# Herpes Simplex Virus Type 1, Apolipoprotein E and Alzheimer's Disease

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**KEY WORDS**

*HERPES SIMPLEX VIRUS ▮ ALZHEIMER'S DISEASE ▮ CENTRAL NERVOUS SYSTEM ▮ APOLIPOPROTEIN E-ε4 ▮ HUMAN HERPESVIRUS TYPE 6 ▮ MANAGEMENT RECOMMENDATIONS*

**SUMMARY**

**Various infectious agents, and viruses in particular, have been proposed as potential causes of Alzheimer's disease. Herpes simplex virus type 1 (HSV-1) is one of the stronger candidates because it is neurotropic, ubiquitous in the general population and able to establish lifelong latency in the host. The body of evidence for the role of HSV-1 in Alzheimer's disease is contentious but centres around its presence in the regions of the brain affected by Alzheimer's disease. The fact that HSV-1 is also present in elderly patients without the disease suggests that the virus is not an independent cause of the condition. The incidence of Alzheimer's disease is highest in carriers of the apolipoprotein (APO) E-ε4 allele who harbour HSV-1 DNA in the CNS, so it is possible that these agents are co-factors for the disease. However, studies investigating this have been small and limited by the need to access brain tissue from non-diseased APOE-ε4 carriers. Human herpesvirus type 6 (HHV-6) is another virus investigated as a potential contributor to Alzheimer's disease. However, it is uncertain whether its presence is a cause or a consequence of the disease and it may be that HHV-6 merely exacerbates the potentially harmful effects of HSV-1 in APOE-ε4 carriers. It is difficult to ascertain the role of an infectious agent in Alzheimer's disease due to the difficulty of establishing the timepoint at which the agent becomes involved. Further research into the possible link between herpesviruses and Alzheimer's disease is therefore required.**

## Introduction

THIS PAPER FOCUSES on herpes simplex virus type 1 (HSV-1) as a potential aetiological factor in the development of Alzheimer's disease. Studies discussed include investigations of the prevalence and distribution of HSV-1 DNA in brain tissue using polymerase chain reaction (PCR), and potential herpesvirus-targeted drug therapies for the prevention and/or management of the disease.

## Potential Role for a Virus in the Aetiology of Alzheimer's Disease

Genetic factors play a significant aetiological role in the development of early-onset Alzheimer's disease (defined as patients aged <65 years).[1] Early-onset disease is inherited as an autosomal dominant and three genes are associated with a subset of cases. These genes code for the amyloid precursor protein and the proteins presenilin-1 and presenilin-2.[2,3] The majority of cases (>90%), however, are late-onset, occurring in patients aged 65 years or older. Over 95% of late-onset cases are sporadic in nature and do not appear to be associated with a known set of genetic markers, suggesting that the disease has a multifactorial aetiology.

An infectious cause of Alzheimer's disease has been proposed to account for several observations of a discordance of occurrence between twins.[4] Viruses have been suggested as an aetiological factor in its pathogenesis because they have been associated with other neurological diseases. In particular, HSV-1 has been implicated, as it is neurotropic – matching the neurological nature of this disease – and ubiquitous within the human population,[5] residing lifelong in the peripheral nervous system, normally in a latent state. However, the role of an infectious agent in the pathogenesis of Alzheimer's disease is difficult to ascertain because of the inherent uncertainty in establishing the precise timepoint when the agent becomes involved. It may plausibly act during the early stages of the disease process, yet no longer be required or evident during later stages, as is the case with human papilloma virus and its relation to cervical cancer.[6]

### PREVALENCE OF HSV-1 IN BRAIN TISSUE

In the acute brain disease herpes simplex encephalitis (HSE), HSV-1 infects and causes pathological changes in the same regions of the brain as those mainly affected in Alzheimer's disease.[7] Even in the absence of disease, virus DNA can be present in the central nervous system (CNS). It has been detected in the brain of many patients with Alzheimer's disease and, importantly, also in normal brains of aged individuals,[8–10] but it is largely absent from younger human brains,[11] (Wozniak *et al.*, unpublished). Hypothetically, virus reaches the brain in older individuals because of a decline in the immune system.[9]

