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Association between apolipoprotein E4 genotype and human immunodeficiency virus–associated dementia in younger adults starting antiretroviral therapy in South Africa

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It is not known whether the apolipoprotein E (ApoE) ε4 allelic variant is associated with human immunodeficiency virus (HIV)-associated dementia (HAD) in a South African population, where HIV clade C is predominant. ApoE genotyping was performed on 144 participants in a larger study of HIV-associated neurocognitive disorders (HAND). There was a lower frequency of the ε2 and ε3 alleles in the HIV-positive group, compared to a group of 300 community-based newborn infants. There were no differences in ApoE genotype across different categories of HAND. The ε4 allelic variant was less common in individuals with HAD than in those without HAD. These findings suggest that the ε4 allelic variant in HIV-positive individuals is not associated with the development of HAD in Southern Africa. *Journal of NeuroVirology* (2010) **16,** 377–383.

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Introduction

Apolipoprotein E (ApoE) is a protein involved in lipid metabolism in both peripheral tissue as well as the central nervous system. It has three major allelic variants: ε2, ε3, ε4. ApoE allelic variants occur with different frequencies across different ethnicities, in particular between individuals of European and African descent (Gerdes, 2003). The ApoE ε4 allelic variant is thought to be the most common in African populations. In one epidemiologic review, the frequency of ε4 in Caucasian samples (defined as the number of ε4 alleles in the total sample) ranged from 0.082 to 0.194, whereas in a Nigerian sample, the reported frequency was 0.310 (Eichner *et al*, 2002). In a study of the Khoi San population of Southern Africa, an allelic frequency of 0.37 was reported (Eichner *et al*, 2002; Sandholzer *et al*,

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1995b). The higher frequency of ApoE4 in individuals of African ethnicity may have substantial implications for the frequency of neurodegenerative conditions in this setting.

The ε4 allelic variant has been shown to increase the risk of developing both Alzheimer's disease (AD) and certain brain disorders (Frisoni *et al,* 1994; Samatovicz 2000). ApoE4 is thought to be involved in the consequences of abnormal lipid metabolism (such as atherosclerosis), as well as in several inflammatory pathways in the central nervous system (CNS) (Eichner *et al,* 2002). The latter pathways include increased microglial responses, oxidative stress, and the production of nitric oxide (Barger and Harmon, 1997; Colton *et al,* 2004; Laws *et al,* 2003). In AD, the ε4 allelic variant is associated with an increased deposition of amyloid plaques (Polvikoski *et al,* 1995). Amyloid production is in part mediated by cytokines secreted from activated microglia, a process that may be accelerated in carriers of the E4 allele (Corder *et al,* 1998). Microglial activation may also occur in infections of the CNS infections, such as human immunodeficiency virus (HIV). The combined effect of certain ApoE allelic variants and HIV infection may result in an additive neurodegenerative effect (Pemberton *et al,* 2008).

There are similarities between neurodegeneration in HIV-associated dementia (HAD) and that of AD. For example, the amyloid precursor protein has been reported in those with HIV encephalitis (An *et al,* 1997). Other clinical-pathological studies among older subjects with HIV infection have identified an association with CNS plaques and elevations of cerebrospinal fluid (CSF) amyloid beta (Esiri *et al,* 1998; Valcour *et al,* 2004). In one report it was noted that a genetic variant of tumor necrosis factor alpha (TNF-α) is associated with HAD (reported as acquired immunodeficiency syndrome [AIDS] dementia complex) (Pemberton *et al,* 2008). Clinical studies of the associations between ApoE and HAD have produced conflicting results, with some reporting increased risk for HAD and peripheral neuropathy and others describing no association (Corder *et al,* 1998; Dunlop *et al,* 1997). Some have noted that the ApoE4 genotype together with advancing age confers an increased risk for HAD (Burt *et al,* 2008; Valcour *et al,* 2004).

To date most studies of ApoE and HIV-associated neurocognitive disorders have been conducted in high-income countries where HIV clade B predominates. It has been proposed that the different HIV clades exert different neurotoxic effects via a functional difference in the transactivator protein (tat) (Mishra *et al,* 2008). Clinical reports from India and South Africa suggest that HIV-associated neurocognitive disorders (HAND) may be as common in regions where clade C predominates (Gupta *et al,* 2007; Joska *et al,* 2009). In these populations, HAND may then involve mechanisms of neurotoxicity other than those mediated by tat. The possible role of other correlates of HAND, such as ApoE4, requires further study. Evidence for the interactive effect of age with ApoE4 on neurodegeneration suggests that this variant is associated with an accelerated disease course and progression to death (Burt *et al,* 2008; Valcour *et al,* 2008). These data are supported by the fact that although HAD has been linked to a higher frequency of ε4 in some reports, this genotype occurs less often in older individuals, even when controlling for ethnicity (Valcour *et al,* 2008).

