplotypes in the Patient and His Parents

Mutation or Polymorphism	CFTR Gene Haplotype[a]		
	Patient	Father of Patient	Mother of Patient
ΔF508	ΔF508/+	ΔF508/+	+/+
F508C	+/F508C	+/+	+/F508C
M470C	+/M470V	+/+	+/M470V

[a] A plus sign (+) denotes presence of the wild-type allele.

Compound heterozygosity for ΔF508 and F508C has been reported in clinically normal individuals (Kobayashi et al. 1990; Macek et al. 1992), in patients with typical CF symptoms (Kerem et al. 1990), and now, for the first time, in a case of CBAVD. The basis for this wide clinical variability is unclear, as is the functional significance of the F508C mutation. The M470V mutation detected in our patient is considered a benign sequence variation without clinical consequences. It cannot be excluded, however, that this mutation contributes to the clinical phenotype of CBAVD if it is inherited together with ΔF508 and F508C. To further clarify this issue, we are currently screening a larger number of patients with congenital anomalies of the Wolffian-duct derivatives, for M470V and F508C mutations.

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