The presence of the virus has been inferred both by direct detection of virus DNA by PCR[8–10] and by the detection of intrathecal antibodies (Wozniak *et al.*, submitted). However, in early studies using relatively insensitive techniques, such as Southern- or dot-blotting or *in situ* hybridization, HSV-1 was often not detected or, if it was found, it was at a lower frequency and with greater variability than in later studies using sensitive PCR assays (Table 1).[8–16] These PCR studies usually sampled the temporal and frontal cortex regions of the brain as, in HSE, HSV-1 is usually present in these areas.[17] The variation in the percentage of individuals found to be HSV-1 DNA-positive is probably due, in part, to the inclusion of subjects of a very wide age range, as well as to one study being conducted with Japanese subjects, who were likely to have a relatively lower prevalence of HSV-1 infection.[14] Furthermore, not all the studies appeared to have checked for the artefacts to which PCR is prone.

In an ongoing study examining synthesis of intrathecal antibodies to HSV, immunoglobulin (IgG) was detected in a similar proportion of patients with Alzheimer's disease and elderly controls (52% and 69%, respectively). These values do not differ significantly (Wozniak *et al.*, submitted). Detection of

## RECOMMENDATIONS AND STATEMENTS

- Further research is required to understand whether there is an association between HSV-1 and the development of Alzheimer's disease (research need recommendation)

RECOMMENDATION AND STATEMENT CATEGORIES

**Category 1**

Consistent evidence from controlled clinical trials. For example, for an antiviral, this would include results from at least one well-designed, randomized, controlled clinical trial, and, in the case of laboratory studies, consistent evidence from comparative studies.

**Category 2**

Evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytical studies (preferably from more than one centre), or from multiple time-series studies or dramatic results from uncontrolled experiments.

**Category 3**

Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports of expert committees.

**Research Need**

Area in which research is warranted.

**Table 1: Studies that have detected HSV-1 DNA using PCR in brain tissue from patients with Alzheimer's disease and controls (non-neurological cases) [8–16]**

| Study | Primers used for PCR | Area of brain sample | HSV-1 DNA-positive individuals: Alzheimer's disease *n* (%) | HSV-1 DNA-positive individuals: Controls *n* (%) |
|---|---|---|---|---|
| Jamieson *et al.*[8] | TK | Temporal and frontal cortex; hippocampus | 8 (100) | 6 (100) |
| Jamieson *et al.*[11] | TK | Temporal and frontal cortex; hippocampus | 21 (67) | 15 (60) |
| Baringer & Pisani[12] | Various | Various | NR | 40 (35) |
| Gordon *et al.*[13] | Various | Hippocampus and frontal cortex | | 30 (27) |
| Itabashi *et al.*[14] | gD | Temporal and frontal cortex | 46 (30) | 23 (22) |
| Itzhaki *et al.*[9] | | Frontal and temporal cortex | 46 (67) | 44 (64) |
| Lin *et al.*[10] | TK | Frontal and temporal cortex | 61 (74) | 48 (63) |
| Bertrand *et al.*[15] | gD | Various | 98 (75) | 57 (72) |
| Cheon *et al.*[16] | gD | Frontal cortex | 10 (100) | 10 (100) |

HSV-1, herpes simplex virus type 1; PCR, polymerase chain reaction; gD, glycoprotein D protein; TK, thymidine kinase; NR, not reported.

the antibodies was not attributable to blood–cerebrospinal fluid (CSF) barrier damage (as indicated by serum and CSF albumin levels). In addition, antibodies to HSV were not detected in the CSF of children, whose brains would be expected to be virus-negative.

LOCATION OF HSV IN BRAIN TISSUE

Alzheimer's disease is histopathologically characterized by intracellular accumulation of neurofibrillary tangles, abnormal extracellular deposition of the β-amyloid protein into focal plaques and extensive neuronal cell death. These changes predominantly occur in limbic brain structures, such as the medial temporal and frontal cortex and the hippocampus.[18]

The HSV-1 genome is detectable by PCR in the temporal and frontal cortex both of patients with Alzheimer's disease and also of age-matched controls without the disease.[8,11] Moreover, virus genome is not detected in the occipital cortex, which is largely spared in Alzheimer's disease.[11] Thus, in keeping with the proposed association of HSV-1 with Alzheimer's disease, HSV-1 DNA is detectable in regions of the brain that are affected by the disease but at no higher frequency than controls. However, the relationship between detection of HSV-1 DNA and the ability of the virus to reactivate from its latent state is not known; for there to be a relationship between HSV-1 and Alzheimer's disease, the virus must presumably reactivate in the CNS.

