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Evidence against an X-Linked Visual Loss Susceptibility Locus in Leber Hereditary Optic Neuropathy

R. M. Chalmers, M. B. Davis, M. G. Sweeney, N. W. Wood, and A. E. Harding

Neurogenetics Section, University Department of Clinical Neurology, Institute of Neurology, London

Summary

Pedigree analysis of British families with Leber hereditary optic neuropathy (LHON) closely fits a model in which a pathogenic mtDNA mutation interacts with an X-linked visual loss susceptibility locus (VLSL). This model predicts that 60% of affected females will show marked skewing of X inactivation. Linkage analysis in British and Italian families with genetically proven LHON has excluded the presence of such a VLSL over 169 cM of the X chromosome both when all families were analyzed together and when only families with the bp 11778 mutation were studied. Further, there was no excess skewing of X inactivation in affected females. There was no evidence for close linkage to three markers in the pseudoautosomal region of the sex chromosomes. The mechanism of incomplete penetrance and male predominance in LHON remains unclear.

Introduction

Leber hereditary optic neuropathy (LHON) causes severe visual loss, most commonly in young adult males. It is associated with pathogenic mutations of mtDNA at positions 11778, 14484, or 3460 bp in nearly all families in the United Kingdom (Riordan-Eva et al. 1995). Other, so-called secondary mutations in mtDNA have been described in LHON, but they occur in the normal population and in association with the 11778, 14484, and 3460 mutations; their significance is unclear.

Some features of LHON cannot simply be explained by mitochondrial inheritance. First, while mtDNA heteroplasmy may be a factor determining the penetrance of the disease, in most cases both patients and their unaffected relatives from LHON families have very high amounts of mutant mtDNA (>95%). Second, there is an excess of males affected by LHON, with a male:female ratio of 4.2:1 in British families (Harding et al. 1995). These findings and analysis of 31 pedigrees from published series led to the suggestion of an interacting X-linked visual loss susceptibility locus (VLSL) (Bu and Rotter 1991). Not all sons of affected females develop the condition, and, therefore, a proportion of affected females must be heterozygous for this putative VLSL with disadvantageous, unbalanced X inactivation. The model predicts a penetrance of .11 in heterozygous females, with 60% of affected females heterozygous and 40% homozygous for the VLSL. The population frequency is predicted to be .08. A similar study in Japanese pedigrees supported this hypothesis, although the predicted penetrance in heterozygous females was higher (.196; Nakamura et al. 1993). Analysis of British pedigrees showed remarkably close accordance with the model proposed by Bu and Rotter (1992; also see Harding et al. 1995).

Previous attempts to demonstrate such a VLSL have been unsuccessful. Initial analysis of Finnish families showed linkage to DXS7 (Vilkki et al. 1991), but studies of the same region of Xp in British and Italian families excluded the presence of a VLSL over a 30-cM region (Sweeney et al. 1992). Analysis of German families (Carvalho et al. 1992) and reanalysis of an expanded Finnish data set (Juvonen et al. 1993) did not show linkage to DXS7. In these studies, varied age-related penetrances were used, and the tested penetrance in female heterozygotes (.01) was lower than that predicted by Bu and Rotter (1991). These linkage data have not excluded a susceptibility locus from the entire X chromosome.

The telomeric pseudoautosomal region (PAR) of the sex chromosomes, comprising 2.6 Mb (short arm) and 0.4 Mb (long arm), are identical on the X and Y chromosomes and escape X inactivation. During male meiosis, an obligatory crossover occurs in the PAR (Rappold 1993). Thus sequences within this region exhibit varying degrees of partial sex linkage, depending on their physical location within the PAR. The male predominance of LHON might therefore be explained by the presence of a VLSL within the PAR, with the VLSL found at higher frequency on the Y chromosome–associated PAR. Such nonrandom distribution of alleles on the X and Y chromosome PAR has been described both close to the pseudoautosomal boundary (Ellis et al. 1990) and 750 kb into the PAR (Decorte et al. 1994).

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Address for correspondence and reprints: Dr. R. M. Chalmers, University Department of Clinical Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom.

