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Optic Atrophy in Leber Hereditary Optic Neuroretinopathy Is Probably Determined by an X-chromosomal Gene Closely Linked to DXS7

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Summary

Leber hereditary optic neuroretinopathy (LHON) is a maternally inherited disease, probably transmitted by mutations in mtDNA. The variation in the clinical expression of the disease among family members has remained unexplained, but pedigree data suggest an involvement of an X-chromosomal factor. We have studied genetic linkage of the liability to develop optic atrophy to 15 polymorphic markers on the X chromosome in six pedigrees with LHON. The results show evidence of linkage to the locus DXS7 on the proximal Xp. Tight linkage to the other marker loci was excluded. Multipoint linkage analysis placed the liability locus at DXS7 with a maximum lod score (Z_{max}) of 2.48 at a recombination fraction (θ) of .0 and with a $Z_{max} - 1$ support interval $\theta = .09$ distal to $\theta = .07$ proximal of DXS7. No evidence of heterogeneity was found among different types of families, with or without a known mtDNA mutation associated with LHON.

Introduction

Leber hereditary optic neuroretinopathy (LHON) is a maternally inherited disease (Nikoskelainen et al. 1987) characterized by acute or subacute visual loss due to severe bilateral optic atrophy. The age at onset of visual impairment is usually between puberty and 30 years. The hypothesis of mitochondrial transmission of the disease was recently supported by the discovery of an mtDNA mutation associated with LHON (Wallace et al. 1988; Singh et al. 1989). The mutation at nucleotide (nt) 11778 converts an evolutionarily conserved amino acid to another in the subunit ND4 of the respiratory complex I. This mutation was found in 11 of 20 Finnish families with LHON (Vilkki et al. 1989).

The clinical expression of LHON varies greatly within and between different families. The interfamilial variation in the expression of LHON may be explained by genetic heterogeneity (different mtDNA mutations are associated with the disease) (Holt et al. 1989; Vilkki et al. 1989). However, the variation in the clinical expression of the disease among members of the same family has remained unexplained. Some of this variation may be due to the segregation of mutant and normal mtDNA along a heteroplasmic maternal lineage (Holt et al. 1989; Vilkki et al. 1990). Nevertheless, individuals with identical mtDNA composition display phenotypes ranging from ophthalmoscopically normal fundi to complete optic atrophy (Vilkki et al. 1989; authors' unpublished data).

Genealogical data from several countries show that approximately 50% of males and approximately 20% of females in LHON maternal lineages have optic atrophy (van Senus 1963; Seedorf 1985; Nikoskelainen et al. 1987). This suggests that the liability to develop optic atrophy may be determined by some X-linked gene(s), even if the primary cause of the disease is maternally inherited. A mutation in mtDNA would lead to optic atrophy when matched with a specific allele of an X-linked susceptibility gene. The other allele(s) combined with the mtDNA mutation would

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lead to microangiopathy or asymptomatic fundi. To test this hypothesis we used 15 known X-chromosomal DNA markers to study linkage relationships in six Finnish lineages with cases of LHON. Both families with and without the ND4 mtDNA mutation were included in the study, in order to see whether the linkage results are affected by the mtDNA-based genetic heterogeneity.

Material and Methods

Subjects

One hundred fifteen individuals from the six pedigrees were included in the linkage analysis (fig. 1). Blood samples were obtained from 82 subjects. Of these, the subjects belonging to the maternal lineages have all passed the age of 30 years and have had a recent ophthalmological examination. Diagnostic criteria of LHON have been described elsewhere (Nikoskelainen 1985). The 29 males married to the lineages have no known ocular disease. The maternal lines in pedigrees 1–4 carry the nt 11778 mutation (Wallace et al. 1988) in their mtDNA, while in the maternal lines of pedigrees 5 and 6 the entire ND4 gene DNA sequence is normal (Huoponen et al. 1990).