PRESENCE OF OTHER HERPESVIRUSES IN THE BRAIN

If herpesviruses other than HSV-1 are present in CNS tissue, they could (theoretically) contribute to the development of Alzheimer's disease. However, varicella zoster virus (VZV) is not found in brain tissue from patients with Alzheimer's disease.[19] Along with HSV-1, both HSV-2 and cytomegalovirus (CMV) occur in a similar proportion in brains from people with or without Alzheimer's disease, but the latter two occur relatively infrequently.[20] Thus, given the relatively high prevalence of Alzheimer's disease, these viruses are not likely associated with this neurological disorder. However, HHV-6 is found in the CNS of more patients with Alzheimer's disease than in age-matched controls.[20] The implications of the occurrence of this herpesvirus in relation to Alzheimer's disease are discussed later in this chapter.

## Evidence for APOE-ε4 Allele Involvement

HSV-1 INFECTION ALONE IS NOT INDEPENDENTLY ASSOCIATED WITH ALZHEIMER'S DISEASE

The ubiquity of HSV-1 infection and the observation that HSV-1 is frequently detected in the brains of elderly individuals with and without Alzheimer's disease, combined with the fact that the disease develops only in a subset of HSV-1-infected individuals, suggest that HSV-1 infection alone is not independently associated with Alzheimer's disease. The likelihood of developing the disease is hypothesized to be increased by an interaction between an APOE allele and HSV-1.[9]

The protein apoE plays an important part in the physiology of neural tissue. It controls neurite outgrowth and branching, microtubular depolymerization, adhesion to basal membrane components and immunomodulation.[21,22] Importantly, apoE binds to the neurofibrillary tangles and is present in the senile plaques that occur in Alzheimer's disease and it binds to β-amyloid,[23] the main component of the plaques.

The human APOE gene has three common alleles, designated ε2, ε3 and ε4. These alleles encode apoE2, E3 and E4 protein isoforms, respectively. The E2 and E4 isoforms differ from E3 by a single amino acid substitution at residue 158 for E2 and 112 for E4.[24] The isoforms also have opposite effects on neuronal outcomes. In neuronal cultures, the presence of E2 or E3 is associated with enhanced neurite outgrowth and

branching, and in contrast, E4 reduces the outgrowth and branching.[21]

The presence of the APOE-ε4 allele has been associated with the development of Alzheimer's disease.[25,26] However, carriage of APOE-ε4 alone is neither essential, nor sufficient, for disease development. Thus, an interaction with another factor likely results in Alzheimer's disease. The possibility of an APOE-HSV-1 association has been investigated in this context.

In studies by Itzhaki and colleagues [9,10] the relative risk of developing Alzheimer's disease for those positive for HSV-1 DNA in brain and who carried an APOE-ε4 allele was 12-fold that of individuals possessing only one or neither of these factors (Table 2). In another study, by Itabashi *et al.*,[14] PCR was used to detect HSV DNA (using a 142 base pair sequence for the glycoprotein D gene of HSV-1 and HSV-2) in brain samples of 46 Alzheimer's disease patients and 23 age-matched controls (mean age 77.25 years). In agreement with the results of Itzhaki and collegues,[9] significantly more patients with Alzheimer's disease were positive for both HSV and the APOE-ε4 allele than patients with one factor alone,[14] although the proportion of patients and of controls who were HSV-1-positive was much lower than in some of the previous studies, and the incidence of Alzheimer's disease among those carrying the APOE-ε4 allele was similar in HSV-positive and HSV-negative subjects.[14] These differences might be at least partly explained by the relatively low prevalence of HSV-1 infection in a Japanese population, as shown by the low proportion with serum antibodies to HSV-1 – less than 50% compared with 80–90% in elderly Europeans and North Americans.[5]

