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Apolipoprotein E and herpes virus diseases: herpes simplex keratitis

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Our previous studies showed that herpes simplex type 1 virus (HSV1) is present in a high proportion of the brain of elderly normal people and Alzheimer's disease (AD) patients. We subsequently discovered that the combination of HSV1 in brain and carriage of the type 4 allele of the gene for apolipoprotein E (*apoE-ε4*) is a strong risk factor for AD, and also that *apoE-ε4* is a strong risk factor for herpes labialis. In this study we have examined apoE genotypes of sufferers from another disorder caused by HSV1, namely, herpes simplex keratitis (HSK), to find if an apoE allele is involved in the disorder. In 46 HSK patients the apoE-ε4 allele frequency was 15%–the same as that found in 238 unaffected controls. The apoE-ε2 allele frequency was 13%–higher than the value of 7% for unaffected people, but the difference is not statistically significant.

Keywords: herpes keratitis; apolipoprotein E; herpes simplex type 1 virus

Introduction

The type 4 allele of the gene for apolipoprotein E (*apoE-ε4*) is a risk factor for cold sores.[1,2] More importantly, herpes simplex type 1 virus (HSV1) is a risk factor for Alzheimer's disease (AD) when present in brain of possessors of an apoE-ε4 allele, the combination of virus and genetic factor being found in almost two thirds of the AD patients studied.[1,2] On the basis of these results in PNS and CNS disease, the apoE genotype of sufferers from a further variant of HSV disease–herpes simplex keratitis (HSK)–was investigated, to determine whether or not the disorder is associated with a specific apoE allele. HSK is the leading infectious cause of corneal blindness in the developed world.

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Subjects and Methods

Forty-six HSK patients comprised 27 males, mean age 58, range 18–89, and 19 females, mean age 60, range 33–87. They were diagnosed on the basis of chronic, unilateral recurrent disease characterised by dendritic or geographical keratitis, and in some cases they progressed to stromal involvement. Non-HSK sufferers ('controls') comprised volunteers from the University and from two local hospitals[2] (referred to as 'volunteer' and 'population' groups, respectively). There were 101 males, mean age 37.5 years, 132 females, mean age 36.5 years, and five of unspecified sex. All patients and controls gave consent for a single blood sample to be taken for research purposes.

Apolipoprotein E genotypes were determined by preparing DNA from peripheral leucocytes obtained from patients' blood,[1] and amplifying a 227 bp sequence in the *apoE* gene,

followed by digestion with *cfo*I and electrophoresis of the digestion products.[3]

Results and Discussion

Figure 1 shows typical results of agarose gel electrophoresis after PCR. Table 1 displays the apoE genotypes and the allele frequencies of the HSK patients. The apoE-ε4 allele frequency of the HSK patients is 15%, which is identical to that of our normal population value of 15% ($P > 0.5$, χ^2 test). The apoE-ε2 allele frequency is 13%, which is higher than our normal population value of 7% but does not reach statistical significance ($P = 0.06$, χ^2 test). Of the HSK sufferers, 33% (15/46) reported that they suffer from cold sores. This proportion is higher than that of our 'controls' (24%), though not significantly so.

The apolipoproteins comprise a class of proteins responsible for transporting lipids in plasma. They are involved also in the repair response to tissue injury in various organs, including nerve injury and regeneration.[4] Attachment of apoEs to cells,[5] and also that of

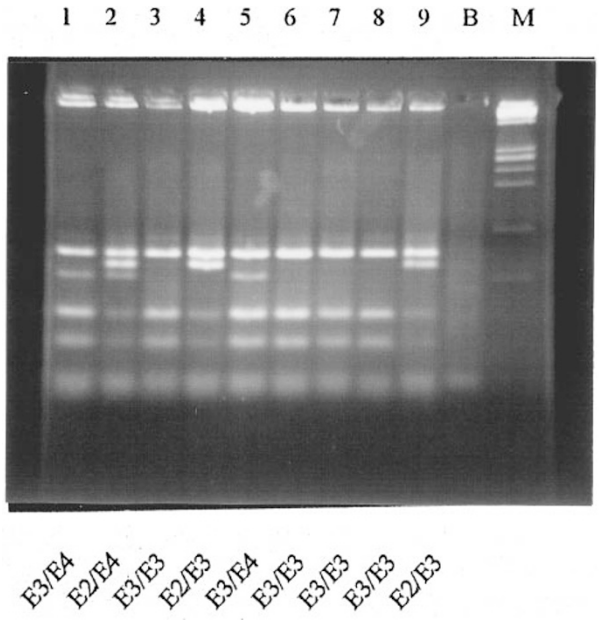

Figure 1 *Agarose gel electrophoresis of amplified products after PCR, for apoE genotyping of HSK patients. M is a molecular weight marker (ΦX174, HaeIII-digested) and B is a reagent blank*

Table 1 Apolipoprotein E genotypes of HSK patients and of local 'normal' subjects