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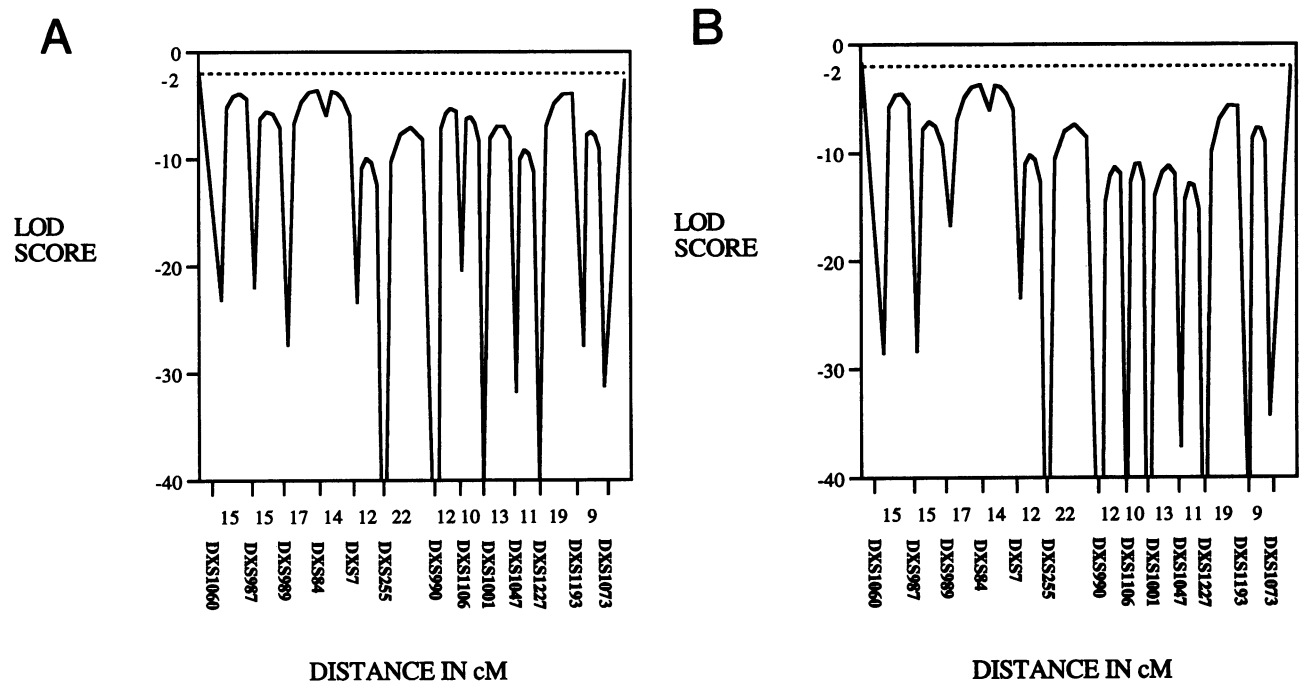

Figure 1 *A*, Multipoint linkage analysis in families with the 11778 mutation. *B*, Multipoint linkage analysis in all LHON families.

Because our British and Italian families show a pattern of inheritance of LHON that closely fits the model of Bu and Rotter (1991), we have sought evidence to support the existence of such a sex-linked VLSL. We have performed linkage analysis using DNA polymorphisms covering the entire X chromosome map and the short arm PAR. We have also looked for evidence of unbalanced X inactivation in affected females.

Patients and Methods

Patients

LHON was defined as in a previous study (Holt et al. 1989). The families studied for linkage analysis are as described in figure 1 of Sweeney et al. (1992). Family 13 has the 14484 mutation; family 11, 12, and part of family 9 have the 3460 mutation; the remaining families have the 11778 mutation. Patients studied for X inactivation were affected females (23 with the 11778 mutation, 2 with the 3460 mutation, and 3 with the 14484 mutation), unaffected females with affected sons (13 with the 11778 mutation, 0 with the 3460 mutation, and 1 with the 14484 mutation), and controls (wives of males with other neurological diseases). Informed consent was obtained from all subjects.

Methods

DNA was extracted from 20 ml of blood from each patient by using standard techniques. mtDNA analysis using standard methods (Riordan-Eva et al. 1995) showed one of the mutations at bp 11778, 3460, or 14484 in all LHON families.