In this study, we sought to establish the distribution and frequency of ApoE allelic variants in a South African HIV-positive clinic population well characterized with respect to HAND status. In particular we evaluate potential associations between ApoE4 and HAD. We hypothesized that the ApoE ε4 allelic variant would occur at higher frequencies similar to that reported in other African populations, and that this allelic variant would be associated with HAD status.

Results

A total of 144 HIV-positive participants were included in the analysis. The majority were women (74%), isiXhosa speaking (88%), and had a median CD4 cell count of 168 (interquartile range [IQR] 115–199). The median CD4 counts for the categories of HAND were 182 (IQR 148–191) for nonimpaired, 181 (IQR 146–235) for asymptomatic neuropsychological impairment (ANI), 180 (IQR 128-241) for mild neurocognitive disorder (MND), and 139 (IQR 75–184) for HIV-associated dementia (HAD). "The mean age was 29.5 (SD 3.65) and level of education was 10 years (SD 1.85) (Table 1)".

The distribution of ApoE alleles were found to be in Hardy-Weinberg equilibrium (HWE) for both the group of controls, as well as the HIV+ participants. Using an exact statistic, the probability of the two groups not being in HWE was .68 and. 55, respectively.

Table 1 Demographic characteristics of HIV-positive participants and HIV-negative adult neuropsychology controls

Characteristic	HIV+ participants (n = 144)	HIV− participants (n = 50)	P value
Mean age (SD)	29.5 (3.65)	25.28 (5.58)	<.001
Women (%)	106 (74)	32 (64)	
Years of education (SD)	10.02 (1.85)	10.82 (1.64)	.642
Speak isiXhosa (%)	127 (88.2)	42 (84)	
CD4 count	188 (118)	—	—
AAN-defined HAD (%)	41 (28.4%)	—	—

Table 2 Allelic distributions for newborns and participants

Alleles	HIV-positive participants *n* (%)	Control newborns *n* (%)	Exact (2-sided)
2,2	2 (1)	18 (6)	.028
2,3	21 (15)	52 (17)	.497
2,4	8 (6)	33 (11)	.079
3,3	50 (35)	78 (26)	.073
3,4	49 (34)	90 (30)	.444
4,4	14 (10)	29 (10)	.999
Total	144	300	

Note. Table *P* value (exact) = .042.

Apolipoprotein E ε alleles in HIV-positive group and the general population

The distribution of ApoE allelic combinations for HIV-positive study participants and a newborn population-based sample for comparison is presented in Table 2. When we compared the overall genotype distributions between two groups, we found significant differences between the genotypes (two-sided exact *P* value = .042). The ε2/ε2 allelic combination was significantly less frequent in the HIV-positive group than the newborn controls.

The allelic frequencies between the HIV-positive and the newborn groups are presented in Table 3. We found that the ε2 and ε3 alleles differed significantly ($\chi^2 = 12.2$, $df = 2$, $P = .002$), with a lower frequency of the ε2 and ε3 alleles in HIV-positive adults than newborn controls.

Apolipoprotein E ε alleles and HAND

There were no differences in the allelic distributions of ApoE across different categories of HAND (Table 4) (overall exact *P* value = .66), nor were there differences in CD4 count for the ApoE genotypes ($P = .14$). The relationship between ε4 status (having one or more ε4 alleles) was compared to participants with and without HAD (Table 5).

Discussion

We report the first study, to our knowledge, evaluating the relationship between ApoE genotype and HAND in southern Africa. In this study, the allelic frequency of the ε4 allelic variant in both the community-based newborns and the HIV-positive adults was similar to reports of other African populations. The frequency of the ε2 allelic variant was significantly lower in the HIV-positive adults compared to newborns. There were no differences in ApoE genotype and category of HAND. We did note a significantly lower frequency of the ε4 allelic variant in adults with HAD, compared with those without HAD.

In this sample, among isiXhosa speakers, we found a high frequency of the ε4 allelic variant amongst newborns, as well as HIV-positive adults (0.302 and 0.297, respectively). The relatively high frequency of the ε4 variant is in keeping with other studies of African populations, where frequencies of ApoE ε4 of 0.31 and 0.20 have been found, and confirms that ApoE ε4 is more common in this ethnic group than in non-African groups (Burt *et al,* 2008; Kamboh *et al,* 1989). In a study of the indigenous Khoi San population in Southern Africa, a slightly higher frequency was found (0.37) (Sandholzer *et al,* 1995). A similar frequency of ApoE ε4 was found also in the control sample (0.30). The implications of this high frequency of ε4 are for increased rates of ε4-related neurodegenerative diseases, including HAD. This is of especial relevance in a regions with a very high HIV seroprevalence and an aging population (Burt *et al,* 2008; Mahley *et al,* 2009).

