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Genome-wide Association Study of Neurocognitive Impairment and Dementia in HIV-Infected Adults

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Abstract

Background—The neuropathogenesis of HIV-associated neurocognitive disorders (HAND) is unclear. Candidate gene studies have implicated genetic susceptibility loci within immune-related genes; however, these have not been reliably validated. Here we employed genome-wide association (GWA) methods to discover novel genetic susceptibility loci associated with HAND, and validate susceptibility loci implicated in prior candidate gene studies.

Methods—Data from 1287 participants enrolled in the Multicenter AIDS Cohort Study between 1985 and 2010 were used. Genotyping was conducted with Illumina 1M, 1MDuo, or 550K platform. Linear mixed models determined subject-specific slopes for change over time in processing speed and executive functioning, considering all visits including baseline and the most recent study visit. Covariates modeled as fixed effects included: time since the first visit, depression severity, nadir CD4+ T-cell count, Hepatitis C co-infection, substance use, and antiretroviral medication regimen. Prevalence of HIV-associated dementia (HAD) and neurocognitive impairment (NCI) was also examined as neurocognitive phenotypes in a case-control analysis.

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Results—No genetic susceptibility loci were associated with decline in processing speed or executive functioning among almost 2.5 million single nucleotide polymorphisms (SNPs) directly genotyped or imputed. No association between the SNPs and HAD or NCI were found. Previously reported associations between specific genetic susceptibility loci, HIV-associated neurocognitive impairment and HAD were not validated.

Conclusions—In this first GWAS of HAND, no novel or previously identified genetic susceptibility loci were associated with any of the phenotypes examined. Due to the relatively small sample size, future collaborative efforts that incorporate this dataset may still yield important findings.

Keywords

HIV; NeuroAIDS; HIV-associated neurocognitive disorder; genome-wide association; HIV-associated dementia

INTRODUCTION

Soon after the first cases of Acquired Immunodeficiency Syndrome (AIDS) were identified in the early 1980s, it became clear that progressive cognitive impairment leading to dementia was a common consequence of the disease. The dementia syndrome associated with advanced infection by HIV, characterized by cognitive impairment, behavioral changes, and progressive central motor abnormalities, was first systematically described and termed AIDS Dementia Complex by Navia and colleagues in 1986 (Navia et al., 1986). More recently, the term HIV-associated neurocognitive disorder, or HAND, has been established to capture the wide spectrum of HIV-related neurocognitive deficits, ranging from asymptomatic neurocognitive deficits without significant impact on day-to-day functioning, to debilitating dementia (Antinori et al., 2007). Although the widespread use of combined antiretroviral therapy has resulted in substantial improvements in life expectancy and lowered incidence of dementia, HAND continues to occur with a high prevalence, although mostly in mild forms (Ances and Ellis, 2007; Dore et al., 2003; Valcour et al., 2004b; Wojna et al., 2006).

The pathogenesis of HAND remains unclear. Evidence suggests that at least the mild and moderate forms of HAND are due to a chronic neuroinflammatory state (Kraft-Terry et al., 2009; McArthur, 2004). Decades of research have led to identification of a widely varying array of cellular and molecular factors associated with HIV-related neuropathology and neurobehavioral dysfunction (Achim and Wiley, 1996; Anthony and Bell, 2008; Borjabad et al., 2010; Boven et al., 2000; Cysique and Brew, 2009; Everall et al., 2005b; Fox et al., 1997; Gelman, 2007; Khanlou et al., 2009; Levine et al., 2009a; Masliah et al., 2000; Pemberton and Brew, 2001; Roberts et al., 2004; Woods et al., 2009; Zheng and Gendelman, 1997; Zhou et al., 2009), yet no reliable biomarkers have been identified. HAND may therefore not be attributable to a single pathogenic process. Instead, HAND may be the behavioral culmination of various pathogenic processes resulting in systemic dysregulation and dysfunction.

Searching for the mechanisms of HAND neuropathogenesis, and identifying early indicators and biomarkers of HAND, are central themes of NeuroAIDS research. Currently, several factors are useful for identifying individuals who are inherently or chronically at risk for HAND, including demographic characteristics (Becker et al., 2004; Cherner et al., 2004; Valcour et al., 2004b), medical comorbidities (Cherner et al., 2005; Lin et al., 2011; Qureshi et al., 1998; Tozzi et al., 2005; Valcour et al., 2005), and lifestyle behaviors, in particular substance abuse (Levine et al., 2006; Martin-Thormeyer and Paul, 2009; Rippeth et al., 2004). In addition, host genetic factors have received attention, both as a means to identify

