Journal of NeuroVirology, 10(suppl. 1): 67–73, 2004
© 2004 Journal of NeuroVirology
ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280490270770

Taylor & Francis
Taylor & Francis Group

Host genetic polymorphisms in human immunodeficiency virus–related neurologic disease

Ramon Diaz-Arrastia,[1] Yunhua Gong,[1] Cynthia J Kelly,[1] and Benjamin B Gelman[2]

[1]*Department of Neurology, University of Texas Southwestern Medical Center, Dallas, Texas, USA;* [2]*Department of Pathology, University of Texas Medical Branch, Galveston, Texas, USA*

The objective of this study was to determine whether host genetic polymorphisms influence the risk of developing human immunodeficiency virus (HIV) encephalitis and vacuolar myelopathy. Allelic association studies were carried out with common polymorphisms in candidate genes that are postulated to play a role in the pathogenesis of HIV-related neurologic complications. The authors studied brains and spinal cords from 270 patients who died of acquired immunodeficiency syndrome (AIDS) from 1989 to 1996. All had complete gross and microscopic pathologic evaluations, and the presence of microglial nodules, multinucleated giant cells, myelin pallor, and vacuolar myelopathy was assessed by an experienced neuropathologist who was blinded to the genotype. DNA was extracted from frozen brain samples, and determination of the presence of the *APOE4*, *TNF-2*, *IL-1B2, *ILIRN**2 polymorphisms was determined by polymerase chain reaction (PCR) and restricted fragment length polymorphism (RFLP) mapping. The authors did not detect a consistent association between inheritance of candidate polymorphic alleles and the pathologic findings of HIV encephalitis or vacuolar myelopathy. Allelic association studies with candidate genes are powerful techniques that have the potential to contribute to understanding the pathophysiology of HIV-related neurodegeneration. This preliminary study, although including a substantial number of patients, was not sufficiently powered to exclude a modest but clinically significant effects. Future studies will require much larger sample sizes and technical advances to allow screening at larger number of candidate loci.** *Journal of NeuroVirology* (2004) **10(suppl. 1),** 67–73.

Keywords: apolipoprotein E; interleukin-1; tumor necrosis factor

Introduction

Neurologic manifestations of human immunodeficiency virus (HIV) infection, such as HIV-associated dementia (HAD), vacuolar myelopathy (VM), and distal sensory polyneuropathy (DSPN), are common and disabling complications of HIV infection that occurs in 10% to 20% of patients with acquired immunodeficiency syndrome (AIDS) (Lipton, 1998; Simpson, 1999; Swindells *et al*, 1999). Although the risk of developing these neurologic complications is associated with HIV viral load, and inversely associated with CD4 count, the correlation is not high, and many patients with high viral loads and low CD4 counts do not develop these disabling neurologic complications (Childs *et al*, 1999; Sacktor *et al*, 2001), although a substantial number of patients with low HIV burdens and normalized CD4 counts do suffer from these serious complications (Sacktor *et al*, 2001). Over the last decade, much experimental and clinical evidence has accumulated, supporting the hypothesis that virally driven induction of inflammatory mediators such as cytokines, chemokines, nitric oxide, eicosanoids, and free radicals is responsible for neurodegeneration in the setting of HIV infection (Griffin, 1997; Lipton, 1998; Mrak and Griffin, 1997; Swindells *et al*, 1999). Recent progress in the Human Genome Project

Address correspondence to Ramon Diaz-Arrastia, MD, PhD, Department of Neurology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9036, USA. E-mail: Ramon.Diaz-Arrastia@UTSouthwestern.edu
This work was supported by NIH grants R03 MH64889 and R01 AG17861 (to R.D.-A), and U01 AI46373, R24 MH59656, and R24 NS45491 (to B.B.G.)
Received 31 January 2003; accepted 7 April 2003.

has identified polymorphisms in several genes involved in mediating inflammatory reactions in the brain, which have been proposed on the basis of experimental observations to play a role in HAD and VM. These polymorphisms were chosen based on a biologic plausibility for a role of the gene in neurodegeneration, based on animal models or observational human studies, and the association of the polymorphism with other human diseases believed to share some of the pathologic features of HIV-associated neurologic disease. These include *APOE4*, *TNF-2*, *IL-1B*2*, *ILIRN*2*. Two of these polymorphisms have been previously studied in the setting of HIV dementia (Corder *et al*, 1998; Quasney *et al*, 2001; Sato-Matsumura *et al*, 1998), using study designs substantially different from those employed here. This study was undertaken to determine whether inheritance of these polymorphic alleles was associated with increased prevalence of pathologic findings of multinucleated giant cells (MGCs), microglial nodules (MGNs), myelin pallor (MP), and VM in an autopsy series of patients dying from AIDS.