DNA Analysis

DNA was extracted from whole blood according to a method described elsewhere (Vilkki et al. 1988). For RFLP analysis, 5 μg of DNA was digested with 2–3 units/μg DNA of the appropriate restriction endonuclease under conditions recommended by the manufacturer. Digested DNA was fractionated in 0.8% agarose gels and was blotted onto nylon membranes (Hybond N; Amersham). The filters were hybridized with probes that spanned the X chromosome at 2–50-cM intervals (table 1) and that were radiolabeled with ^{32}P by either nick-translation or random hexanucleotide priming.

Linkage Analysis

The 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, and multipoint analyses were conducted with the program LINKMAP. The RFLP allele frequencies used are from Kidd et al. (1989), and their order and distances are from Keats et al. (1989). The computer program HOMOG (Ott 1985) was used to perform heterogeneity testing of multipoint linkage data.

The males married to the maternal lineages were defined as unknown for their affection status, since, having normal mtDNA, they do not express LHON, irrespective of the putative X-chromosomal factor. In the present study, only individuals with evident signs of visual loss were defined as affected, whereas individuals with more subtle ocular manifestation without visual impairment (microangiopathy) were defined as unaffected. The susceptibility gene was assumed to be X-linked recessive. Different allele frequencies (range .05–.95) at the liability locus were tested. All Z values were computed in two ways: first, the disease penetrance was set to 1.0, and, second, the penetrance was set to .9. Lower penetrance values were not used, since all individuals included in the study had passed the median age for visual loss. In both cases a penetrance of .01 for optic atrophy was given to heterozygous females, to cover the possible effect of the inactivation of the X chromosome.

To assess (1) the effect of maximizing Z over 19 susceptibility-gene frequencies and (2) two penetrance values for the comparison of the susceptibility gene versus DXS7, computer simulation was carried out according to methods described elsewhere (Ott 1989; Weeks et al. 1990). A total of 2,000 replicates were generated under the assumption of absence of linkage. In each replicate, for family members available for testing, genotypes were randomly generated for a marker with DXS7 allele frequencies and were analyzed in two ways, (1) by maximizing the Z over recombination fraction (θ) values only, assuming a susceptibility-gene frequency of .50 and a penetrance of 1, and (2) by maximizing it also over the 38 parameter values. The resulting 2 × 2,000 respective values of $Z1_{max}$ and $Z2_{max}$ were used to calculate approximate empirical significance levels associated with the Z_{max} values actually observed in this study.

Results

Two-point linkage data between the liability to develop optic atrophy and the 15 X-chromosomal RFLP markers are shown in table 1. Positive Z were found for five marker loci on the proximal Xp: DXS41, DXS84, DXS7, DXS255, and DXS14. Recombinations have occurred between the putative susceptibility locus and every marker except DXS7. The results imply tight linkage to the locus DXS7, while tight linkage to the 14 other marker loci is excluded.

Highest positive Z values for the susceptibility locus versus DXS7 were obtained with the susceptible-allele