risk factors and to determine the neuropathogenesis of HAND. To date, only a few genetic susceptibility loci have been identified that alter the risk for HIV-associated dementia or neurocognitive impairment among those with HIV (Bol et al., 2012; Boven et al., 1999; Corder et al., 1998; Gonzalez et al., 2002; Levine et al., 2009b; Quasney et al., 2001; Singh et al., 2004; Spector et al., 2010; Valcour et al., 2004a; van Rij et al., 1999). Importantly, none of the associations between these polymorphisms and HAND-related phenotypes have been consistently replicated. One possible reason for this lack of replication is the use of different neurobehavioral phenotypes across studies. Most used the diagnosis of HIV-associated dementia (HAD) as the phenotype (Boven et al., 1999; Gonzalez et al., 2002; Levine, 2009; Pemberton et al., 2008; Valcour et al., 2004a), which is a largely unreliable phenotype due to poor agreement among diagnosticians (Woods et al., 2004). A minority has simply averaged all scores from a battery of individual neuropsychological tests to arrive at a composite “global” neurocognitive functioning score (Singh et al., 2003; Singh et al., 2004; Spector et al., 2010). The major drawback of these phenotypes, particularly HAD, is that they are complex, influenced by a large number of environmental, psychometric, and other exogenous and endogenous factors. The numerous non-genetic contributors to variance in these measures, including measurement error, makes them less suitable targets for genetic analysis, especially when effect sizes for genetic associations are expected to be low. As it has no known biomarker, HAD is never a definitive diagnosis, but is instead based primarily on exclusion of other etiological possibilities. In making the diagnosis, clinicians differentially weigh demographic, neuropsychological, psychiatric, substance use, medical comorbidites, and neuroimaging data, to name a few. As such, true group membership is never certain. Furthermore, global measures of neurocognitive functioning run the risk of missing domain-specific associations with genes of interest. Therefore, cognitive domain-specific scores (e.g., memory or processing speed) may be more suitable cognitive phenotypes for use in genetic analysis. Composite domain scores (i.e., those arrived at by averaging scores of individual tests that compose that domain) have greater test-retest reliability when compared to individual measures, making them more attractive for creating progression phenotypes. In addition, the use of neurocognitive measures, many of which have demonstrated heritability, offers more precision and a more easily replicable phenotype across studies as compared to diagnostic categories.

Ever improving technological advances in genotyping coupled with refinement of phenotype measurement has recently made the application of genome wide association studies (GWAS) potentially fruitful for neuroAIDS. GWAS have identified additional risk variants associated with HIV disease progression, viral set point, rapid progressors, and other disease-related phenotypes, as previously reviewed (An and Winkler, 2010; Aouizerat et al., 2011). Not surprisingly, susceptibility loci identified to date are largely associated with innate and adaptive immunity. In the case of other neurologic conditions, GWAS have recently proven to be valuable, even when the disease examined was already relatively well-characterized. For example, a recent GWAS of late-onset Alzheimer’s disease, a neurodegenerative condition with well-established genetic risk factors, recently led to the identification of several new susceptibility loci (Belbin et al., 2011; Braskie et al., 2011; Laumet et al., 2010; Seshadri et al., 2010). In the case of HAND, for which the neuropathogenesis is less understood, GWAS may hold even greater promise. While it is unlikely that one or a few genetic susceptibility loci confer a large proportion of the risk for HAND, the genetic variance explained by GWAS help to achieve an improved mechanistic understanding of the disease. Genes identified through GWAS, even common alleles with weak statistical effects, can provide important information about biological pathways (Ho et al., 2010), and ultimately lead to targets for pharmaceutical treatment and prevention.

GWAS require large ethnically homogeneous samples, detailed phenotype information, and tissue or fluid samples that will yield DNA of sufficient quality. While large cohorts of HIV-

infected individuals exist, few have detailed information about neurocognitive functioning that would allow examination of HAND-related genetic susceptibility loci. As such, for now GWAS of HAND must rely on relatively small samples. Here, we detail the results of the first GWAS of HAND. This study leveraged the resources of the Multicenter AIDS Cohort Study (MACS), which included serial neurocognitive testing and neuromedical examinations since its launch in 1984. This enabled us to examine a variety of neurocognitive phenotypes for association. In order to replicate findings from previous candidate gene studies, we examined the prevalence of HAD and neurocognitive impairment cross-sectionally. In addition, we examined domain-specific neurocognitive changes over time.

MATERIAL AND METHODS

Participants

Genotype, behavioral, medical, and virologic data were obtained from participants in the Multicenter AIDS Cohort Study, or MACS. The MACS is a multicenter epidemiological study of the natural history of HIV infection in homosexual men, conducted in four U.S. cities (Baltimore, Chicago, Pittsburgh, and Los Angeles). Recruitment procedures are detailed elsewhere (Miller et al., 1990). The MACS participants are generally evaluated at semiannual intervals, although neurocognitive testing occurs every two years. Evaluations included physical examinations, HIV testing, laboratory testing, structured clinical interviews, and neuropsychological testing. After quality control procedures (described below), the final sample included 1287 individuals of Northern European ancestry evaluated between 11/8/1985 and 9/27/2010. Participant characteristics are summarized in Table 1.