Results

As anticipated, the prevalence of neuropathologic findings in this autopsy series was higher in the earlier cohort (1989–1993) than in the later cohort (1994–1996). Figure 1 shows the prevalence of MGNs, MGCs, MP, and VM in the two cohorts. In the earlier cohort, the prevalence of neuropathologic findings was in the range of 30% to 40%, whereas in the latter cohort, neuropathologic findings were significantly less common, approximately 10%. There was no difference between the two cohorts in the frequency of opportunistic infections in the brain. This result confirms the finding of clinically based studies that have identified a decrease in incidence of HIV-related neurologic disease in the era of active antiretroviral therapy (Monforte *et al*, 2000; Sacktor *et al*, 2001).

Table 1 summarizes the results of the allelic association analysis with the four candidate polymorphic alleles. The uncorrected *P* values (Fisher's exact test) are listed for each cohort. For five of the comparisons, the uncorrected *P* value reached nominal significance ($P < .05$). However, in each case, the association was not replicated in the other cohort. Further, the relative risks for the significant associations found in a single cohort were generally modest, ranging from 2.7 to 3.2. For the association between risk of VM and *APOE4*, the direction of the relative risk (0.44) is different from what would be expected given the expected biologic role of the *E4* allele. The most likely explanation for these results is that the apparent significant associations are false-positive findings resulting from performing multiple analysis (Lander and Kruglyak, 1995; Plomin *et al*, 1994).

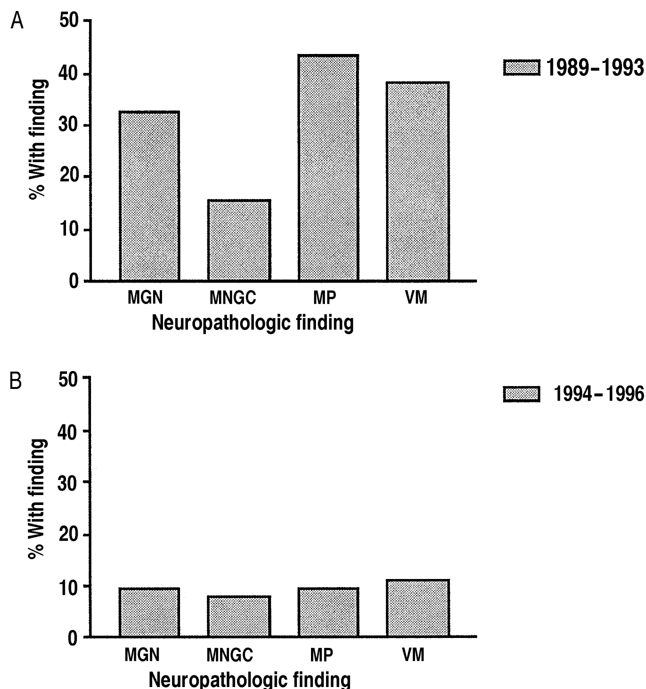

Figure 1 Prevalence of microglial nodules (MGN), multinucleated giant cells (MNGC), myelin pallor (MP), or vacuolar myelopathy (VM) in two consecutive series of patients dying of AIDS in two periods (**A**, 1989–1993; **B**, 1994–1996).

Discussion

The progression from HIV infection to neurodegeneration is highly variable and may depend on many factors, such as systemic and/or central nervous system (CNS) HIV viral burden, neurotropism of particular HIV strains, integrity of the blood-brain barrier, or the genetic background of the infected host (Zink *et al*, 1999). The latter possibility is the focus of the current study. Recent progress in the Human Genome Project offers an approach to dissect host contributions to HIV neuropathogenesis, by exploiting the fact that many genes that have been postulated, on the basis of animal models or observational studies in humans, to play a role in inflammation and neurodegeneration in the CNS are polymorphic, and that many of these polymorphisms result in biologically relevant changes in the function of the gene. Thus, an attractive approach to determine whether certain genes play a role in HAD is to determine whether polymorphisms in those genes are associated with HAD (Risch and Merikangas, 1996).