These results suggest that either the presence of HSV-1 in the brain or possession of the APOE-ε4 allele alone does not appear to be a significant predictor for developing Alzheimer's disease. Rather, it is the combination of the two factors that is likely to have a stronger association with the development of Alzheimer's disease. However, these studies were conducted with relatively small sample sizes, APOE-ε4 carriage is not high, particularly in elderly people, and most elderly individuals harbour HSV-1 in the brain. As a consequence, it would be desirable to obtain samples from elderly individuals without dementia who carry the APOE-ε4 allele in order to determine whether or not they are HSV-1-positive in brain; *de facto*, these requirements limit the size of this group and hinder comparisons. Therefore, to evaluate further, and in a more robust manner, the association of HSV and APOE-ε4 in Alzheimer's disease, a larger number of samples will have to be analysed. The HSV-1–APOE-ε4 combination accounts for about 60% of the patients investigated;[10] risk factors for the remaining 40% are unknown, but as mentioned earlier, the aetiopathogenesis of the disease is very probably multifactorial.

OTHER INTERACTIONS WITH APOE

The association between Alzheimer's disease and infection with HSV-1 combined with carriage of the APOE-ε4 allele has led to the investigation of the role of APOE in other diseases. Data suggest that susceptibility to facial herpes may be influenced by the same APOE allele.[9,10] There is also an association of the APOE-ε4 allele with protection from severe damage by hepatitis C virus (HCV) in the liver.[27] Further, preliminary results indicate that age of first infection by the malarial protozoon is determined by APOE.[28] As a whole, these studies suggest that the possible role of APOE in disease is worthy of further investigation.

**Interactions with APOE and HSV in patients with facial herpes:** As with the relationship observed between HSV-1 in the CNS and Alzheimer's disease, a high proportion of HSV-1-infected individuals who have recurrent episodes of HSV-1 facial herpes (due to reactivation of the virus in the peripheral nervous system) carry the APOE-ε4 allele (Table 3). Analysis indicated that the APOE-ε4 allele frequency was significantly higher (29.7% versus 8.4%; $P<0.0001$) among those with recurrent facial herpes than those without.[9,10] This may be because HSV-1 can reactivate and cause more damage in APOE-ε4 carriers during stress or immunosuppression than in those with other alleles; the same may apply also to the CNS in respect to Alzheimer's disease.

**Interactions between APOE and HIV, and APOE and hepatitis C virus:** Individuals with HIV infection (pre-AIDS) who carry the APOE-ε4 allele show increased rates of reversible dementia and peripheral neuropathy.[29] The APOE-ε4 allele appears to be associated with a lower risk of liver damage in hepatitis C virus-positive individuals, which is in contrast to observations in Alzheimer's disease, in which carriage of the APOE-ε4 allele is associated with the disease. More individuals graded 0–1 on the Knodell score (fibrosis and necroinflammation) of liver damage carried the APOE-ε4 allele than those with a score ≥9 ($P<0.001$).[27] Further data are required to explore the relationship between APOE and hepatitis C virus, and to address the underlying molecular mechanisms that differentiate the interactions of hepatitis C virus and HSV with the APOE gene products.

In summary, the above studies showing that APOE determines the outcome of (or susceptibility to) infection by very diverse pathogens strongly, even if indirectly, support the concept that HSV-1, together with APOE-ε4, is involved in the aetiology of Alzheimer's disease. This is supported further by a study of APOE transgenic mice in which, 6 days after HSV-1 infection, the viral load in the brain was much greater in APOE-ε4 than in APOE-ε3 animals, whereas there was no difference in viral load in peripheral

**Table 2: Prevalence and frequency of APOE-ε4 allele and effect of HSV-1 status in the brain, comparing HSV-1 positive and HSV-1 negative patients with Alzheimer's disease and age-matched healthy controls.[10] Values in parentheses are group numbers for prevalence, allele numbers for allele frequency**

| HSV-1 status in brain | Number of subjects | Prevalence of APOE-ε4 allele (%) | Frequency of APOE-ε4 allele (%) |
|---|---|---|---|
| **Patients with Alzheimer's disease** | 61 | | |
| Positive | 45 | 80* (36) | 52* (47) |
| Negative | 16 | 25 (4) | 13 (4) |
| **Controls** | 48 | | |
| Positive | 30 | 10 (3) | 5 (3) |
| Negative | 18 | 17 (3) | 8 (3) |

*$P<0.0001$.
APOE-ε4, apolipoprotein E-ε4; HSV-1, herpes simplex virus type 1.