For linkage studies on the X chromosome, the following markers were used: DXS1060, DXS987, DXS989, DXS990, DXS1106, DXS1001, DXS1047, DXS1227, DXS1193 (Gyapay et al. 1994), and DXS1073 (Dib et al., in press). Linkage studies employed the model of Bu and Rotter (1991). This model predicts that, in maternally related LHON pedigrees, females homozygous for the X-linked VLSL and males inheriting the VLSL develop LHON. Females heterozygous for the VLSL develop LHON only if subject to inappropriate X inactivation, and the predicted penetrance in such females is .11. Age-related penetrances should be applied; this study employed values as described in Sweeney et al. (1992). The population frequency of the LHON VLSL was assumed to be .08. Data from Sweeney et al. (1992) using probes L1:28 (DXS7), L754 (DXS84) and M27Beta (DXS255) were included in multipoint linkage analysis.

In addition, in order to reduce the problems of heterozygosity and age-related penetrance, a subset of the families with the 11778 mutation was defined; this subset included only the affected males and the females necessary to link them. Pairwise LOD scores were obtained and multipoint analysis was performed using this subset and the parameters described above.

For linkage studies on the PAR the following markers were used: MIC2 (Schmitt et al. 1993), DXYS17 (Decorte et al. 1994), and DXYS14 (Le Roux et al. 1994). Age-related penetrance values were as described above,

Table 1

Pairwise LOD Scores for Putative LHON VLSL and X Chromosome Polymorphisms

Locus and mtDNA Mutation	LOD Score at θ = 0	.01	.05	.10	.20	.30	.40
DXS1060:							
11778	−18.81	−7.68	−4.34	−2.86	−1.52	−.82	−.34
3460	−6.93	−1.65	−.81	−.41	−.04	.08	.08
14484	1.22	1.20	1.10	.98	.71	.42	.14
Total	−24.52	−8.13	−4.05	−2.29	−.85	−.32	−.12
DXS987:							
11778	−14.45	−4.54	−2.48	−1.48	−.85	−.71	−.46
3460	−6.47	−1.25	−.60	−.37	−.20	−.11	−.03
14484	.12	.12	.13	.13	.10	.06	.02
Total	−20.80	−5.67	−2.95	−1.72	−.95	−.76	−.47
DXS989:							
11778	−13.09	−7.42	−3.93	−2.46	−1.29	−.80	−.43
3460	−11.89	−2.2	−.83	−.28	.14	.25	.17
14484	.00	.00	.00	.00	.00	.00	.00
Total	−24.98	−9.62	−4.76	−2.74	−1.15	−.55	−.26
DXS990:							
11778	−36.72	−7.06	−3.46	−2.04	−.97	−.55	−.28
3460	−17.84	−4.26	−2.20	−1.32	−.52	−.16	−.01
14484	−4.50	−2.01	−1.16	−.74	−.33	−.13	−.03
Total	−59.06	−13.33	−6.82	−4.10	−1.82	−.84	−.32
DXS1106:							
11778	−15.36	−4.52	−1.90	−.90	−.17	.03	.02
3460	−17.84	−4.32	−2.25	−1.37	−.56	−.18	−.02
14484	.00	.00	.00	.00	.00	.00	.00
Total	−33.20	−8.84	−4.15	−2.27	−.73	−.15	.00
DXS1001:							
11778	−36.81	−9.08	−4.79	−2.92	−1.24	−.49	−.14
3460	−17.32	−3.22	−1.88	−1.27	−.61	−.26	−.07
14484	−4.50	−1.06	−.42	−.19	−.04	.00	.00
Total	−58.63	−13.36	−7.09	−4.38	−1.89	−.75	−.21
DXS1047:							
11778	−17.66	−6.59	−3.21	−1.81	−.63	−.17	.01
3460	−8.92	−2.11	−1.33	−.92	−.43	−.16	−.02
14484	−5.36	−1.53	−.83	−.52	−.23	−.09	−.02
Total	−31.94	−10.23	−5.37	−3.25	−1.29	−.42	−.03
DXS1227:							
11778	−37.65	−12.00	−6.40	−3.98	−1.74	−.70	−.18
3460	−13.81	−3.33	−1.84	−1.16	−.49	−.18	−.03
14484	−5.36	−1.03	−.39	−.17	−.02	.01	.01
Total	−56.82	−16.36	−8.63	−5.31	−2.25	−.87	−.20
DX1193:							
11778	−26.78	−4.43	−2.14	−1.13	−.25	.03	.10
3460	−1.10	−.38	.22	.39	.38	.25	.07
14484	−5.36	−1.99	−1.14	−.74	−.33	−.13	−.03
Total	−33.24	−6.80	−3.06	−1.48	−.20	.15	.14
DXS1073:							
11778	−31.38	−9.02	−4.86	−3.17	−1.55	−.73	−.22
3460	−2.69	−.12	.48	.65	.67	.53	.29
14484	.27	.27	.27	.25	.18	.10	.02
Total	−33.80	−8.87	−4.11	−2.27	−.70	−.10	.09

