

ELECTRONIC LETTER

The role of *APOE* in the phenotypic expression of Leber hereditary optic neuropathy

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Leber hereditary optic neuropathy (LHON, MIM 535000) is a mitochondrial genetic disease that causes blindness in young adults, with an estimated prevalence of 1 in 25 000 in the north east of England.¹ It classically presents as bilateral subacute loss of central vision owing to the focal neurodegeneration of the retinal ganglion cell layer. There is marked cell body and axonal degeneration, with associated demyelination and atrophy observed from the optic nerves to the lateral geniculate bodies.^{2,3} Over 95% of cases are principally the result of one of three "primary" mtDNA point mutations, G3460A, G11778A, and T14484C, which all involve genes encoding complex I subunits of the mitochondrial respiratory chain.⁴ However, only ~50% of male and ~10% of female LHON carriers will develop the optic neuropathy.⁵ This marked incomplete penetrance and gender bias clearly indicates that additional factors are required for the phenotypic expression of the pathogenic mtDNA mutations in LHON. Segregation analysis initially suggested that a nuclear encoded modifier locus on the X chromosome could account for these intriguing features.^{6,7} However, attempts to map this X linked susceptibility locus using standard linkage analysis have so far not been successful.⁸⁻¹⁰ Furthermore, the Bu and Rotter segregation model could not be replicated in every LHON population.¹¹ It is therefore likely that the phenotypic expression of the primary mtDNA LHON mutations is dependent upon the complex interaction of multiple susceptibility factors, which may include nuclear modifier genes and environmental agents.¹²

There is a strong association between *APOE* genotype and a number of common neurodegenerative conditions, including Alzheimer disease and dementia with Lewy bodies. The *APOE* $\epsilon 4$ allele is associated with an increased lifetime risk and an earlier age of onset for these disorders, whereas the $\epsilon 2$ allele appears to have a protective effect.¹³ The mechanisms behind these effects remain unclear, but there is emerging evidence that *APOE* has a wide range of important functions in the central nervous system (CNS) besides its postulated role in amyloid plaque and neurofibrillary tangle formation. It has been implicated in the modulation of neuronal response to oxidative stress and also as an important neurotrophic factor inducing recovery after acute neuronal injury.¹⁴ *APOE* is produced by Muller cells within the retina and then secreted into the vitreous humour, after which it is taken up by retinal ganglion cells and rapidly transported to the optic nerve.^{15,16} It is therefore possible that *APOE* genotype could modulate the expression of the primary mtDNA LHON mutations. Here, we

Key points

- Leber hereditary optic neuropathy (LHON) is primarily the result of one of three pathogenic mitochondrial DNA (mtDNA) mutations, but additional unknown genetic and/or environmental factors influence the expression of the primary mtDNA defect.
- We determined the apolipoprotein E (*APOE*) genotype in affected and unaffected subjects in 16 English and 44 Italian pedigrees with LHON.
- Logistic regression analysis showed no statistically significant effect of *APOE* genotype upon disease penetrance in LHON, with an odds ratio for the $\epsilon 4/-$ genotype of 1.80 ($p > 0.05$, 95% CI 0.62 to 5.22) and for the $\epsilon 2/-$ genotype of 0.52 ($p > 0.05$, 95% CI 0.19 to 1.44). There was no significant difference in the age of onset of blindness between patients with $\epsilon 4/-$ and non- $\epsilon 4/-$ genotypes ($\chi^2 = 0.79$, $p > 0.05$ by log rank test).
- We found no evidence that *APOE* is a visual loss susceptibility gene in LHON. *APOE* genotype also does not appear to influence the age of onset of visual failure.

present the results of a family based association study that investigated whether *APOE* genotype influenced the risk of visual loss and/or the age of onset in LHON pedigrees from two different background populations.

METHODS

Case ascertainment

This study included 16 LHON pedigrees from the north east of England and 44 from Italy, ascertained through a clinically affected proband (table 1). Affected subjects were diagnosed as having LHON by an experienced ophthalmologist based upon the classical phenotype and molecular confirmation of a primary mtDNA mutation. Unaffected LHON carriers were classified as family controls only if they were > 30 years of age and the proportion of mutant mtDNA (mutation load) was $> 60\%$ in blood DNA. Thirteen unaffected pedigree members from the north east of England and 19 from Italy did not fulfil these inclusion criteria and they were excluded from the analysis.

Table 1 LHON pedigrees included in this study

Cohort	Pedigrees				Subjects	
	Total	G3460A	G1178A	T14484C	Cases	Controls
England	16	5	10	1	30	33
Italy	44	4	37	3	51	51

Table 2 Summary of *APOE* genotype in our north east of England and Italian cohorts

Cohort	Status	No	Genotype						Group			
			ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	ε4/-	Non-ε4/-	ε2/-	Non-ε2/-
England	Cases	30	0	4	0	18	7	1	8	22	4	26
	Controls	33	0	7	0	25	1	0	1	32	7	26
Italy	Cases	51	0	5	0	39	7	0	7	44	5	46
	Controls	51	0	9	3	36	3	0	6	45	12	39

Table 3 Logistic regression analysis

Predictors	Coefficient	SE	Z	p	Odds Ratio	95% CI
Constant	-2.26	1.50	-1.51	0.13	-	-
Age	-0.99	0.40	-2.49	0.01	0.37	0.17 to 0.81
Gender	2.73	0.42	6.48	0.00	15.33	6.72 to 35.01
Mutation	0.05	0.35	0.13	0.89	1.05	0.53 to 2.08
Ethnic origin	-0.48	0.43	-1.13	0.26	0.62	0.27 to 1.43
ε4/- genotype	0.59	0.54	1.08	0.28	1.80	0.62 to 5.22
ε2/- genotype	-0.65	0.52	-1.26	0.21	0.52	0.19 to 1.44

SE: Standard error.