**Table 3: APOE genotypes of individuals with and without facial herpes[10]**

| | Facial herpes (*n*=69) | No facial herpes (*n*=77) |
|---|---|---|
| **Genotype** | | |
| E2/E2 | 0 | 1 |
| E2/E3 | 4 | 11 |
| E2/E4 | 6 | 0 |
| E3/E3 | 27 | 52 |
| E3/E4 | 29 | 13 |
| E4/E4 | 3 | 0 |
| **Allele number** | | |
| E2 | 10 | 13 |
| E3 | 87 | 128 |
| E4 | 41 | 13 |
| APOE-ε4 (%) | 29.7* | 8.4 |

APOE, apolipoprotein E; *$P$<0.0001 versus no facial herpes.

tissues.[30] Despite the route to the brain being haematogenous, which is unlikely to be the case in humans, this result indicates that entry into brain was earlier, and/or replication or spread of the virus was greater, in the APOE-ε4 transgenic animals. The role of HSV-1 in Alzheimer's disease is also supported by another approach: in three epidemiological studies, one showed that vaccination against various viruses is protective against the disease,[31] and the others found that cognitive decline occurs in Alzheimer's disease patients for at least 2 months after a systemic infection,[32] and that in elderly cardiovascular patients, cognitive decline is associated with viral burden (HSV-1 alone and with HSV-2 and CMV).[33]

**Possible mechanisms of an APOE-ε4 and HSV-1 interaction:** The evidence indicating the possibility of an interaction between HSV-1 and APOE-ε4 would be strengthened if the mechanisms by which the virus or lipoprotein can potentiate Alzheimer's disease were known. Several mechanisms are postulated for the contribution of APOE-ε4 and HSV-1, either together or alone, in the development of the disease. One suggestion involves competition between APOE-ε4 and HSV-1 at the plasma membrane of neurons.[9] Both HSV-1 and apoE bind to heparan sulphate proteoglycan (HSPG) molecules in cell plasma membranes.[34,35] ApoE, which is synthesized by astrocytes, is taken up by neurons in the brain, where it is involved in nerve regeneration. Thus, HSV-1 may compete with APOE-ε4 for HSPG at the cell surface, thereby influencing the extent to which the virus enters a neuron and hence influencing virus spread and consequent damage. Alternatively, since apoE4 appears to be less proficient at repairing neuronal injury, it might allow more damage to persist after virus action.

The apparent paradox, that APOE-ε4 confers risk of Alzheimer's disease, facial herpes and dementia and neuropathy in an individual with HIV, but is protective in hepatitis C virus-induced liver damage, could be explained by a cell- or tissue-type dependence of binding and entry into cells of both virus and apoE. In the case of hepatitis C virus, cell entry occurs via one of the family of receptors to which apoE attaches. Studies on the affinity of the different apoE isoforms for this receptor show that apoE4 has the highest affinity in the case of hepatoma cells in culture.[36,37] Thus, apoE4 could compete more strongly than the other isoforms with hepatitis C virus for attachment and entry into liver cells, hence minimizing virus spread and damage. The isoform effect is probably dependent on cell type: in the only other type studied, fibroblasts, no difference in affinity amongst the isoforms was found. This might explain why no significant dependence on APOE was found in a study of another HSV-1 disease, herpes simplex keratitis, in which the main cell type infected (in the cornea) is fibroblast-like.[38] In the case of the malaria protozoon, this pathogen too binds to HSPG and also to a specific apoE receptor in the hepatocyte cell membrane. Therefore, as with the above viruses, competition for cell entry between pathogen and apoE would account for our findings.[28]