The selection of polymorphisms to study is inherently somewhat arbitrary. The number of potential candidates is very high—probably in the thousands. Given current technical limitations, we chose to study four genes for which a substantial amount of prior work had indicated (1) a biologic plausibility for the role of the gene in neurodegeneration, based

Table 1 Prevalence of neuropathologic findings, by genotype

Pathologic finding	*Cohort A (1989–1993)*		P	*Cohort B (1994–1996)*		P
	ApoE4+ *(n)*	ApoE4− *(n)*		ApoE4+ *(n)*	ApoE4− *(n)*	
MGN+	12	31	1	2	10	.21
MGN−	24	64		44	71	
MGC+	5	14	1	4	6	1
MGC−	30	74		42	75	
MP+	12	38	.83	3	4	.68
MP−	17	48		21	45	
VM+	8	37	.01	4	8	1
VM−	29	43		37	62	
	TNF*2+ *(n)*	TNF*2− *(n)*		TNF*2+ *(n)*	TNF*2− *(n)*	
MGN+	10	31	.68	7	5	.01
MGN−	25	63		25	90	
MGC+	7	14	.60	6	4	.02
MGC−	28	76		26	91	
MP+	14	38	.83	5	2	.01
MP−	16	49		15	51	
VM+	13	37	1	4	10	.75
VM−	20	52		24	73	
	IL1B*2+ *(n)*	IL1B*2− *(n)*		IL1B*2+ *(n)*	IL1B*2− *(n)*	
MGN+	15	16	.35	10	2	.03
MGN−	33	54		60	55	
MGC+	8	6	.24	5	5	.75
MGC−	39	64		65	52	
MP+	19	19	.30	5	2	.43
MP−	24	49		33	33	
VM+	11	26	.10	5	7	.36
VM−	34	38		57	42	
	IL1RA*2,3,4+ *(n)*	IL1RA*2,3,4− *(n)*		IL1RA*2,3,4+ *(n)*	IL1RA*2,3,4− *(n)*	
MGN+	10	21	.83	3	9	.37
MGN−	31	57		46	69	
MGC+	4	10	.77	4	6	1
MGC−	37	67		45	72	
MP+	13	25	1	2	5	1
MP−	23	42		25	41	
VM+	14	23	.83	5	7	1
VM−	25	47		40	59	

on animal models and observational human studies; (2) a polymorphism is present at a appropriate allele frequency in the population (typically 0.1 to 0.75); and (3) the polymorphism results in a biologically relevant change in the function of the gene; and (4) the polymorphism has been associated with other human diseases believed to share some pathophysiologic features with HIV-associated neurologic disease. In this study, we report our findings with four polymorphic alleles. *APOE4* was chosen based on its known association with Alzheimer's disease (Corder *et al*, 1993; Mayeux *et al*, 1993), traumatic brain injury (Friedman *et al*, 1999; Jordan *et al*, 1997; Teasdale *et al*, 1997), and multiple sclerosis (Chapman *et al*, 2001; Fazekas *et al*, 2001). The *TNF*2* polymorphism is located at nucleotide −308 relative to the transcriptional start site of the gene affects, and regulates tumor necrosis factor (TNF)-α secretion (Wilson *et al*, 1997). *TNF*2* is overrepresented in children dying from cerebral malaria (McGuire *et al*, 1994) and meningococcal meningitis (Nadel *et al*, 1996). *IL1B*2* is a polymorphism at position +3953 in exon 5 of the *IL1B* gene, which results in an increased production of interlenkin (IL)-1β *in vitro* (Pociot *et al*, 1992). Finally, *IL1RN*2* is a variable number of tandem repeats polymorphism, which is associated with increased production of a dysfunctional IL1-RA protein (Danis *et al*, 1995), and has been associated with a number of autoimmune diseases, including multiple sclerosis (Crucius *et al*, 1995; de la Concha *et al*, 1997), Sjögren's syndrome (Perrier *et al*, 1998), inflammatory bowel disease (Mansfield *et al*, 1994), and systemic lupus erythematosus (Blakemore *et al*, 1994).