Table 2

Pairwise LOD Scores for Putative LHON VLSL and Pseudoautosomal Sex Chromosome Polymorphisms

Locus and mtDNA Mutation	LOD Score at Male θ = 0	.01	.05	.10	.20	.30	.40
	VLSL Assumed to Act as Dominant Trait						
MIC2:							
11778	−2.49	−1.17	−.51	−.24	−.03	.02	.03
3460	−.81	−.78	−.65	−.50	−.29	−.16	−.09
14484	.00	.00	.00	.00	.00	.00	.00
Total	−3.30	−1.95	−1.16	−.74	−.32	−.14	−.06
DXYS17:							
11778	−12.02	−5.70	−2.98	−1.82	−.80	−.36	−.14
3460	−2.26	−1.92	−1.36	−1.02	−.64	−.40	−.21
14484	−3.59	−2.25	−1.24	−.76	−.33	−.13	−.03
Total	−17.87	−9.87	−5.58	−3.60	−1.77	−.89	−.38
DXYS14:							
11778	−8.51	−5.40	−3.13	−2.04	−1.01	−.52	−.28
3460	−.93	−.90	−.81	−.72	−.57	−.47	−.38
14484	.03	.02	.02	.02	.01	.00	.00
Total	−9.41	−6.28	−3.92	−2.74	−1.57	−.99	−.66
	VLSL Assumed to Act as Recessive Trait						
MIC2:							
11778	−4.40	−3.49	−2.13	−1.46	−.87	−.61	−.45
3460	−.17	−.15	−.10	−.06	.02	.06	.08
14484	.01	.01	.00	.00	.00	.00	.00
Total	−4.56	−3.63	−2.23	−1.52	−.85	−.55	−.37
DXYS17:							
11778	−11.34	−4.97	−2.97	−1.95	−.95	−.46	−.15
3460	−1.98	−1.54	−.91	−.55	−.16	.04	.13
14484	−1.91	−1.51	−.93	−.61	−.28	−.11	−.03
Total	−15.23	−8.02	−4.81	−3.11	−1.39	−.53	−.05
DXYS14:							
11778	−7.87	−5.38	−3.45	−2.38	−1.42	−.95	−.65
3460	−.53	−.52	−.46	−.38	−.26	−.17	−.11
14484	.61	.61	.59	.57	.54	.50	.46
Total	−7.79	−5.29	−3.32	−2.19	−1.14	−.62	−.30

and the frequency of the LHON VLSL was assumed to be .01. Statistical analysis followed the strategy of Ott with a female:male map distance ratio of .066 (Ott 1991); analysis was performed using both dominant and recessive modes of inheritance of the putative VLSL.

Linkage analysis was performed using the FASTLINK version of the LINKAGE program package (Cottingham et al. 1993; Schäffer et al. 1994).

For studies of unbalanced X inactivation, the methylation pattern of the first exon of the human androgen receptor locus was analyzed. The methylation of *Hpa*II sites close to the highly polymorphic trinucleotide repeat in this exon correlates with X inactivation. As *Hpa*II is methylation sensitive, digestion of genomic DNA with *Hpa*II and subsequent PCR amplification of the first exon allow quantification of the degree of skewing of X inactivation (Pegoraro et al. 1994). The distributions of skewing of X inactivation in affected females, unaffected females with affected sons, and controls were compared using the Mann-Whitney U test.

Results

Linkage Analysis

Pairwise LOD scores for the putative VLSL and X chromosome markers are shown in table 1, with families grouped according to mtDNA mutation. Variation of the tested gene frequency between .05 and .20 did not significantly alter pairwise LOD scores (data not shown). Multipoint analysis excluded the presence of a

Table 3

Distribution of Skewed X Inactivation in Affected Females, Unaffected Females with Affected Sons, and Controls

	No. of Informative (No. Studied)		
Mutation	Affected Females	Unaffected Females	Controls
11778	19 (23)	12 (13)	
3460	1 (2)	0	
14484	3 (3)	1 (1)	
All groups	23 (28)	13 (14)	15 (16)
	Median Degree of Skewing (Range)		
11778	60 (50–90)	70 (55–95)	
3460	65		
14484	70 (70–75)	65	
All groups	65 (50–90)	65 (55–95)	60 (50–85)

VLSL between these markers both in families with the 11778 mutation (fig. 1*A*) and when the three mutation groups were analyzed together (fig. 1*B*).