Neurocognitive Phenotypes

Rate of neurocognitive decline—Rate of decline in two neurocognitive domains were examined as phenotypes. Processing speed (SPEED) consisted of the Stroop Color Naming trial (Comalli et al., 1962) and the Symbol Digit Modalities Test (Smith, 1982). Executive functioning (EXEC) consisted of the Stroop Interference trial (Comalli et al., 1962) and Form B of the Trail Making Test (Reitan, 1958). These domains were chosen as they are commonly affected in HIV+ individuals (Heaton et al., 2011; Woods et al., 2009), and because these tests were most reliably administered to the MACS participants across the duration of the study. Heritability for the Trail Making Test has been reported to be 34–68% (Swan and Carmelli, 2002). Heritability for a measure analogous to the Symbol Digit Modalities Test is 76% (Posthuma et al., 2001). Heritability for Stroop Color trial is estimated to be 70% (Stins et al., 2004), and the Interference trial 53–74% (Stins et al., 2004; Taylor, 2007). Standardized Z-scores for each test were based on normative data derived from the MACS HIV-seronegative participants, and adjusted for age and education (Selnes et al., 1991). An average domain Z-score was calculated and used in the analysis. The decision to use domain scores, derived by averaging Z-scores of their constituent tests, was made due to the psychometric stability and reliability of domain scores over individual test scores.

We used a linear mixed model (LMM) to determine subject-specific slopes for change in SPEED and EXEC, including (as fixed effects) the time (in years) since the first visit and the covariates described below. Subject-specific slopes for change in SPEED and EXEC with time were determined for each individual using data from multiple clinic visits. We applied the following exclusion criteria to visits: (1) visits where the phenotype of interest was not assessed were excluded, (2) visits in which the participant was HIV-seronegative were excluded, (3) visits in which the participant presented with an opportunistic infection of the CNS, and all subsequent visits, were excluded. Provided that a participant still had at least

two visits remaining after these exclusions, we then constructed phenotypes that estimated the change in SPEED or EXEC with time, measured in years. The LMM included as fixed effects time (in years) since the first visit, depression severity, antiretroviral therapy, alcohol and drug use, nadir CD4 cell count, and Hepatitis C infection (see below "Covariates" section). Random effects for the intercept and time were also included. The estimate of the best linear unbiased predictor for the random effect of time for each participant was used as the estimate of the subject -specific change in SPEED or EXEC with time.

Prevalence of HIV-associated dementia (HAD)—The MACS participants are queried at every visit about new diagnoses or medical symptoms. When new medical conditions are reported or detected on the MACS physical exam, medical charts are requested and formal diagnoses are recorded in the MACS database. Participants who show neurocognitive decline as determined via neuropsychological testing or who reported new neurologic symptoms were referred for a neurological exam with the study neurologist. A structured neurological exam was then used to document neurologic abnormalities and outcomes. The MACS neurologists and neuropsychologists reviewed all available data (medical charts, NP testing, neurologic exams, neuroimaging, etc.) to arrive at consensus diagnoses based on established criteria (AAN, 1991). Diagnosis of HAD at any study visit was considered a positive indicator for this phenotype.

Prevalence of neurocognitive impairment—Determination of neurocognitive impairment (NCI) was based on a comprehensive neuropsychological battery administered as part of the standard MACS protocol, and described elsewhere (Miller et al., 1990). NCI was determined at each visit according to an algorithm derived from the 2007 criteria from the Frascetti conference (Antinori et al., 2007). Functional domains that were assessed, along with measures, are shown in Table 2. Using the 2007 Frascetti conference criteria, at each visit participants were classified as normal, mild-to-moderate NCI, or moderate-to-severe NCI. A diagnosis of mild-to-moderate or moderate-to-severe NCI at any study visit was considered a positive indicator for this phenotype.

All neurocognitive phenotypes relied heavily upon neuropsychological tests for which heritability information is listed in Table 2.

Covariates

As mentioned previous in section, the following were considered in the analysis of progression of neurocognitive decline in EXEC and SPEED. All were time-varying with the exception of nadir CD4.

Nadir CD4 was the lowest measured CD4 count before 1996. Drug and alcohol use was recorded at each visit in terms of frequency and quantity of use during the previous six months. For drugs, we chose to examine only cocaine and amphetamine (including methamphetamine) use, as these drugs appear to have the greatest impact on neurocognitive and neurophysiological functioning in those with HIV (Everall et al., 2005a; Gaskill et al., 2009; Jernigan et al., 2005; Levine et al., 2006; Nath et al., 2002; Rippeth et al., 2004). Using frequency and quantity data from the 6 months preceding each visit, participants were classified based on their pattern of drug use over all visits examined. Specifically, they were classified as having *moderate or heavy* use of a substance if they reported either daily or weekly use, on average, across all visits. They were classified with *none or minimal* use if they reported monthly or less use on average across all visits. For alcohol, a total cumulative score was calculated based on information obtained at each visit (frequency × quantity for the 6 month period preceding each visit). Hepatitis C Infection (HCV) status was obtained, as it is believed to affect HIV disease progression (Jaen et al., 2008) and to compound

neurocognitive deficits in those with HIV (Cherner et al., 2005; Parsons et al., 2006). HCV status in the MACS participants was determined via blood testing. Participants were classified as HCV-negative if their viral testing was not indicative of HCV infection. Participants were classified as HCV-positive if they were found to be in the process of seroconversion, acute infection, chronic infection, clearing (between RNA+ and RNA−), or previously HCV-positive but now being clear of HCV RNA. Depression was determined with the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977). Scores on the CES-D are entered for each visit in a continuous manner. Higher scores indicated greater depression. Antiretroviral (ARV) Regimen use was determined for each study visit. Participants were classified as using *no therapy, monotherapy,* or combined/potent therapy.