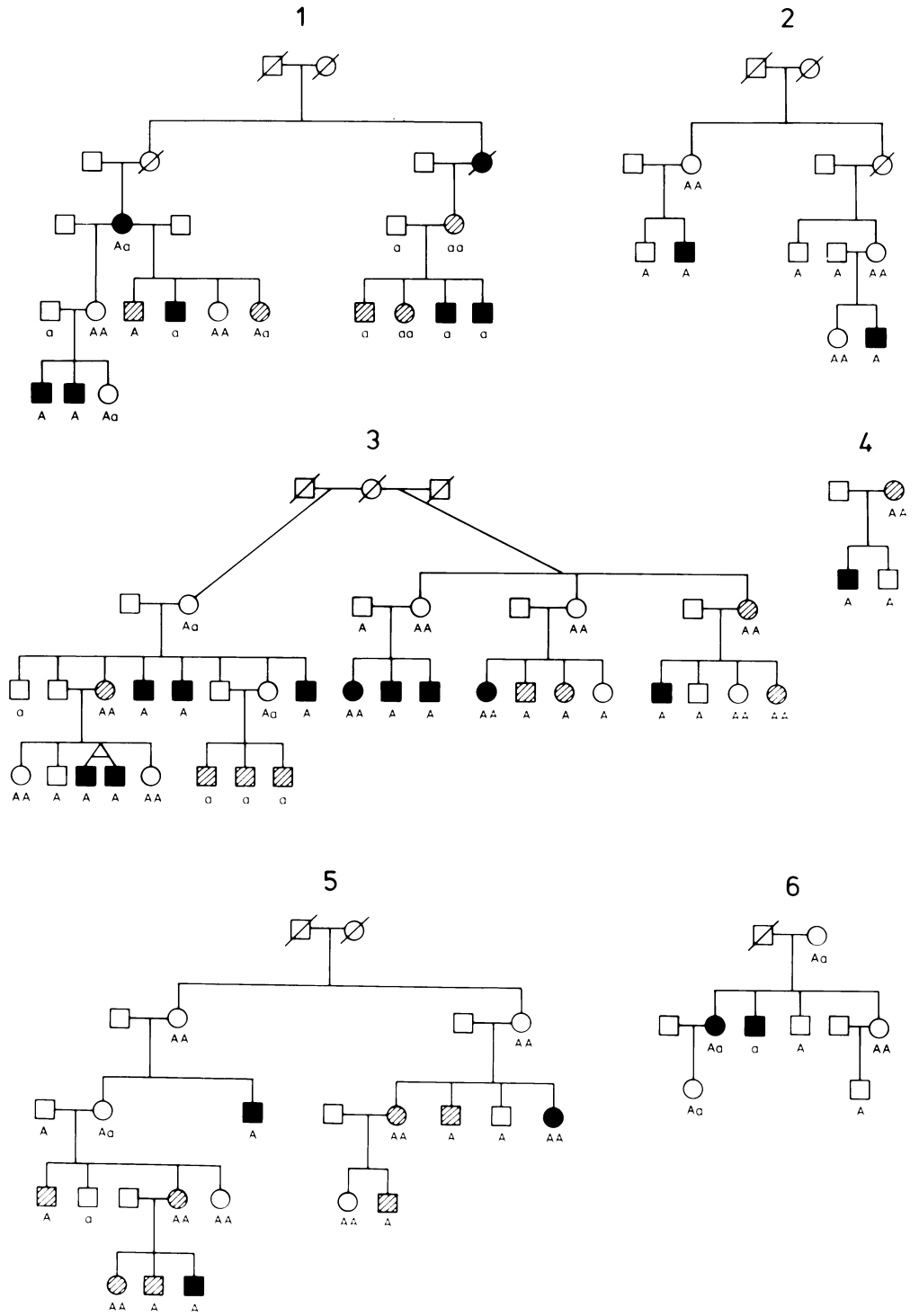

Figure 1 Pedigree of LHON families used in study. Only individuals who were included in the linkage analysis are shown. ● and ■ = subject with visual loss, ⊘ and ▨ = subject with peripapillary microangiopathy; ○ and □ = ophthalmoscopically normal subject; ∅ and ⍁ = deceased. The alleles for the locus DXS7 (A is 12 kb; a is 9 kb) are shown for each subject studied. Families 1–4 have the nt 11778 mutation in the ND4 gene in mtDNA; in families 5 and 6 the nature of the mtDNA defect is unknown.

Table 1

Two-Point *Z* Values for Linkage between Liability to Develop Optic Atrophy and 15 X-chromosomal Marker Loci, with Frequencies of .5 at Susceptibility Locus and Assumption of Disease Penetrance of 1 in Homo- and Hemizygotes