Pairwise LOD scores obtained using the subset of those families with the 11778 mutation (containing affected males only) also provided no evidence for linkage. Multipoint analysis excluded the presence of a VLSL over 65% of the X chromosome (data not shown).

Pairwise LOD scores for short arm PAR markers are shown in table 2. There was no evidence for a VLSL tightly linked to these regions.

X Inactivation Studies

Results from X inactivation studies are shown in table 3. Seven females (five affected females, one unaffected female, and one control) were homozygous at the androgen receptor locus and were hence uninformative for X inactivation quantification. There was no significant difference in skewing of X inactivation between affected females and controls ($P = .92$) or between affected and unaffected females ($P = .11$).

Discussion

Pedigree analysis of our LHON families closely fits the model of Bu and Rotter (1991; also see Harding et al. 1995). However, linkage analysis and studies of X inactivation in British and Italian families do not support this hypothesis.

First, linkage studies exclude the presence of this VLSL in 12 British and 1 Italian family with LHON between the telomeric markers DXS1060 and DXS1073, which cover 169 cM of the X chromosome. The majority of these families carry the commonest LHON mutation at bp 11778, and our data exclude the presence of the VLSL when these families alone are analyzed. There are insufficient data to exclude such a locus in our families with LHON mutations at bp 3460 or 14484.

Second, while the model of an X-linked VLSL predicts that 60% of affected females will show unbalanced, inappropriate, X inactivation, this study has shown no difference between the degree of unbalanced X inactivation seen in the leukocytes of affected females; unaffected females who, by virtue of having affected sons, would be predicted to be carriers of a VLSL; and controls.

We have also found no evidence for a VLSL in the PAR of the sex chromosomes. Because of the high rate of recombination in the PAR, this is not excluded by our data.

The existence of an X-linked VLSL in some cases of LHON cannot entirely be ruled out. LHON may be genetically heterogeneous. Thus, families with the bp 11778 mutation from other populations or families carrying the bp 3460 or 14484 mutation could theoretically develop LHON because of such a VLSL. These and previous data do not support such a contention, although it has not been excluded. Further, our data do not exclude the presence of two or more separate susceptibility loci on the X chromosome.

In addition, exclusion mapping can be inaccurate if the model applied is incorrect. The two-locus model of Bu and Rotter (1991) predicts a gene frequency of .08; variation of the gene frequency used in this study did not significantly alter the pairwise LOD scores. Further, in order to reduce the problems of genetic heterogeneity, heterozygosity, and age-related penetrance in the analysis of the two locus model, we examined a subset of the families with the 11778 mutation containing only affected males. Pairwise LOD scores provided no evidence for linkage in this subset, and multipoint analysis excluded the presence of a VLSL over 65% of the X chromosome.

The absence of unbalanced X inactivation in the leukocytes of females with LHON does not exclude its occurrence in the optic nerve (Bu and Rotter 1992). However, there is evidence that the degree of unbalanced inactivation in peripheral blood is a generally similar to or greater than that seen in other tissues. One study of X inactivation that examined a variety of tissues including blood, skin, muscle, and colon found no cases in which skewing of inactivation was observed in other tissues but not in blood (Gale et al. 1994). Further, we have studied a female with McLeod syndrome and 100% skewed inactivation of brain tissue, in whom the degree of skewing in blood was also 100% (Ho et al., in press).

If the model of Bu and Rotter (1991) is incorrect, some other mechanism must underlie the reduced penetrance and male predominance of the condition. There are a number of other possible explanations for the development of blindness in LHON. First, environmental factors have been proposed. There is anecdotal evidence that tobacco and alcohol use can advance the course of LHON, and this was a striking feature of some families

with the 3460 and 14484 mutations in our series (Riordan-Eva et al. 1995). Second, a number of features of LHON are surprising for a genetic disorder, and an autoimmune component has been suggested (Harding et al. 1992). This suggestion is supported by the demonstration in rodents of mtDNA-encoded peptides that are presented by classical and "neoclassical" class I major histocompatibility complex molecules and act as transplantation antigens (Loveland et al. 1990; Davies et al. 1991) and of antibodies to optic nerve proteins in patients with LHON (Smith et al. 1995).

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