Genetic Data

Data sets—Three data sets were analyzed, all using DNA derived from the MACS. The first was created by the Center for HIV-AIDS Vaccine Immunology (CHAVI) and generously provided by Jacques Fellay, M.D. This data set consisted of 1439 males genotyped on the Illumina 1M, 1MDuo platform (972 samples) or the Illumina 550K platform (467 samples). CHAVI targeted for genotyping individuals who seroconverted after enrolling in the MACS. The second was generously provided by Stephen O'Brien, Ph.D., of the Laboratory for Genetic Diversity (LGD) at the National Cancer Institute, and consisted of 1,877 males genotyped on the Affymetrix 6.0 platform. In addition to seroconverters, the LGD sample also consisted largely of seroprevalent individuals (i.e., already HIV+ at time of enrollment into the MACS). The final data set was created and generously provided by James I. Mullins, Ph.D., of the University of Washington (herein referred to as the Mullins data set) and consisted of 191 males genotyped on the Affymetrix 500K platform. This sample consisted of triplets of slower progressors (with regards to immune suppression), moderate progressors, and fast progressors.

Quality Control—Initial quality control was applied to individual data sets. All analyses were restricted to SNPs on chromosomes 1–22 and X. Genotyping completeness by sample and SNP were evaluated, and SNPs or samples missing >5% of data were excluded. Samples were checked to verify that the genetic sex was male. Each data set was merged with HapMap2 data release 23 and a principal components analysis was performed using EIGENSTRAT (Price et al., 2006) to identify population subgroups. An analysis of identity-by-descent (IBD) was conducted using PLINK (Purcell et al., 2007) to identify duplicated samples and related individuals. Concordance was checked for all sample duplicates and SNPs with high levels of discordance between duplicates identified and excluded from further analysis. Hardy-Weinberg Equilibrium (HWE) was checked separately by data set and population subgroup.

After removing one member of each duplicate pair, allele frequencies of SNPs in common in the Illumina platform sample (CHAVI) and the two Affymetrix platforms (LGD and Mullins) were compared using PLINK (Purcell et al., 2007), separately by ancestry group. SNPs showing high levels of discordance between duplicate data sets were excluded prior to analysis. The genomic control parameter lambda (Devlin and Roeder, 1999) was calculated for each allele frequency comparison.

Imputation—Imputation was performed using MACH (Li et al., 2010), and all data were imputed to the forward/positive strand. Strand ambiguous AT/GC SNPs were excluded. HapMap2 was used as the reference population (Frazer et al., 2007). SNPs with an imputation quality score (r^2) greater than 0.3 were retained for analysis.

Association Analysis

Rate of neurocognitive decline in specific domains (SPEED and EXEC): Participant-specific slopes derived from the LMM described above were used as a continuous phenotype in the association analysis, where the slope was modeled as a function of SNP genotype (considered to act additively) and the first three principal components from the EIGENSTRAT analysis. Genotyped SNPs were analyzed using PLINK (Purcell et al., 2007), and the dosage from imputed SNPs was analyzed using probABEL (Aulchenko et al., 2010).

Prevalence of HAD: All HIV+ participants were coded as ever having a diagnosis of HAD or not, and a logistic regression was used to model this binary outcome as a function of SNP genotype (considered to act additively), participant age at the first visit and the first three principal components from the EIGENSTRAT analysis. Genotyped SNPs were analyzed using PLINK, and the dosage from imputed SNPs was analyzed using probABEL (Aulchenko et al., 2010).

Prevalence of neurocognitive impairment (NCI): All HIV+ participants were coded as *ever impaired* or *never impaired*, and a logistic regression was used to model this binary outcome as a function of SNP genotype (considered to act additively) and the first three principal components from the EIGENSTRAT analysis. Genotyped SNPs were analyzed using PLINK, and the dosage from imputed SNPs was analyzed using probABEL (Aulchenko et al., 2010).

RESULTS

Genotyping Quality Control (QC)

Genotyping completeness in the CHAVI data set averaged >99%; all 1439 participants were missing < 5% of data. In the 550K CHAVI SNP set, 33,091 SNPs were missing >5% of data, leaving 519,478 SNPs for analysis. In the 1M and 1MDuo CHAVI SNP sets, 218,406 SNPs were missing >5% of data, leaving 946,551 SNPs for analysis. In the LGD data set, three participants were found to be genetically female and 25 were missing >5% of data, leaving 1849 participants for analysis. Genotyping completeness in these 1849 participants averaged >99%. There were 82,420 SNPs missing >5% of data, leaving 822,597 SNPs for analysis. There were 5 participants in the Mullins data set missing >5% of data, leaving 186 participants for analysis. Genotyping completeness in these 186 subjects averaged >99%. There were 43,518 SNPs missing >5% of data, leaving 456,929 SNPs for analysis.