In addition, we found that the ε2/ε2 genotype occurred significantly less frequently among HIV-positive adults compared to community-based newborns, whereas the individual ε2 and ε3 allelic variants specifically were less frequent in this group. We noted also the distribution of ApoE alleles compared with other studies suggests higher frequencies of the 2/3 and 2/4 combination, and a lower frequency of the 3/3 combination (Eichner *et al,* 2002; Sandholzer *et al,* 1995; Valcour *et al,* 2004). Some have proposed that the ε4 variant is associated with increased rates of HIV acquisition (Kaslow *et al,* 2005). In the absence of ApoE genotype of the adult HIV-negative controls, and survival data on our HIV-positive group, we are unable to draw conclusions regarding the role that ApoE allelic variants might have on acquisition of HIV infection in our population. Larger cross-sectional and seroconversion studies are needed to provide evidence for this link.

In this sample of young adults entering HIV care, we report that there were no differences in allelic distributions across HAND categories. However, we

Table 3 Allelic frequencies for control newborns and HIV-positive participants

	300 newborns		144 HIV-positive adults		
Alleles	*n*	Frequency (95% CI)	*n*	Frequency (95% CI)	*P* value
ε2	121	0.202 (0.170–0.236)	35	0.114 (0.081–0.155)	.001
ε3	298	0.497 (0.455–0.537)	180	0.588 (0.531–0.643)	.01
ε4	181	0.302 (0.265–0.340)	91	0.297 (0.247–0.352)	.876
Total	600		288		

Table 4 Allelic frequencies of HIV-positive participants across neurocognitive disorders

	Neurocognitive disorder category				
Genotype	Nonimpaired (n = 29)	ANI (n = 18)	MND (n = 56)	HAD (n = 41)	Exact (2-sided)
22	1 (50%)	0	0	1 (50%)	.47
23	2 (9.5%)	2 (9.5%)	8 (38.1%)	9 (42.9%)	.381
24	2 (25%)	1 (12.5%)	3 (37.5%)	2 (25%)	.999
33	7 (14%)	9 (18%)	21 (42%)	13 (26%)	.31
34	12 (24.49%)	4 (8.2%)	19 (38.9%)	14 (28.6%)	.627
44	5 (35.7%)	2 (14.3%)	5 (35.7%)	2 (14.3%)	.351

Note. Exact P value for table = .66.

did find a significantly lower frequency of the ε4 allelic variant in individuals diagnosed with HAD, compared with those without HAD. Our sample size was likely too small to detect differences in genotype across the four groups. In our two-by-two analysis, we found a significantly lower frequency of ε4 status among HIV-positive adults with HAD. This finding is in contrast to that of Corder and colleagues who found that in a sample of 44 HIV+ participants followed prospectively from around the time of seroconversion, mild suspected dementia was nearly twice as frequent in the ε4-bearing individuals (Corder *et al,* 2008). Our sample was too small to separate ε4 status in hetero- and homozygous groups, although others have noted that a dose-dependent relationship of ε4 with the development of AD (Burt *et al,* 2008; Mahley *et al,* 2009).

Our findings are consistent with the report of Dunlop and the younger group in the Valcour study (Dunlop *et al,* 1997; Valcour *et al,* 2004). Our finding that the ε4 allelic variant was negatively associated with HAD could be explained by the relatively small sample size, or it could suggest a nonassociation between ε4 status and the development of HAD. Further conclusions regarding the effect of ε4 on survival require follow-up survival data. The negative impact of ε4 has been proposed by the Valcour group. A longitudinal study design would be needed to provide data for this explanation.

Our study was limited by relatively small numbers of participants and by the absence of other data on vascular risk factors. Nonetheless, we believe that our findings warrant scrutiny, given the burden of disease of infection with clade C HIV and the frequency of ApoE4. Many of these issues would be clarified by larger prospective cohort studies where recently diagnosed isiXhosa speakers are genotyped. To our knowledge, this is the first study of ApoE genotype in a well-characterized group of HIV plus; individuals with predominantly clade C HIV about to commence HAART. These findings are of relevance given the high prevalence of HIV in Southern Africa, the growing evidence that clade C HIV may be as neurotoxic as other clades, and the changing phenotype of HAND in the era of HAART.

Table 5 Comparison between ε4 status with HAD and non-HAD clinical groups

	HIV-positive (n = 102)		
	With ε4 allele	Without ε4 allele	Total
HAD	18	33	51
No neurocognitive disorder	29	22	51
Total	47	55	102

Note. Exact P value for table = .029 (i.e., 18/51 is significantly lower than 29/51).

