SHORT REPORT

Herpes simplex encephalitis: involvement of apolipoprotein E genotype

W-R Lin, M A Wozniak, M M Esiri, P Klenerman, R F Itzhaki

Abstract

It was previously found that herpes simplex type 1 virus (HSV1) when present in the brain, is a risk factor for Alzheimer's disease in carriers of the type 4 allele of the gene for apolipoprotein E (apoE ε4), and apoE ε4 is a risk factor for herpes labialis. Whether a specific allele of the gene is involved in susceptibility to another disorder caused by HSV1—herpes simplex encephalitis (HSE)—has now been investigated. DNA was prepared from formalin-fixed, paraffin-embedded blocks of specimens from the brain or spleen of 14 United Kingdom patients with HSE, confirmed by necropsy, and from the CSF of seven United Kingdom clinical patients with HSV1 in their CSF detected by polymerase chain reaction (PCR). ApoE genotype of the DNA from blocks was determined by seminested PCR, and of the DNA from CSF by one step PCR, followed by restriction endonuclease digestion. The apoE allele frequencies were compared with values previously obtained for 238 normal people from the United Kingdom. The apoE ε2 allele frequency of the patients with HSE was 26%, significantly higher than the value of 7% for the normal subjects (OR=4.6, 95% confidence interval (95% CI) 2.0–10.8). The apoE ε3 and ε4 allele frequencies did not differ significantly between the two groups. Thus, it seems that apoE ε2 is a risk factor for HSE.

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Herpes simplex encephalitis (HSE) is a necrotising, inflammatory brain disease caused by lytic infection with herpes simplex virus (HSV) (usually type 1). Its incidence is very low—about 1 to 3 cases/million/year—despite the fact that a high proportion of adults are infected with the virus, often in infancy, and harbour it lifelong in latent form in their peripheral nervous system. It is uncertain whether HSE results from entry of virus into the brain from the peripheral nervous system, from reactivation at a site of latency in the brain, or from de novo infection via the olfactory tract.[1] Susceptibility factors for HSE are not defined but possibly one or more genetic factors are involved.

In studies on possible risk factors in Alzheimer's disease, we established by polymerase chain reaction (PCR) that HSV1 is present in a high proportion of brains from patients with Alzheimer's disease and elderly normal subjects,[2] and we subsequently showed, using multiple logistic regression analyses, that HSV1 is a strong risk factor for Alzheimer's disease when present in the brain of carriers of the type 4 allele of the gene for apolipoprotein E (apoE ε4).[3 4] We found also that apoE ε4 is a risk factor for herpes labialis.[3 4] ApoE is a protein that functions as a component of plasma lipoproteins in the transport of lipids to cells and tissues, and is involved also in nerve cell regeneration. There are three common isoforms—2, 3, and 4—and the type 4 allele of the apoE gene had previously been found to be a susceptibility factor for Alzheimer's disease,[5] although neither essential nor sufficient to cause the disease. Based on our findings in Alzheimer's disease implicating apoE ε4 and HSV1 as a combined risk factor, we considered that apoE might be a factor also in HSE. The present study indicates that in HSE, another apoE allele is involved.

Methods

We obtained formalin-fixed, paraffin-embedded specimens of postmortem brain or spleen from 14 patients (all white, mean age 40 years) with untreated, necropsy confirmed HSE; all of these were from the Radcliffe Infirmary Neuropathology Department brain archive, Oxford, and had been collected before the introduction of treatment with acyclovir (ACV). Also, we obtained CSF from seven recent patients with HSE (mean age 42 years) with HSV1 detected in CSF by polymerase chain reaction (PCR), who were subsequently treated with ACV. HSV1 was confirmed as the cause in the necropsied patients: in the brain of those dying in the first month after the onset of symptoms, immunocytochemistry for HSV was positive. Subjects with longer survival periods showed at necropsy the characteristic bilateral temporal lobe necrotising damage seen in HSV1 encephalitis and they had previously had an acute illness typical of this condition.