Examination of plots of principal component for each sample (Figure 1), overlaid with HapMap2 samples, showed that all three data sets were comprised of persons of Northern European ancestry (NE; CHAVI: n=1,221, LGD: n=1,693, Mullins: n=154) and African ancestry (A; CHAVI: n=143, LGD: n=147, Mullins: n=23), and a few individuals of possibly Asian or other ancestry (ASO; CHAVI: n=75, LGD: n=9, Mullins: n=9). In the principal component analysis, samples of Northern European (NE) ancestry were defined as samples with PC1<0.01 and PC2<0.01. Samples of African (A) ancestry were defined as samples with PC1>0.025. ASO individuals did not fall into either of these principal components.

IBD analysis revealed a number of individuals who were genetically identical in multiple data sets: 167 participants were identical in both CHAVI and Mullins, 146 participants were identical in both LGD and Mullins, and 1029 participants were identical in both CHAVI and LGD. Additionally, there were two pairs of duplicate samples within CHAVI, one pair within LGD, and one pair of brothers within LGD. Genotype concordance by SNP was

assessed for duplicate samples as another SNP QC metric. There were 40,466 SNPs that had one or more discordant calls (range 1–217) in the three comparisons of duplicate samples. The distribution of the number of discordant calls per SNP showed that 80% of SNPs had fewer than six discordant calls; the 6824 SNPs with six or greater discordant calls were excluded from further analysis.

Some of the identical samples were known duplicates, in that they had the same identification numbers (GWAS IDs). In such cases, one member of each duplicate sample was excluded from further analysis. The duplicated sample was retained in the data set that contained the most SNPs. There were some pairs of identical persons with different GWAS IDs, and some pairs of persons with the same GWAS IDs that were not genetically identical. As these pairings indicate likely sample mix-ups, both persons in such pairings were excluded from analysis.

A total of 2247 participants were retained for analysis, 1058 from CHAVI, 1180 from LGD and 9 from Mullins. The 9 remaining Mullins samples were combined with the 1180 LGD samples for further analysis. The distribution of ancestry groups in each set of retained samples were CHAVI NE: 852, A: 133, ASO: 73; LGD+Mullins NE: 1099, A: 82, ASO: 8. Due to the small sample sizes, A and ASO individuals were not used in further analyses.

Evaluation of HWE was done separately by data set. In the CHAVI NE data set, 112 SNPs were out of HWE at $p<10^{-6}$, respectively. In the LGD+Mullins NE data set, 2,055 were out of HWE at $p<10^{-6}$, respectively.

Using only SNPs in HWE at $p>10^{-6}$ in all data sets, and excluding discordant SNPs as described above, allele frequencies were compared between CHAVI and LGD+Mullins. Under the null hypothesis of no difference in allele frequencies between data sets, the genomic control parameter (λ) should be 1.0. The estimate of λ for participants of NE ancestry was 1.027. In light of these differences in allele frequencies, imputation was done separately for Illumina and Affymetrix data sets. NE ancestry data sets were imputed using HapMap CEU as reference. There were 2,511,469 and 2,487,515 autosomal SNPs imputed with $r^2>0.30$ in CHAVI and LGD+Mullins, respectively.

Phenotype data were available for 1,464 of the 1,951 individuals of NE ancestry. There were 177 persons that were HIV negative for all clinic visits and were excluded from all further analyses. Analysis of change in SPEED functioning excluded 135 persons who did not have two or more visits with phenotype data following seroconversion, 1 person who did not have two or more visits with phenotype data after excluding visits with an opportunistic infection of the CNS, and 20 persons who did not have two or more visits with phenotype data and complete covariate data, leaving maximally 1131 persons for analysis of SPEED. Analysis of change in EXEC functioning excluded 57 persons who did not have two or more visits with phenotype data following seroconversion, 2 persons who did not have two or more visits with phenotype data after excluding visits with an opportunistic infection of the CNS, and 90 persons who did not have two or more visits with phenotype data and complete covariate data, leaving maximally 1138 persons for analysis of EXEC. HAD and NCI analyses excluded only the 177 persons who were HIV negative throughout the course of study; the sample size for analysis of both binary phenotypes was maximally 1287. Of these, 71 had a diagnosis of HAD, and 863 were diagnosed with NCI.

GWA Analyses

Association analysis of slope of change in the domains of SPEED and EXEC, as well as prevalence of HAD and NCI, to genotyped and imputed SNPs was done separately by cohort (CHAVI and LGD+Mullins) and then combined via meta-analysis. Meta-analysis

combining results from the two samples was done using METAL (http://www.sph.umich.edu/csg/abecasis/metal/) by calculating a Z-statistic that was a weighted average of sample-level statistics, where the weights were proportional to the square root of the number of individuals examined in each sample, and selected so that the squared weights summed to 1. SNPs that were monomorphic in the subset of persons with phenotype data were not analyzed. The total number of imputed and genotyped SNPs analyzed for association to each phenotype is summarized in Table 3. The genomic control parameters for SPEED, EXEC and NCI showed no inflation over the null expectation of 1.0. In the analysis of HAD, imputed SNPs show moderate inflation of the genomic control parameter (Table 3). None of the phenotypes resulted in genome-wide significance ($p < 10^{-8}$) across the nearly 2.5 million SNP markers analyzed.