Another hypothesis is that cleaved HSV-1 glycoprotein B (gB) might serve as a potent initiator of the cascade of β-amyloid fibril formation and deposition. A sequence of gB is highly homologous to the β-amyloid domain thought to be involved in neurotoxicity, nucleation and assembly.[39] A relevant gB peptide (residues 22–42), but not a scrambled version thereof, forms fibrils similar to the β-amyloid fibrils found in Alzheimer's disease tangles and plaques,[39] and is more neurotoxic on a molar basis than β-amyloid.[39] Thus, if cleaved appropriately, this peptide could initiate β-amyloid fibril formation. Interestingly, HSV-1 has been found to bind to the various lipoproteins in serum and this occurs via gB.[40] Another relevant finding is that HSV-1 affects the degradation of the amyloid precursor protein (APP), the precursor of β-amyloid (Dobson *et al.*, unpublished). Infection of cultured human neuroblastoma cells causes a large increase in a 55kDa C-terminal component (although as expected during a lytic infection, levels of APP and most other proteins decrease). Further, APP has been shown to have a role in the fast anterograde transport of HSV-1.[41]

A further mechanism by which HSV-1 may be associated with Alzheimer's disease is through induction of interleukin (IL)-6. HSV-1 can induce IL-6 expression in animal models, and overexpression of IL-6 can lead to cerebral neurodegeneration in mice.[42,43] This neurotropic cytokine is elevated in amyloid plaques of Alzheimer's disease brains; it has been detected in 10 out of 10 Alzheimer's disease brains compared with only three of nine age-matched healthy controls,[16,44] and a raised IL-6 level in severe Alzheimer's disease correlates with level of neurofibrillary tangle pathology,[45] suggesting an adverse effect of this cytokine.

The above studies on gB and β-amyloid, on HSV-1 and APP degradation, and on APP and HSV-1 in anterograde transport, all implicate HSV-1 in Alzheimer's disease. However, none yet indicates the role of APOE. Nonetheless, a combination of some of the mechanisms proposed here provides a speculative sequence for the development of Alzheimer's disease. Stressful events, including peripheral infections, might activate latent HSV-1 in the brain, perhaps via entry of cytokines and subsequent inflammation in the latter organ,[32,33] the magnitude of the resulting damage depending on APOE;

the actual production of damage might involve interference with APP metabolism (Dobson *et al.*, unpublished) and/or seeding of amyloid plaques by viral glycoproteins.[39] As for the results of the study of APOE-transgenic mice,[30] indicating a greater viral load or earlier viral entry in brain of HSV-1-infected APOE-ε4 transgenic mice, an analogous process in APOE-ε4 humans would account for their earlier age of onset of Alzheimer's disease and for the greater deposition of β-amyloid in their brains.

In summary, several mechanisms have been proposed to account for the potential contribution of HSV-1 to the pathogenesis of Alzheimer's disease. However, further research is required to establish any pathogenic mechanisms that involve the virus.

## Potential HSV-1-specific Strategies to Prevent the Development of Alzheimer's Disease

If HSV-1 is proven to play a role in the development of Alzheimer's disease, two potential strategies may either prevent disease or retard its progression. These are the use of antiviral drugs or immunization against HSV-1 (or even HHV-6 if further evidence confirms this as a risk factor). The latter option requires the development of an effective vaccine.

POTENTIAL USE OF ANTIVIRAL DRUGS

It is too early to predict the impact of antiviral therapy on the development of Alzheimer's disease. The pathogenesis of the disease and, particularly, any role of HSV-1 should first be assessed. If HSV-1 infection in the CNS is an early event, then long-term suppressive therapy will be required. Moreover, identification of suitable candidates is problematic because of the invasive nature of sampling to determine infection of the CNS. Furthermore, seropositivity does not correlate with HSV-1 infection of the CNS, the majority of elderly individuals being HSV-1 seropositive. An alternative approach would be to identify those individuals who harbour HSV-1 in the brain by seeking antibodies to HSV-1 in the CSF, although this would not identify all, since the proportion with intrathecal antibodies is somewhat lower than the proportion of brains in which HSV-1 DNA has been detected (Wozniak *et al.*, submitted). However, as this is an invasive procedure, further study of the potential association between HSV-1 and Alzheimer's disease would be required before such a suggestion could be made, even in the setting of preliminary research.

