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Letter to the Editor

Association of HSV1 and apolipoprotein E-ε4 in Alzheimer's disease

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The results of Marques *et al* (2001) are very surprising in that only one brain specimen was found to be positive for herpes simplex virus type 1 (HSV1) in a group of 15 Alzheimer's disease (AD) patients, and only one in 15 age-matched controls. Obviously, therefore, the association of HSV1 with the type 4 allele of the apolipoprotein gene (apoE-ε4) in AD patients that we discovered (Itzhaki *et al*, 1997) could not be tested in their specimens. This association between HSV1 and apoE-ε4, which confers a high risk of AD, is one of several such interactions between viruses and lipoprotein components (Dobson and Itzhaki, 1999). Other viral disorders involving apoE include herpes labialis (Itzhaki *et al*, 1997; Lin *et al*, 1998) and herpes simplex encephalitis (Lin *et al*, 2001)—via types 4 and 2 alleles, respectively—and dementia and peripheral neuropathy in HIV-infected subjects (pre-AIDS)—via type 4 (Corder *et al*, 1998).

The findings of Marques *et al* (2001) differ from those of previous studies seeking HSV1 in human brain by PCR, all of which revealed the virus in a much higher proportion of both normals and patients—and were done on a relatively large number of subjects. This higher proportion of virus-positives was apparent even in cases where people of a range of ages were investigated. On the basis of our studies showing absence of the virus in young and infant brains (Jamieson *et al*, 1992; Wozniak, Lin, Cairns, Mann, Itzhaki, to be published), inclusion of the latter brains would yield a lower value for proportion infected. After our initial study (Jamieson *et al*, 1991) establishing the presence of virus in elderly brains, Bertrand *et al* (1993) detected HSV1 in 75% of 98 AD brains and 72% of 57 elderly control brains. These values agree well with our current estimates of about 74% and 63% (Lin *et al*, 1998), based on 61 patients and 48 nonneurological age-matched controls. Baringer and Pisani (1994) examined brains from 40 nonneurological subjects with a range of ages and found 35% were virus-positive. In two subsequent smaller studies, Gordon *et al* (1996) examined 30 nonneurological controls with a range of ages and detected HSV1 in 27%, and Sanders *et al* (1996) found HSV in 29% of 17 elderly nonneurological controls and 23% of 22 AD patients.

The striking difference between these values and those of Marques *et al* (2001) suggests that the overall procedures of the latter group have led to an inability to detect virus-positive specimens, despite their checks for the presence of inhibitors of PCR, including usage of an internal control with an amplified product longer than the HSV1 amplicon. However, their internal control would presumably be less susceptible to any inhibitor, because of its relatively high concentration, than any HSV1 DNA in brain. Also, they did not verify the actual brain DNA product by using primers for a human gene. In our publications we recommended the latter procedure as well as several other precautions (Jamieson *et al*, 1991; Lin *et al*, 1998). Other factors that would presumably contribute to the problems of detecting reproducibly the very low levels of HSV1 DNA in human brain, and might thus account for the variety of values of virus-positivity cited above are: usage of different brain regions, and of fixed or frozen specimens, as well as age of subject. To these should be added the inherent problems of PCR for detection of low-level sequences. However, we suspect that main reason for the very low detection level found by Marques *et al* (2001) (and the variability of values found in other studies) might be an inability to extract the viral

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DNA. Indeed, we are currently examining different preparative methods in the hope of elucidating why these yield different proportions of viral DNA in the final DNA product.

We previously offered help to Marques and coworkers when they contacted us to request relevant information and we gladly offer it again in the hope of clarifying the issue, thus providing useful information for those in the field.

Note in proof

We recently detected intrathecal antibodies to HSV (these are known to be very long-lived in cerebrospinal fluid) in almost 50% of AD patients and of age-matched normals (Wozniak *et al*, to be published). This suggests the presence of the virus in the brain and indicates the possibility that it reactivated there.

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