Other investigators have previously studied polymorphisms in *APOE* and *TNF* in association with HIV-related neurologic disease. Corder *et al* (1998) looked at the distribution of apoE alleles in patients with HIV infection. They studied a total of 44 patients with HIV infection, the majority of which (61%) were asymptomatic. Thirty-three subjects were apoE4 negative, whereas the other 11 were apoE4 heterozygotes. Although there was no difference in the neuropsychologic scores between the two groups at the time of entry into the study, the apoE4+ subjects did not show the expected improvement (resulting from the practice effect on retaking the neuropsychologic tests) in subsequent visits. This was interpreted as

evidence of slightly worse dementia in the apoE4+ subjects. Using pathologic rather than clinical examinations, we were unable to reproduce the findings of Corder *et al* (1998). The different conclusions between the two studies may be a result of the differences in diagnostic criteria (clinical versus pathologic). Our findings are in agreement with Dunlop *et al* (1997), who did not find a effect of *APOE4* in the prevalence of HIV dementia (diagnosed clinically) or HIV encephalopathy (diagnosed pathologically).

Quasney *et al* (2001), using primarily clinical criteria, found a substantially increased risk (O.R. 10.3, 95% C.I. 2.6–39.9) of HAD in patients heterozygous for the *TNF*2* polymorphism. Differences in diagnostic technique (clinical versus pathological) between Quasney's study and ours may explain the contradictory results, although generally there is an excellent association between clinical and pathologic diagnosis of HAD. Sato-Matsumara *et al* (1998) looked for the distribution of the *TNF2* polymorphism in 44 brains with HIV encephalopathy or leukoencephalopathy, compared to 30 pathologically normal brains, and failed to find an increased risk of HIV-related neuropathologic changes in *TNF2*-positive individuals. Our findings confirm these conclusions.

Relatively little is known about differences in allelic frequencies of the polymorphisms studied between different ethnic and racial groups. Of the four alleles studied here, good data are available only for *APOE4* (Tang *et al*, 1996) . The E4 allele is significantly more common in African Americans, compared with Caucasians or Hispanics. Additionally, among populations of European descent, a north-south gradient has been identified (Lucotte *et al*, 1997), with higher E4 prevalence in Northern Europe compared to Southern Europe. Comparably detailed information is not available for the other three polymorphisms studied. Although such differences in baseline allelic frequencies between ethnic groups has the potential to confound our study, we believe this is unlikely, as there was no difference in our cohort in the prevalence of neuropathologic findings by ethnicity.

There are several important limitations to our study. First, although we studied a large autopsy series, the total number of subjects was relatively low for an allelic association study. Estimates of the number of subjects needed to obtain acceptable levels of false positive and false negative errors ($\alpha = 0.00005$ and $\beta = 0.8$) indicate that approximately 200 affected subjects (and a similar number of controls) are needed to either include or exclude a biologically relevant relative risk of 2.5-fold (Lander and Kruglyak, 1995; Risch and Merikangas, 1996). Further, if the effect of the polymorphism is genetically recessive (i.e., inheritance of two copies of the polymorphic allele are required to produce a biologic effect), the number of affected subjects required is higher. The small number of HIV encephalopathy cases in the latter cohort may have limited the power of our study. Collection of such a large cohort of subjects is likely beyond the ability of a single institution, and multi-institutional collaborations will be needed to perform such studies in the future. Second, the lack of neurologic examinations performed during life in the patients in our cohort is an important limitation. Although there is a correlation between the pathologic findings of MGCs, MGN, MP, and clinical dementia, up to 45% of neurocognitively normal individuals have pathologic findings of HIV encephalitis (Cherner *et al*, 2002). Additionally, we have very limited information regarding details such as the interval between diagnosis and death in our two cohorts, although we expect that the latter cohort had a longer duration of illness. Finally, we screened only a small number of candidate genes. The number of genes that have a potential role in inflammation and neurodegeneration in the CNS is very large, numbering at least several hundred (Emahazion *et al*, 1999). Screening large number of patients with several hundred polymorphic alleles is a major technical undertaking, but advances in technology and in understanding the structure of the human genome will likely make such studies possible in the near future (Goldstein, 2001).

In summary, we did not detect allelic association between polymorphisms in the *APOE*, *TNF*, *IL1B*, and *IL1RN* genes in a cohort of neuropathologically characterized individuals who died of AIDS. Important limitations inherent to these studies make it impossible to exclude a role for these genes in HIV-associated neurodegeneration. Further studies in this area will likely require multi-institutional collaborations in gathering and characterizing patient material, as well as technical advances in genotyping methods to lower expense and increase throughput.