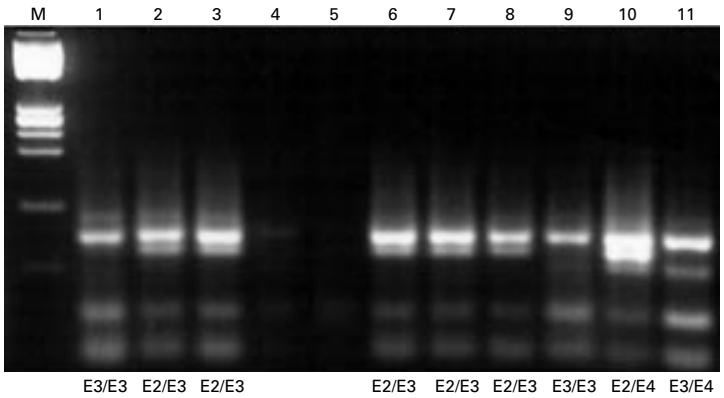
Characteristics of the cases of HSE

Case	*Sex*	*Age (y)*	*Survival*	*Genotype*
		Necropsy confirmed cases		
1	F	51	3 weeks	ε3/ε3
2	M	18	5 days	ε2/ε3
3	F	19	10 days	ε2/ε3
4	M	43	5 days	ε3/ε3
5	M	40	15 months	ε3/ε3
6	M	47	5 days	ε3/ε4
7	M	4	1 y	ε2/ε2
8	M	42	2 weeks	ε3/ε3
9	M	11	2 weeks	ε2/ε3
10	F	64	2 weeks	ε2/ε3
11	F	51	2 weeks	ε3/ε3
12	M	50	3.5 weeks	ε2/ε3
13	F	81	10 days	ε2/ε3
14	M	38	11 y	ε3/ε3
		CSF cases		
A	M	35	NA	ε3/ε3
B	M	38	NA	ε3/ε4
C	M	48	NA	ε2/ε3
D	M	49	NA	ε2/ε3
E	M	1.5	NA	ε2/ε4
F	F	63	NA	ε3/ε3
G	F	61	NA	ε2/ε3
ApoE allele	*Allele number*		*Allele frequency (%)*	
ε2	11		26	
ε3	28		67	
ε4	3		7	
Total	42			

NA=Not available.

DNA was extracted from scrapings, in the case of the paraffin blocks, using a fresh scalpel blade for each case to prevent cross contamination. Paraffin was removed with xylene and the xylene removed with ethanol, and then aqueous suspensions of the scrapings were incubated with protease K (10 μg) for several days at 37°C, to digest proteins; the enzyme was subsequently inactivated by heating at 95°C for 10 minutes. In the case of CSF, DNA was prepared as described.[6]

For apoE genotyping, a sequence in the apoE gene was amplified by PCR. For the CSF DNA, we used our normal one step PCR,[7] amplifying a 227 bp DNA product. As the quality of the DNA extracted from the paraffin blocks was usually very poor, we used seminested PCR[8] to increase the yield of amplified product and hence the sensitivity, before digestion with a restriction endonuclease,[7] *cfo*I, which enables the genotypes to be identified. In the seminested PCR, the first PCR used the same outer primers and the same conditions as in our one step procedure. The second PCR used 1μl of the initial PCR product, diluted 10-fold, as a template; one of the primers (downstream) was the same as in the first PCR, the other (upstream) primer was 5'-CTGGGCGCGGACATGGAG-3', and reaction conditions were as described.[8] All genotyping was done "blind". Odds ratios and 95% confidence intervals (95% CIs) for the allele frequencies were calculated for statistical analysis.



Agarose gel electrophoresis of DNA after seminested PCR and cfoI digestion for apoE genotyping. M=marker DNA; lanes 1–9=DNA from HSE specimens in paraffin blocks; lanes 10 and 11=DNA from frozen brain of known apoE genotype. Numbers 4 and 5 were unsuccessful on this and subsequent attempts to genotype the DNA, and so could not be included in the analyses. The 21 other cases described in the text were successfully genotyped.

Results

The table shows the characteristics of the patients. As a check on the accuracy of the seminested PCR we used it also for DNA of known apoE genotype previously determined by the usual one step PCR method[7] from two frozen brain specimens. As a further check on the whole procedure, we prepared and genotyped DNA from formalin-fixed, paraffin-embedded blocks of brain or spleen tissue from five normal subjects from whom frozen brain material was also available, allowing comparison of apoE genotyping performed from the two sources.