A small number of SNPs associated with SPEED, and one with NCI, did have p-values less than 10^{-6} (Table 4). Upon further examination, it was found that all SNPs on chromosome 10 listed in Table 4 were in high LD ($R^2 = 1.0$), and thus reflect the same signal. Importantly, these largely occurred in an intergenic region. Further, as with the NCI association on chromosome 1, the distance of these SNPs from the nearest exonic region suggests that they are not in LD with any genes.

Fixed Effects Analyses

Results for the fixed effects portion of the LMM for SPEED and EXEC are displayed in Table 5. Depression was associated with significantly lower SPEED and EXEC scores. Use of alcohol was associated with higher SPEED scores and lower EXEC scores, the latter being only marginally significant. Moderate to heavy use of drugs did not significantly affect SPEED or EXEC scores. Nadir CD4 count increased with SPEED and EXEC scores, the latter being only marginally significant. Having a comorbid diagnosis of HCV did not significantly affect SPEED or EXEC. Use of monotherapy was associated with significantly lower SPEED scores over that observed with no therapy, but did not impact EXEC. Use of combination/potent therapy did not significantly impact SPEED but did increase EXEC scores relative to no therapy. Histograms of the participant-specific changes with time in SPEED and EXEC are estimated from the random effects component of the LMM and are presented in Figure 2.

Examination of SNPs identified in previous candidate gene association studies

Where possible, we determined *p*-values and odds ratios (OR) for SNPs previously implicated in HAND or neurocognitive impairment in HIV+ participants. These SNPs are listed in Table 6 along with the phenotypes used in previous studies and relevant findings. As shown, none of the SNPs were validated in the current analysis.

Note that the candidate gene association studies sometimes used an alternative model of analysis, where the effect of two copies of the “risk” allele was contrasted against the effect of having 0 or 1 copy of the risk allele (recessive model), or where the effect of 1 or 2 copies of the risk allele was contrasted against having no copies of the risk allele (dominant model). Because some of the SNPs in the current study were imputed, thus producing an allele “dosage” rather than a genotype – the dosage measure takes into account the uncertainty in imputing an unobserved genotype. As such, allele dosage data cannot be analyzed as a recessive or dominant mode of inheritance, as was done in some previous studies.

rs1130371 (MIP1-α)—This SNP was imputed from the CHAVI dataset and genotyped directly in LGD dataset. Using an additive model in the combined analysis, the risk allele increased the chance of HAD in both datasets (OR for CHAVI = 1.36, OR for LGD = 1.39). As such, one copy of the risk allele increased risk of HAD 1.38x, whereas 2 copies increased

risk 1.38^2x. This did not reach statistical significance (p=0.102). In the genotyped LGD sample, testing the effect of the HAD-related T/T genotype vs. others also produced a non-significant p-value (*p*=0.42, OR=1.68; T/T increases risk of dementia 1.68x over the other two possible genotypes.).

rs1800629 (TNF-α)—This SNP was genotyped in CHAVI and imputed in LGD. Using an additive model in the combined analysis, the risk allele decreased risk of dementia in the CHAVI sample and increased risk in the LGD sample to produce an overall *p*-value of 0.98 in the meta-analysis. Testing a dominant model in the genotyped CHAVI sample alone also results in a non-significant result (*p*=0.93).

rs1024611 (MCP-1)—This SNP was genotyped in both the CHAVI and LGD cohorts. Using an additive model, the risk allele did increase the risk of dementia; however, the effect was non-significant (meta analysis p-value = 0.31, OR for CHAVI=1.43, OR for LGD=1.01, these OR estimate the increase in risk for 1 copy of the risk allele, two copies of the risk allele increase odds of dementia 1.43^2 and 1.01^2, respectively). An additional test was performed in each cohort to contrast the effect of homozygotes for the risk allele to the other two genotypes. In CHAVI, the OR for risk allele homozygotes was 1.90 (p=0.19) and in LGD the OR for risk allele homozygotes was ~1.0 (p=0.99).

rs1801157 (SDF-1)—This SNP was imputed in CHAVI and genotyped directly in LGD. In our meta-analysis of the binary outcome of NCI, using an additive model resulted in a non-significant *p*-value of 0.302. The risk allele (A) decreased the odds of impairment for CHAVI and increased the odds of impairment for LGD [CHAVI OR = 0.97; LGD OR = 1.30].

rs2839619 (Prep1)—This SNP was directly genotyped by the Affymetrix platform. In our meta-analysis of the binary outcome of HAD, using an additive model, resulted in a non-significant p-value of 0.80. The C allele increased the odds of dementia for CHAVI and LGD by 1.05x and 1.03x, respectively.

rs1800450, rs1800451, and rs5030737 (MBL haplotype)—The haplotypes formed by alleles at SNPs rs1800451, rs1800450 and rs5030737 are typically coded as “wild type” (G-G-C haplotype) or “mutant” (anything other than G-G-C). Constructing haplotypes requires genotype data rather than imputed dosage data. The CHAVI cohort was genotyped directly for all three SNPs and haplotypes in that cohort were estimated using the program BEAGLE (Browning and Browning, 2007). Each person was coded as having 0, 1 or 2 copies of a mutant haplotype.