Methods

Patients

Patient material used was archived frozen brain tissue from the 270 patients who died from complications of AIDS, collected by one of the authors (BBG) from 1989 to 1996. Comprehensive clinical information is not available for most of these cases, but the majority of these patients received antiretroviral therapy with a single drug or at most two drugs. The patients were analyzed in two separate cohorts: Cohort A consisted of 134 patients who died between 1989 and 1993, during the era of monotherapy. Cohort B consisted of 136 patients who died between 1994 and 1996, during the era of dual therapy. Two cohorts were used to allow for the second to function as a second independent test of the hypothesis, as has been suggested for allelic association studies (Plomin *et al*, 1994). Inclusion of patients in the sample was not influenced by the presence of neurologic symptoms.

Neuropathologic assessments
All patients underwent autopsy examinations, including gross pathological and microscopic studies. A complete survey of all brain regions was examined in every case, including a minimum of 12 histological sections. The examination of HIV-associated pathologic findings (MGNs, MGCs, MP, and VM) was performed by a board-certified neuropathologist who was blinded to the DNA genotype, according to previously published methods (Gelman *et al*, 1996). A positive histological finding was assigned when the diagnosis was either present or absent. For MGN, the diagnosis was assigned when one of more of these structures was encountered in any of the sections but there was not enough evidence for a more specific diagnosis with respect to cause. One MGN was sufficient for the diagnosis. MP was assessed in each case and in every section by staining with luxol fast blue (LFB). Paleness of the color intensity of LFB staining was a judgment made by the same neuropathologist (BBG) in all cases, taking into consideration the intensity of all slides. HIV encephalitis was diagnosed whenever multinucleated giant cells were identified in the setting in which HIV encephalitis occurs (i.e., in conjunction with microglial nodules of poliodystrophy or MP or white matter astrogliosis, leukoencephalopathy, or lymphocytic infiltration of the brain). In a few cases, HIV encephalopathy was diagnosed even though MNG cells were not present. In those cases, at least three features named above were highly prevalent findings, and no other infectious pathogen was present to account for the severe changes.

Genotype determinations
DNA was extracted from approximately 10 mg of brain tissue using a silica gel binding method (QIAamp mini kit; Qiagen, Valencia, CA) according to the manufacturer's instructions. Genotyping at the *APOE* locus carried out by a modification of the method of Chapman *et al* (1996). The upstream primer was 5′-TCCAAGGAGCTGCAGGCG GCGCCA-3′ and the downstream primer was 5′-ACA GAATTCGCCCCGGCCTGGTACACTGCCCA-3′. The 227-bp polymerase chain reaction (PCR) product was separately digested by *Afl III* (New England Biolabs), which digests $\varepsilon2$ and $\varepsilon3$ into 177- and 50-bp fragments, but does not hydrolyze $\varepsilon4$, and by *Hae II* (New England Biolabs), which digests $\varepsilon3$ and $\varepsilon4$ into 195- and 32-bp fragments, but does not hydrolyze $\varepsilon2$. Genotyping the *TNF* locus was done by the method of Huang *et al* (1997). PCR was used to amplify a 345-bp fragment of the *TNF-α* promoter region, from −331 to +14, using the following oligonucleotides: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-GAGC GTCTGCTGGCTGGGTG-3′, and the product digested with *Nco* I. Under these conditions the *TNF1* product is not digested (345 bp), whereas *TNF2* allele is digested into two fragments (325 and 20 bp). Genotyping at the *IL-1B* locus was by the method of Bioque *et al* (1995). A 249-bp fragment from exon 5 of the gene was amplified with the following oligonucleotide primers: 5′-GTTGTCATCAGACTTTGACC-3′ (upstream) and 5′-TTCAGTTCATATGGACCAGA-3′ (downstream). The PCR product was digested with *Taq* I, which leaves intact the product of the *IL-1B*2* allele, but cleaves the *IL-1B*1* product into fragments of 135 and 149 bp. Genotyping the *IL1-RN* locus will be done by the method of Bioque *et al* (1995). The region within the second intron of the *IL1-RA* gene, which contains variable numbers of 86 bp variable number of tandem repeats (VNTR), was amplified by PCR using the primers 5′-CTCAG CAACACTCCTAT-3′ and 5′-TCCTGGTCTGCAGGT AA-3′. The product of the *IL1RN*1* allele, consisting of four repeats, is 172 nucleotides longer than the product from the *IL1RN*2* allele, which contains only two repeats.

Statistical analysis
Categorical data were analyzed using Fisher's exact test. Statistical analysis was performed using the InStat program v. 3.0 (GraphPad Software, San Diego, CA).