The figure shows typical gel electrophoresis results after seminested PCR and *cfo*I digestion and the table shows apoE genotypes for DNA from both the fixed samples and the CSF. We found that 9/21 (6/14 necropsy cases, 3/7 clinical cases) of the patients with HSE carried one apoE ε2 allele and another necropsy patient was an apoE ε2 homozygote. The apoE ε2 prevalence was thus 48% and the allele frequency 26%—significantly higher than the allele frequency of 7% obtained in our laboratory for a group of 238 normal people (mean age 37 years)[9] (OR for ε2 allele frequency= 4.6, 95% CI 2.0–10.8). The apoE ε3 and apoE ε4 allele frequencies were not significantly altered in HSE. The reliability of the procedure for preparing and genotyping DNA from fixed specimens was satisfactory: the brain DNA samples from frozen tissue yielded the expected genotypes, and those from fixed and frozen tissue showed the same apoE genotypes as each other, except in one case where the genotyping from fixed material was equivocal.

Discussion

We have previously shown[3 4] that possession of an apoE ε4 allele and presence of HSV1 in brain confers a high risk of Alzheimer's disease, and also that apoE ε4 is a risk factor for herpes labialis, both of which suggest that the interaction of HSV1 with the nervous system is constrained by apoE genotype. Possession of an apoE ε4 allele also confers risk of dementia and peripheral neuropathy in HIV-infected pre-AIDS patients.[10] These results, along with our Alzheimer's disease and herpes labialis data, add to the increasing volume of literature describing lipoprotein-virus interactions.[11] Our present preliminary findings are yet another example of lipoprotein-virus interactions and they exemplify the role apoE has in the interaction of HSV1 with the nervous system.

Interestingly, apoE has been implicated also in cerebral amyloid angiopathy related haemorrhage, in two studies the association being with apoE ε4 allele[12 13] but in another, with apoE ε2.[14]

Our present results contrast with our previous data on Alzheimer's disease: whereas we have shown that apoE ε4 is a risk factor for Alzheimer's disease when HSV1 is present in the brain, and is a risk also for herpes labialis, our new findings show that apoE ε2 but not apoE ε4 confers a risk of the rare disease HSE.

As to the interaction between apoE and HSV1 in HSE, it is worth noting that the disturbed apoE frequencies in HSE are apparent in both the untreated subjects, whose survival times after onset of disease varied considerably (table), and in the subjects treated with ACV. Of course we cannot make deductions relating to survival from the treated patients, but the variability of survival times in the untreated, and the similarly high ε2 allele frequency of untreated and treated patients suggest that apoE may influence susceptibility to HSE rather than survival. Susceptibility in turn may be determined by the ease of virus reactivation (if HSE is due to reactivation from latent infection), or by the neuroinvasiveness/neurovirulence of the virus for CNS cells—perhaps involving cell surface interactions. HSV1 binds to heparan sulphate proteoglycan (HSPG) molecules on the cell surface,[15] as do apoEs,[16] the second subsequently entering cells via the receptors of the low density lipoprotein receptor (LDLR) family, which includes LDLR and LDLR related protein (LRP). Based on the fact that the affinity of apoE2 is lower than that of the other isoforms for these receptors,[17] and on the suggestion that rare HSV1 mutants (highly neurovirulent) cause HSE,[18] we speculate that if such mutants were to access cells via one of these LDLR family receptors instead of HSPG, they would compete with apoEs; apoE2 would compete to a lesser extent—hence allowing more entry and spread of virus. In Alzheimer's disease, we have proposed that apoEs and HSV1 compete for binding to HSPG molecules in the cell surface,[3 4] apoE4 doing so less efficiently—thus allowing more virus spread—as its entry via HSPG is known to be less (in neuronal cells) than that of the other isoforms.[16] Interestingly, the interaction of apoE ε4 and HIV in HIV-induced dementia and peripheral neuropathy might also be mediated by HSPG.[19]

Clearly, the next step is to examine the apoE genotypes of a much larger number of patients with HSE, preferably using fresh or frozen tissue, CSF (from which cell DNA can be prepared), or blood specimens. If these confirm our preliminary data, it would strengthen the case for a vaccine against HSV1, particularly for apoE ε4 carriers and for apoE ε2 carriers (in the case of Alzheimer's disease and HSE, respectively).

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