The frequency of impairment in persons with 0 copies of a mutant haplotype is 71.9%, whereas the frequency of impairment in persons with 2 copies of a mutant haplotype is 70.7% (Table 7). These are clearly not different. Treating the number of copies of a mutant haplotype additively in a logistic regression to predict the probability of impairment resulted in a *p*-value of 0.43.

DISCUSSION

HIV infection leads to neurocognitive dysfunction in almost half of infected individuals (Heaton et al., 2010). By and large, the biological mechanisms underlying HAND remain poorly understood. Data-driven GWA studies have proven useful in identifying common genetic variants associated with HIV disease factors, thereby allowing further delineation of biological factors underlying progression. This paper describes the first GWAS of HIV-related neurocognitive impairment and disorders.

Because of the heterogeneity of HIV-associated neurocognitive disorders (Antinori et al., 2007), we examined a variety of neurocognitive phenotypes: domain-specific decline over time, prevalence of HIV-associated dementia, and prevalence of NCI. We also considered as covariates factors that have been most consistently associated with HAND in previous studies: nadir CD4, ARV regimen, substance use, alcohol use, HCV co-infection, and depression. Our analyses did not identify any genetic susceptibility loci among almost 2.5 million SNPs to domain-specific decline over time, prevalence of HAD, or prevalence of NCI.

With regards to NCI, the majority of the MACS participants who were genotyped, including those who were HIV-seronegative, met our criteria for NCI. Our analyses show that 55% of the HIV-seronegative participants who underwent genome-wide genotyping met criteria for NCI, as compared to 67% of HIV+ participants. This suggests that NCI was caused by factors other than HIV (e.g., substance use, depression) in most cases. As such, NCI was not a particularly good phenotype for HIV-related neurocognitive impairment in this sample. Yet even when HAD was considered, a diagnosis based on more extensive evaluation and after ruling out other potential causes, no significant associations emerged.

The null findings suggest that the relatively common genetic variants examined do not have an appreciable impact on risk for HAND among Caucasian men. If true, this would also suggest that findings from some previous genetic association studies are erroneous, perhaps explaining the difficulty of validating positive findings from such studies. Indeed, our SNP-specific analyses did not find strong associations between previously identified genetic susceptibility loci and the neurocognitive phenotypes, even without the strict GWA threshold for statistical significance. The MIP1α and MCP-1 polymorphisms showed the largest odds ratios (1.68 and 1.90, respectively), but these were not statistically significant at even a nominal .05 threshold. Among alleles not examined here, due to lack of coverage on the genotyping platforms and inadequate LD which would have allowed imputation, were the CCR5 32bp deletion, CCR2-64-I, and ApoE4, for which mixed results have been reported in genetic association studies with HAND and related factors (Andres et al., 2011; Bol et al., 2012; Boven et al., 1999; Burt et al., 2008; Chang et al., 2011; Corder et al., 1998; Dunlop et al., 1997; Pomara et al., 2008; Singh et al., 2003; Spector et al., 2010; Valcour et al., 2004a).

An alternative yet more likely explanation is that the sample size in the current study was too small to detect true associations. For analyzing the progression of neurocognitive decline, using an alpha (type I error) = 5×10^{-8}, in order to have 80% power to detect an additive association to a SNP, the SNP must account for 3.5% of the variability in the phenotype. Our strongest hits occurred for SPEED (Table 4), with none accounting for >3% of the variability (data not shown). Given our sample size of 1287 men for the analysis of NCI and HAD, we were well powered to detect an OR of at least 2.25 and 3.86, respectively (data not shown). As we did not find significant associations, if genetic variants exist that affect the probability of NCI/HAD in HIV+ men, their individual effect sizes are likely to be less than these ORs. While other GWAS have used even smaller samples and have identified novel SNPs associated with HIV disease related factors, they utilized biological phenotypes that were likely more heritable than those used here(Fellay et al., 2007). Conversely, HAND (including HAD) is a heterogeneous syndrome likely to have a varied pathogenesis influenced by myriad immunologic, virologic, neurotrophic, and other factors (Kraft-Terry et al., 2009). As such, a sample size considerably larger than the current one may be required to determine the extent to which functional genetic variants in such genes affects risk for HAND. As shown above, in this cohort targeting only specific aspects of HAND as phenotypes (i.e., neurocognitive decline) is confounded by the impact upon neurocognitive

functioning of lifestyle behaviors, psychiatric, and other factors common among homosexual HIV+ individuals.