References

Bioque G, Crusius JBA, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SGM, Pena AS (1995). Allelic polymorphism in IL-1beta and IL-1 receptor antagonist (IL-1Ra) genes ininflammatory bowel disease. *Clin Exp Immunol* **102:** 379–383.

Blakemore AIF, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW (1994). Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum* **37:** 1380–1385.

Chapman J, Estupinan J, Asherov A, Goldfarb LG (1996). A simple and efficient method for apolipoprotein E genotype determination. *Neurology* **46:** 1484–1485.

Chapman J, Vinokurov S, Achiron A, Karussis DM, Mitosek-Szewcyk K, Birnbau M, Michaelson DM, Korczyn AD (2001). *APOE* genotype is a major predictor of long-term progression of disability in MS. *Neurology* **56:** 312–316.

Cherner M, Masliah E, Ellis RJ, Marcotte TD, Moore DJ, Grant I, Heaton RK (2002). Neurocognitive dysfunction predicts postmortem findings of HIV encephalitis. *Neurology* **59:** 1563–1567.

Childs EA, Lyles RH, Selnes OA, Chen B, Miller EN, Cohen BA, Becker JT, Mellors J, McArthur JC (1999). Plasma viral load and CD4 lymphocytes predict HIV-associated dementia and sensory neuropathy. *Neurology* **52:** 607–613.