Methods

Subjects

From a larger primary study, we invited 283 HIV-infected individuals at three primary health care centers in poor communities in Cape Town, South Africa, to participate from February 2008 through August 2009 (Joska *et al,* 2010). Of these, 144 attended two full study visits, during which detailed a sociodemographic, medical, and neuropsychological assessment were conducted, and laboratory measures (including genotyping) were completed. Primary reasons for this loss to follow-up included financial constraints, casual employment on clinic days, migration between cities, and the need to attend other clinic appointments. Individuals included in this study ranged from 18 through 40 years in age. All were naïve to highly active antiretroviral therapy (HAART). Cases were excluded if they had a severe psychiatric disorder, recent history of substance (including recent alcohol) abuse, or other significant neurological disorder. Control data for neuropsychological testing were obtained from 50 HIV-negative participants. These were recruited from Voluntary Counseling and Testing Services at the same primary care clinics. Other than being HIV negative, as confirmed by a recent rapid HIV test and confirmatory serological test, inclusion and exclusion criteria were identical to HIV-positive participants.

Participant CD4 cell counts were obtained from the clinic records. Although clade sequencing was not available on this sample, previous reports have noted that 89% of infected individuals in Cape Town area

are infected with clade C virus (Jacobs *et al,* 2009). Furthermore, although hepatitis C status was not tested, the prevalence of hepatitis C in South Africa is extremely low (Fernhaber *et al,* 2008).

Background population prevalence of ApoE genotype was obtained from a sample of 300 infants born to isiXhosa-speaking mothers in an area of Cape Town where the majority of the clinical sample above was drawn. The blood from these infants was drawn from 2002 through 2004 and stored in accordance with a previously approved Research Ethics Committee submission, in order to allow for analysis of background prevalence of genes under study. The HIV serostatus of this group was not known. It was assumed to reflect the background population prevalence in newborns at the time. According to local surveys, the seroprevalence amongst mothers in this community in 2006 was 15% (Department of Health, 2008). With untreated mother-to-child transmission rates of 10% to 30%, we estimated the seroprevalence in this infant sample to be between 1.5% and 5%. This rate was felt to be unlikely to affect these data.

We obtained written informed consent from all included participants, including separate consent for genomic analysis. Approval to conduct the study was obtained from the Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, and from the relevant health authorities.

Neuropsychological test battery
A neuropsychological testing battery assessed specific domains of attention, concentration, language, learning, memory, psychomotor speed, and executive function. We translated the instructions and content of all instruments into local languages—isiXhosa and Afrikaans. The instructions were back-translated for fidelity. The battery comprised the following tests: Attention (the Mental Alternation Test and the Mental Control Test), learning and memory (the Hopkins Verbal Learning Test and the Brief Visuospatial Memory Test), motor (Finger Tapping and Grooved Pegboard—both dominant and nondominant hands), psychomotor speed (Trail-Making Part A, Color Trails 1, and Digit Symbol coding), executive function (Colour Trails 2, the Stroop Color Word Test, the Wisconsin Card-Sorting Test, and the Rey Complex Figure), and language (Category Fluency Animals and Category Fluency Fruit and Vegetables).

Determination of neurocognitive disorder status
We used the above neuropsychological test battery, together with scores from the medical assessment and self-reported functional assessment, to classify participants into one of four HAND categories, based on the updated American Academy of Neurology (ANN) criteria (Antinori *et al,* 2007): no impairment, asymptomatic neuropsychological impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HIV-D). Using the modified AAN classification, participants who scored >2 SDs below the control-derived means cut-offs on at least two domains of function were rated as having HAD; those who scored between 1.0 and 2 SDs on two domains of function, or >2 SDs and 1–2 SDs, were rated MND or ANI, depending on their loss of function. The final classification was conducted by a consensus panel comprising two HIV neuropsychiatrists (J.J., J.H.) and a neurologist (M.C.).

Genotyping
DNA was isolated by standard procedures and the method of Hixson and Vernier was used for Apolipoprotein E (ApoE) genotyping (Hixson and Vernier, 1990). We did not include genotyping data of the HIV-negative control adults, as this was available on only 10 participants.

Statistical analysis
Data were analyzed using STATA 10.0 (Stata Corporation, College Station, Texas, USA). In order to establish whether the allelic frequency was randomly distributed in the sample of control newborns and the HIV-positive participants, we conducted an analysis of the Hardy-Weinberg equilibrium (HWE). This principle asserts that mating is random, there is no migration or inbreeding and no selective survivorship (Mayo, 2008). Calculation of the HWE allows the investigator to report with some confidence that external factors have not altered the gene frequency in the population to any significant degree, and therefore that the observed frequency is not an artifact of these external factors. The allelic distributions between the newborn and HIV-positive adult groups were compared using exact statistics, whereas allelic frequencies were calculated to include 95% binomial confidence intervals in addition to chi-square tests. Similarly, we compared allelic distributions across HAND categories. Finally, we compared HAD and non-HAD groups with respect to the presence of the ε4 allelic variant using a table exact statistic.

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