POTENTIAL USE OF A VACCINE

If the link between HSV-1 and Alzheimer's disease were proven, vaccination would be a potential strategy to prevent the development of Alzheimer's disease. However if HSV-1 infection in childhood were an important factor, a vaccine would have to be administered in infancy in order to be effective. One preliminary study has investigated the use of a mixed HSV-1 glycoprotein vaccine prior to infection of mice with HSV-1.[46] Several months after infection, HSV-1 DNA was detected using PCR in the brain tissue of 41% of the unvaccinated mice but in only 7% of vaccinated mice ($P<0.001$). Thus, the vaccine appeared to prevent latent HSV-1 infection of the CNS.[46] However, although mice have been used as a model for reactivation of the virus in the peripheral nervous system of humans, this strategy would first require a vaccine that is protective in humans.

## Relationship between HHV-6 Infection and APOE-ε4

HHV-6 is significantly more common in brain tissue from patients with Alzheimer's disease than normal controls.[20] In a retrospective study of 50 individuals who had Alzheimer's disease, the frequency of HHV-6 was 70%, compared with 40% in 35 age-matched controls.[20] The predominant HHV-6 form was type B in the patients with a lesser predominance (over type A) in the controls. In contrast to the results seen with HSV-1,[9,10] the APOE-ε4 allele frequency was significantly higher in both the HHV-6-positive and HHV-6-negative patients than in the HHV-6-positive and negative controls. Thus, the combination of HHV-6 in the brain and APOE-ε4 does not confer a risk of Alzheimer's disease. Whether HHV-6 presence in the brain is a cause or consequence of the disease is unknown at present. However, in AIDS and progressive multifocal leucoencephalopathy, two diseases known to be caused by viruses, HHV-6 has been suggested to accelerate the disease, and in animal and cell culture studies this virus, when used as a co-infectant with HSV-1 and HIV, respectively, has been found to augment the damage; therefore, in Alzheimer's disease, HHV-6 might exacerbate the harmful effects of HSV-1 in APOE-ε4 carriers. Clearly though, larger sample sizes are required to determine the significance of HHV-6 in the development of this neurodegenerative disease.

## Conclusions

HSV-1 DNA is present in the brain of a high proportion of both elderly people and patients with Alzheimer's disease, although in only very few younger individuals (without Alzheimer's disease). The incidence of Alzheimer's disease is highest in carriers of the APOE-ε4 allele who harbour HSV-1 DNA in the CNS. However, the incidence of this neurodegenerative disease is not increased in those with HSV-1 DNA or APOE-ε4 alone. Furthermore, the incidence of APOE-ε4 is higher in those who have recurrent facial herpes than in those who do not. Interestingly, individuals with HIV infection (pre-AIDS) who carry APOE-ε4 have excess dementia and peripheral neuropathy. In contrast, APOE-ε4 carriage in individuals with hepatitis C infection appears to be associated with a lesser extent of liver damage. Thus, APOE determines the outcome of infection by three very different viruses. The allelic effect on the development of Alzheimer's disease appears to be cell- or tissue-dependent.

Interestingly, HHV-6 DNA is present in a high proportion of Alzheimer's disease brains, although whether this virus is involved in the pathogenesis of Alzheimer's disease remains to be elucidated.

In contrast to the effects of systematic infection on cognitive function in elderly patients[32,33] (many of whom would harbour HSV-1 DNA in brain, which could be reactivated by inflammation, etc.), a very recent study on bipolar disorder patients shows that in middle aged controls, there is no such decline[47]. This is very consistent with our findings that HSV-1 DNA is rarely present in the brains of younger people,[11] (Wozniak *et al.*, unpublished), and it suggests that inflammation alone in brain is insufficient to affect cognitive function; instead a factor such as HSV-1 presence in brain may be necessary.

## Acknowledgements:

I thank Drs MA Wozniak and CB Dobson for their very helpful comments.

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**Received for publication: 29 September 2003**
**Accepted for publication: 11 December 2003**

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