		HSK patients		*Manchester 'Control' subjects*	
		Total	*33%*	*Total*	
			With cold sores	*'population' group*	*University 'volunteer' group*[a]
Genotype	E2/E2	1	0	1	1
	E2/E3	8	3	9	13
	E2/E4	2	1	3	6
	E3/E3	24	9	54	78
	E3/E4	10	2	30	40
	E4/E4	1	0	0	3
	Total	46	15	97	141
Allele number	E2	12	3		
	E3	66	24		
	E4	14	3		
	Total	92	30		
Allele frequency	ApoE2%	13.0	10.0	7.3[b]	
	ApoE3%	71.7	80.0	77.9[b]	
	ApoE4%	15.2	10.0	14.8[b]	

[a]In the 'volunteer' survey, cold sore sufferers were specifically sought, so the proportion of sufferers was high (48%) and therefore non-representative of the general population. To obtain 'Manchester' allele frequency values using as large a number of people as possible, values for the 'volunteer' group and the 'population' group were combined, as described in footnote b.
[b]The proportion of cold sore sufferers in the population survey, which was found to be 24%, was used to calculate appropriate percent allele frequencies for the University 'volunteers', i.e., values for the University sufferers were multiplied by 0.24 and for the non-sufferers by 0.76, and were then added together. The frequencies for the combined University plus population groups were then calculated on a weighted basis, i.e., by correcting each percent allele frequency for the relative numbers in the two groups.

HSV1,[6] is mediated by heparan sulphate proteoglycans in the cell membrane. In neuronal cells in culture the amount of apoE4 that enters the cells is less than that of apoE3.[7] We have therefore suggested a possible mechanism for the combined risk conveyed by HSV1 and apoE-ε4: that on stress or immunosuppression, reactivation of the latent HSV1 occurs in brain (if present) and the number of cells infected by the virus, and hence the extent of damage, is greater in apoE-ε4 possessors than in those with the other alleles.[1]

The lack of involvement of apoE-ε4 in HSK contrasts with its apparent role in AD and in herpes labialis. It is a strong risk factor in both–in the former when combined with HSV1 in brain, and in the latter when combined with HSV1 in the PNS.[1,2] (In the AD study, the odds ratio for apoE-ε4 was 12.0 (CI 3.44–42.06) for HSV1-positive patients and 1.57 (OR 0.32–7.63) for HSV1-negative patients.[2]) On the basis of our findings in AD and in herpes labialis, we postulated that the combination of HSV1 and apoE-ε4 damages the nervous system. Corneal cells are mainly fibroblast-like, and a study of fibroblasts in culture showed that fewer apoE2 molecules than the other isoforms entered the cells.[8] Our present results on HSK indicate that apoE-ε4 is not involved in this disorder but that the apoE-ε2 allele frequency increases slightly. This is consistent with the concept of a non-neuronal site of latency, and hence of reactivation, in this disorder.[9] ApoE2 might compete less effectively than the other apoE isoforms with HSV1 for attachment to corneal cells, and hence entry of the virus and subsequent damage could be greater in apoE-ε2 possessors. Alternatively, the receptors in corneal cell membranes might differ from those of neuronal cells in respect of attachment to apoEs and HSV1.

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References

1 Itzhaki RF, Lin WR, Shang D, Wilcock GK, Faragher B, Jamieson GA: Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. *Lancet* 1997; **349**: 241–2.
2 Lin WR, Graham J, MacGowan S, Wilcock GK, Itzhaki RF: Alzheimer's disease, herpes virus in brain, apolipoprotein E4 and herpes labialis. *Alz Repts* 1998; **1**:
3 Wenham PR, Price WH, Blundell G: Apolipoprotein E genotyping by one-stage PCR. *Lancet* 1991; **337**: 1158–1159.
4 Mahley RW: Apolipoprotein E: structure and function in lipid metabolism and neurobiology. In: Rosenberg RN, Prusiner SB, DiMauro S, Barchi RL (eds). *The Molecular and Genetic Basis of Neurological Disease.* Butterworth Heinemann: Newton, MA, 1997, pp 1037–1050.
5 Ji ZS, Fazio S, Lee YL, Mahley RW: Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heparan sulfate proteoglycans. *J Biol Chem* 1994; **269**: 2764–2772.
6 Herold BC, WuDunn D, Soltys N, Spear PG: Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *J Virol* 1991; **65**: 1090–1098.
7 Nathan BP, Chang K-C, Bellosta S *et al*: The inhibitory effect of apolipoprotein E4 on neurite outgrowth is associated with microtubule depolymerization. *J Biol Chem* 1995; **270**: 19791–19799.
8 Weisgraber KH, Innerarity TL, Mahley RW: Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to Cysteine-Arginine interchange at a single site. *J Biol Chem* 1982; **257**: 2518–2521.
9 Cook SD, Hill JH: Herpes simplex virus: molecular biology and the possibility of corneal latency. *Survey Opthalmol* 1991; **36**: 140–148.