Molecular genetic analysis

APOE genotype was determined by PCR amplification of a 227 bp fragment spanning the two polymorphic sites (codons 112 and 158) that determine *APOE* genotype, with subsequent overnight incubation at 37°C with the restriction enzyme *HhaI* (New England Biolabs, UK). The digested products were electrophoresed at 100 V on a 4.01% Nusieve™ 3:1 gel (Boehringer Mannheim, UK) and then visualised under ultraviolet light to determine the restriction pattern and hence the genotype.^{17, 18}

Statistical analysis

A logistic regression model was also used to study the relationship between *APOE* genotype and disease penetrance in LHON. This form of analysis assumes that the logarithm of the odds ratio is a linear function of the predictor variables included in the model:

$$\text{Log}(p/1-p) = B_0 + B_1X_1 + B_2X_2 + \dots + B_nX_n$$

where p is the probability of a LHON carrier going blind, X_1, X_2, \dots, X_n represent the chosen predictor variables, and B_0, B_1, \dots, B_n are coefficients reflecting the nature of each predictor.¹⁹ With logistic regression, it becomes possible to analyse simultaneously all of the following variables and their interactions: age, gender, mutational status (G3460A, G11778A, or T14484C), ethnic origin (north east of England or Italy), and *APOE* genotype (ε4/-: at least one ε4 allele is present, ε2/-: at least one ε2 allele is present). This approach also allows for potential confounding factors such as differences in the age or gender distribution within the study groups, therefore minimising the chance of detecting a spurious statistical association. The effect of *APOE* genotype on age of onset was assessed with Kaplan-Meier survival analysis. The frequency of ε4 heterozygotes in controls from the north east of England is 0.25²⁰ and this figure was used for power calculations.

RESULTS

Both cases and controls were in Hardy-Weinberg equilibrium at the *APOE* locus (table 2). Logistic regression analysis confirmed the well established gender bias in LHON (OR=15.33; 95% CI 6.72 to 35.01, table 3). There was no statistically significant effect of ethnic origin (OR=0.62, 95% CI 0.27 to 1.43), or the specific mtDNA mutation (OR=1.05, 95% CI 0.53 to 2.08), on the risk of visual failure. There was no evidence of a significant effect of *APOE* genotype upon disease

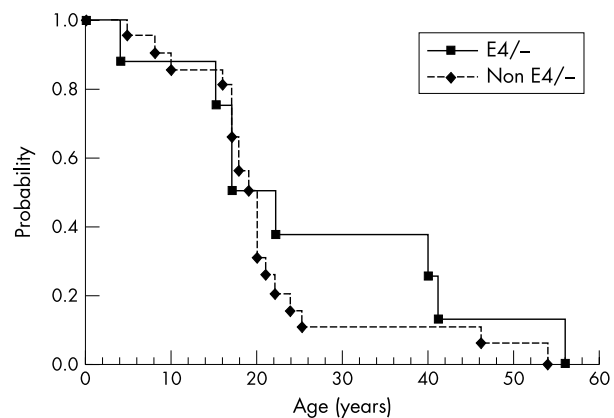


Figure 1 Effect of *APOE* ε4 allele on age of onset of visual failure in LHON pedigrees from the north east of England (Y axis indicates probability of remaining unaffected).

penetrance in LHON, with an odds ratio for the ε4/- genotype of 1.80 (95% CI 0.62 to 5.22) and for the ε2/- genotype of 0.52 (95% CI 0.19 to 1.44) (table 3). The age at which visual loss occurred was available for 28 of the 29 LHON patients from the north east of England. There was no significant difference in the age of onset of blindness between patients with ε4/- and non-ε4/- genotypes ($\chi^2=0.79$, $p>0.05$ by log rank test, fig 1).

DISCUSSION

We have shown that *APOE* genotype does not have a major effect on the phenotypic expression of LHON or the age of onset of visual failure. Power calculations indicate that our study sample had an 88% power to detect an odds ratio of 2.5 or greater for the ε4 allele at the 0.05 significance level. Therefore, if *APOE* does influence the penetrance of the primary mtDNA LHON mutations, the effect is only likely to be modest. It does remain possible that *APOE* might influence the LHON phenotype in a different, more subtle manner, possibly affecting the chance of visual recovery after the acute phase,⁵ but investigating this possibility will not be easy given the relatively low prevalence of primary LHON mutations in the general population. The reduced penetrance of the primary LHON mtDNA mutations remains unexplained, and further

studies are needed to determine the role of nuclear genes and the environment in modulating the expression of this mitochondrial genetic disorder.³

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