Corder EH, Robertson K, Lannfelt L, Bogdanovic N, Eggertsen G, Wilkins J, Hall C (1998). HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. *Nat Med* **4:** 1182–1184.
Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261:** 921–923.
Crucius JBA, Pena AS, van Osten BW, Bioque G, Garcia A, Dijkstra CD, Polman CH (1995). Interleukin-1 receptor antagonist gene polymorphism and multiple sclerosis. *Lancet* **346:** 979–980.
Danis VA, Millington M, Hyland VJ, Grennan D (1995). Cytokine production by normal humanmonocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. *Clin Exp Immunol* **99:** 303–310.
de la Concha EG, Arroyo R, Crusius JBA, Campillo JA, Martin C, Varela de Seijas E, Pena AS, Claveria LE, Fernandez-Arquero M (1997). Combined effect of HLA-DRB1*1501 and interleukin-1 receptor antagonist allele 2 in susceptibility to relapsing-remitting multiple sclerosis. *J Neuroimmunol* **80:** 172–178.
Dunlop O, Goplen AK, Liestol K, Myrvang B, Rootwelt H, Christopherson B, Kvittingen E-A, Maelen J (1997). HIV dementia and apolipoprotein E. *Acta Neurol Scand* **95:** 315–318.
Emahazion T, Jobs M, Howell WM, Siegfried M, Wyoni P-I, Prince JA, Brookes AJ (1999). Identification of 167 polymorphisms in 88 genes from candidate neurodegeneration pathways. *Gene* **238:** 315–324.
Fazekas F, Strasser-Fuchs S, Kollegger H, Berger T, Kristoferitsch W, Schmidt H, Enzinger C, Schifermeier M, Schwarz C, Kornek B, Reindl M, Huber K, Wimmer G, Vass K, Pfeiffer KH, Hartung HP, Schmidt R (2001). Apolipoprotein E4 is associated with rapid progression of multiple sclerosis. *Neurology* **57:** 853–857.
Friedman G, Froom P, Sazbon L, Grinblatt I, Shochina M, Tsenter J, Babaey S, Ben Yehuda A, Groswasser Z (1999). Apolipoprotein E-epsilon4 genotype predicts a poor outcome insurvivors of traumatic brain injury. *Neurology* **52:** 244–248.
Gelman BB, Dholakia S, Casper K, Kent TA, Cloyd MW, Freeman D (1996). Expansion of the cerebral ventricles and correlation with acquired immunodeficiency syndrome neuropathology in 232 patients. *Arch Pathol Lab Med* **120:** 866–871.
Goldstein DB (2001). Islands of linkage disequilibrium. *Nat Genet* **29:** 109–111.
Griffin DE (1997). Cytokines in the brain during viral infection: clues to HIV-associated dementia. *J Clin Invest* **100:** 2948–2951.
Huang S-L, Su C-H, Chang S-C (1997). Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. *Am J Respir Crit Care Med* **156:** 1436–1439.
Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, Gandy S (1997). Apolipoprotein E ε4 associated with chronic traumatic brain injury in boxing. *J Am Med Asso* **278:** 136–140.
Lander ES, Kruglyak L (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* **11:** 241–247.
Lipton SA (1998). Neuronal injury associated with HIV-1: approaches to treatment. *Ann Rev Pharmacol Toxicol* **38:** 159–177.
Lucotte G, Loirat F, Hazout S (1997). Pattern of gradient of apolipoprotein E allele *4 frequencies in Western Europe. *Hum Biol* **69:** 253–262.
Mansfield JC, Holden H, Tarlow JK, Francesco S, Glovine D, McDowell DL, Wilson AG (1994). Novel genetic association betweenulcerative colitis and the antiinflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* **106:** 637–642.
Mayeux R, Stern Y, Ottman R, *et al* (1993). The apolipoprotein epsilon 4 allele in patients with Alzheimer's disease. *Ann Neurol* **34:** 752–754.
McGuire W, Hill AVS, Allsopp CEM, Greenwood BM, Kwiatkowski D (1994). Variation of the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature* **371:** 508–511.
Monforte AD, Duca PG, Vago L, Grassi MP, Moroni M (2000). Decreasing incidence of CNS AIDS-defining events associated with antiretroviral therapy. *Neurology* **54:** 1856–1859.
Mrak RE, Griffin WST (1997). The role of chronic self-propagating glial responses in neurogeneration: implications for long-lived survivors of human immunodeficiency virus. *J NeuroVirol* **3:** 241–246.
Nadel S, Newport MJ, Booy R, Levin M (1996). Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. *J Infect Dis* **174:** 878–880.
Perrier S, Coussediere C, Dubost JJ, Albuisson E, Sauvezie B (1998). IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjogren's syndrome and rheumatoid arthritis. *Clin Immunol Immunopathol* **87:** 309–313.
Plomin R, McClearn GE, Smith DL, Vignetti S, Chorney MJ, Chorney K, Venditti CP, Kasarda S, Thompson LA, Detterman DK, Daniels J, Owen M, McGuffin P (1994). DNA markers associated with high versus low IQ: the IQ quantitative trait loci (QTL) project. *Behav Genet* **24:** 107–118.
Pociot F, Molving J, Wogensen L, *et al* (1992). TaqI polymorphism in the human interleukin-1β gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Inves* **22:** 396–402.
Quasney MW, Zhang Q, Sargent S, Mynatt M, Glass J, McArthur JC (2001). Increased frequency of the tumor necrosis factor-α -308 A allele in adults with human immunodeficiency virus dementia. *Ann Neurol* **50:** 157–162.
Risch N, Merikangas K (1996). The future of genetic studies of complex human diseases. *Science* **273:** 1516–1517.
Sacktor N, Lyles RH, Skolasky R, Kleeberger C, Selnes OA, Miller EN, Becker JT, Cohen BA, McArthur JC (2001). HIV-associated neurologic disease incidence changes: multicenter AIDS cohort study, 1990–1998. *Neurology* **56:** 257–260.
Sato-Matsumura K, Berger J, Hainfellner JA, Mazal P, Budka H (1998). Development of HIV-encephalitis in AIDS and TNF-α regulatory elements. *J Neuroimmunol* **91:** 89–92.
Simpson DM (1999). Human immunodeficiency virus-associated dementia: review of pathogenesis, prophylaxis, and treatment studies of zidovudine therapy. *Clin Infect Dis* **29:** 19–34.
Swindells S, Zheng J, Gendelman HE (1999). HIV-associated dementia: new insights into disease pathogenesis and therapeutic interventions. *AIDS Patient Care STDs* **13:** 153–163.

Tang MX, Maestre G, Tsai WY, Liu XH, Feng L, Chung WY, Chun M, Schofield P, Stern Y, Tycko B, Mayeux R (1996). Relative risks of Alzheimer disease and age-at-onset distributions, based on *APOE* genotypes among elderly African Americans, Caucasians, and Hispanics in New York City. *Am J Hum Genet* **58:** 574–584.
Teasdale GM, Nicoll JAR, Murray G, Fiddes M (1997). Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* **350:** 1069–1071.
Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW (1997). Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A* **94:** 3195–3199.
Zink WE, Zheng J, Persidsky Y, Poluektova L, Gendelman HE (1999). The neuropathogenesis of HIV-1 infection. *FEMS Immunol Med Microbiol* **26:** 233–241.