Locus, Location (probe)	Z at θ of					
	.0	.05	.1	.2	.3	.4
DXS143, p22.3 (dic56)	−∞	−4.94	−2.79	−.96	−.24	−.00
DXS85, p22.2 (782)	−∞	−1.35	−.87	−.41	−.18	−.06
DXS43, p22.2 (D2)	−∞	−4.47	−2.49	−.79	−.08	.10
DXS41, p22.1 (99-6)	−∞	−.51	.15	.53	.49	.28
DXS84, p21.1 (754)	−∞	−.48	.35	.79	.62	.27
DXS7, p11.3 (L1.28)	2.29	2.05	1.80	1.22	.61	.07
DXS255, p11.22 (M27B)	−∞	−2.00	−.33	.67	.58	.19
DXS14, p11.21 (58.1)	−∞	−1.05	−.47	−.02	.09	.08
DXS1, q11.2-q12 (p8)	−∞	−1.71	−.95	−.34	−.09	−.00
DXYS1, q21.31 (DP34)	−∞	−4.90	−2.99	−1.25	−.46	−.09
DXYS2, q21.3 (7b)	−∞	−2.93	−1.86	−.86	−.37	−.11
DXYS12, q21.33 (St25)	−∞	−4.62	−2.98	−1.29	−.49	−.11
F9, q26.2-q27 (P1)	−∞	−1.33	−.80	−.32	−.10	−.01
F8C, q28 (F8A)	−∞	−3.04	−1.87	−.86	−.40	−.14
DXS15, q28 (DX13)	−∞	−6.01	−3.83	−1.65	−.62	−.14

frequency of either .05, if penetrance was set at .9 (Z_{max} = 2.28), or .35, if penetrance was set at 1.0 (Z_{max} = 2.32). The *Z* values are decreased by approximately 1 unit of *Z* under the assumption of high frequencies (.90 and .95) for the susceptible allele. (In the two-point and multipoint analyses, arbitrarily set values of .5 gene frequency and 1.0 penetrance were used.)

To assess the effect of effectively maximizing the *Z* over parameters of disease inheritance (19 gene frequencies and two penetrance values), computer simulation was carried out for disease susceptibility versus a marker analogous to DXS7. In 2,000 replicates, under absence of linkage and under fixed gene frequency of .50 and penetrance of 1, the observed $Z1_{max}$ of 2.292 was exceeded by chance twice; that is, the empirical significance level associated with $Z1_{max}$ is approximately equal to .0010. With maximization over gene frequencies and penetrances, the $Z2_{max}$ of 2.323 that was obtained for disease susceptibility versus DXS7 was exceeded by chance in three of 2,000 replicates, leading to an associated empirical significance level P_2 value of .0015 with an upper 95% confidence limit of 0.0038. This empirical significance level is much smaller than 10^{-2} (= .01), which is the upper limit to the significance level associated with a critical *Z* of 2. The observed linkage between the postulated disease-susceptibility locus and DXS7 is thus statistically significant. In X linkage a $Z_{max} \geq 2$ is generally accepted as strong evidence for linkage.

Three marker loci with observed positive *Z* values (DXS7 and the flanking loci) were used in multipoint linkage analysis. Multipoint *Z* values for each family, obtained from the LINKMAP results, were used in the HOMOG program. The null hypothesis for homogeneity (H_1) is that the genetic distance of the liability locus to a fixed marker locus is the same in each family. The heterogeneity hypothesis (H_2) assumes two family types—type 1, characterized by a θ value, and type 2, assumed to be unlinked. The hypothesis of homogeneity and absence of linkage is H_0. The homogeneity test supports the hypothesis of linkage with one family type (H_1), while the heterogeneity hypothesis (H_2) is not supported (table 2).

Because there is no evident heterogeneity in the present data, the multipoint *Z* values for each family were combined, to infer the most likely location of the susceptibility locus with respect to a map of the known loci. The combined results of multipoint linkage analysis are graphed in figure 2. The multipoint analysis yielded the highest probability (Z_{max} = 2.48) for the location of the locus for the liability to develop optic atrophy at the locus DXS7. The Z_{max} − 1 support limits are θ = .09 distal and θ = .07 proximal of DXS7,

Table 2

Components of χ^2 in Homogeneity Test

Source	df	χ^2	P
H_2 vs. H_1 heterogeneity	1	.000	.5000
H_1 vs. H_0 linkage	1	11.835	.0003
H_2 vs. H_0 total.....	2	11.835	.0013

under the assumption that disease penetrance in homo- and hemizygotes is 1.0 and that allele frequencies at the susceptibility locus are .5.

Discussion

The results of the present study show that in families with LHON the liability to develop optic atrophy is linked to the DXS7 locus at proximal Xp. Recently, Chen et al. (1989*a*) reported preliminary exclusion of an X-linked gene in LHON in three families from Tasmania. However, they analyzed their data without considering the mutant mtDNA as the primary cause of LHON. Because all asymptomatic subjects were classified as normals for their disease status, the males married to the maternal lines were probably misinterpreted during linkage analysis to have a "no disease" allele on their X chromosomes. Either there exists no X-chromosomal allele predisposing to optic atrophy in the Tasmanian population, or reanalyzing the Tasmanian data may reveal X linkage for liability to develop optic atrophy.

Even if there is no evident heterogeneity in the present multipoint linkage data, homogeneity cannot be unequivocally proved, because some of the pedigrees are not informative enough for each of the loci used. However, both families with and families without the ND4 mtDNA mutation showed X linkage for the liability to develop optic atrophy. Thus it seems that the X-chromosomal effect is not confined to one mtDNA mutation only.

Linkage studies have assigned the gene loci for three other hereditary eye diseases—Norrie disease (NDP) (Ngo et al. 1989; Sims et al. 1989), X-chromosomal congenital stationary night blindness (CSNBX) (Gal et al. 1989; Musarella et al. 1989), and one locus for X-linked retinitis pigmentosa (XLRP2) (Chen et al. 1989*b*)—to the same region. In addition, human monoamine oxidase (MAO) A and B genes (Lan et al. 1989) and an ornithine aminotransferase pseudogene (Barrett et al. 1987) map to Xp11.2. The regional

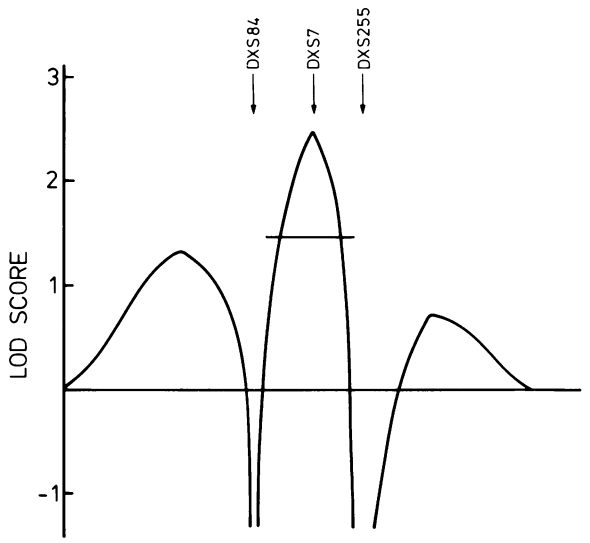

Figure 2 Multipoint linkage analysis vs. map of known loci. The positions of the fixed loci are based on the following θ values: DXS84–.16–DXS7–.14–DXS255. Z_{max} (in the six pedigrees combined) for the location of the locus for liability to develop optic atrophy is 2.48, at DXS7. The Z_{max} – 1 support interval for the location is indicated in the graph by a horizontal line at the Z_{max} – 1 unit.

clustering of the genes that affect the eyes suggests that the liability gene may be a gene that specifically affects ocular function.

On the other hand, the tissue specificity of LHON disease might be explained by the tissue-specific interaction of nucleocytoplasmic proteins. The liability gene may code for a mitochondrial isoenzyme form expressed in tissues affected by the disease. One allele of this isoform could be incompatible with mutations in some respiratory-enzyme subunits encoded by the mitochondrial genome.

In conclusion, the results indicate that a locus genetically determining the development of optic atrophy in LHON should be assigned to the proximal Xp. New markers within the area, such as the MAO loci, will be used to localize the gene more accurately and to allow for the isolation and characterization of the gene(s).

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