Another possible explanation for the null findings is informative censoring; that is, it is possible that individuals who were becoming increasingly cognitively impaired were more likely to opt out of undergoing neuropsychological testing. As such, individuals with greater impairment may have been less likely to undergo evaluation, and were therefore left out of our analyses. Because there is no data to examine this (i.e., there is no way to confirm the degree of impairment in an individual who refused testing), it remains an uncertainty.

It is worth discussing the SLC8A1 and NALCN, both of which had p-values just below the strict GWAS threshold. SLC8A1 (solute carrier family 8 [sodium/calcium exchanger] member 1) is involved in ion channel/ion transporter activity in plasma and mitochondrial membranes. SLC8A1 and NALCN (sodium leak channel, non-selective) are both involved in sodium transport across cellular and intracellular membranes. Loss of mitochondrial membrane potential and other effect of ion transport dysfunction have been implicated in HAD (Benos et al., 1994; Gelman et al., 2004; Noorbakhsh et al., 2010). While these SNPs were associated with change in information processing speed over time rather than HAD (and therefore may not be HIV-specific), these findings are noteworthy.

While not the focus of the current analysis, the covariates considered in our analysis largely provided confirmation of previous studies, although there were exceptions. Perhaps the most unexpected was alcohol use, which had opposite effects on processing speed and executive functioning over time, showing improvement in the former among heavier users and decline in the latter. Most previous studies have found additive or synergistic effects of alcohol use and HIV serostatus upon processing speed (Fama et al., 2009; Rothlind et al., 2005; Sassoon et al., 2007). However, these studies generally included heavy drinkers, or stratified their sample based on alcohol use (e.g. light vs. heavy drinkers) (Rothlind et al., 2005; Sassoon et al., 2007), whereas we used a continuous measure. Also somewhat surprising, moderate-to-heavy use of cocaine and/or methamphetamines did not have a significant impact on neurocognitive ability over time, although the relationship was in the expected direction. The vast majority of research has demonstrated additive effects of stimulants and HIV, as well as poorer neurocognitive functioning among HIV-infected stimulant users as compared to non-drug using counterparts (Gonzalez and Cherner, 2008; Martin-Thormeyer and Paul, 2009; Nath et al., 2002; Rippeth et al., 2004). One possible explanation for the current findings is that the neurocognitive domains affected by stimulant use in HIV-infected individuals were not adequately sampled in our analysis (Levine et al., 2006). Greater degree of depression, as reported on the CES-D, was associated with significant decline in both processing speed and executive functioning. In fact, of all the fixed factors examined, depression had the greatest impact on neurocognitive functioning over time. Higher nadir CD4 was associated with improved performance in both neurocognitive domains. This was expected, as low nadir CD4 has perhaps been the most consistent parameter predictive of neurocognitive impairment and neuropathology in HIV infection (Cysique et al., 2010; Cysique et al., 2006; Everall et al., 2009; Heaton et al., 2010; Robertson et al., 2007). Co-infection with HCV did not have a significant effect on neurocognitive functioning over time. While HCV co-infection has recently been shown in some studies to worsen HIV-related neurocognitive deficits (Hinkin et al., 2008; Martin-Thormeyer and Paul, 2009), this has not been examined longitudinally to our knowledge. Here we present, in a longitudinal examination, evidence that co-infection does not hasten neurocognitive decline in the domains assessed. Note however that in our analysis, we created a dichotomous variable for HCV, with seronegative individuals in one group and the other group consisting primarily of individuals with acute or chronic infection, but also some who were clearing (between RNA + and RNA−) (N=11), cleared (N=34), and with prior infection whose current RNA status

was unknown (N=7). There are no guidelines in this somewhat nascent line of inquiry to classify individuals in these later groups as HCV+ or HCV−, as one could argue that even in cleared individuals, the neuropathological consequences of co-infection may be lasting. Finally, the impact of ARV regimen on neurocognitive functioning over time was revealing. Use of monotherapy was associated with decreased processing speed performance over time, as compared to no therapy. However, it did not impact executive functioning. Conversely, use of combination/potent therapy, a modality initiated in the early 1990s, was not associated with processing speed but was associated with improved performance on measures of executive functioning relative to no ARV therapy. The relationship between ARV regimen and neurocognition is complex; early treatments were used primarily for seriously ill patients, while later treatments were more likely to be initiated based on immune system functioning rather than general systemic health. Also, long term use of some ARV regimens (particularly early monotherapies) may be neurotoxic despite eradicating the virus from the brain. A recent classification scheme to estimate the efficacy of individual ARVs in the CNS will help in further clarifying this relationship (Letendre et al., 2008).

The current findings should be interpreted with the following additional caveats. Firstly, this was a convenience sample, and therefore not all variables were operationalized and measured in an ideal fashion. In addition, the MACS participants chosen for genotyping belonged to specific subgroups of infected individuals, including seroconverters and those with varying levels of disease progression. As such, this sample may not be representative of the general HIV+ population. Secondly, as described above, some variables, such as HCV infection and ARV use, were collapsed into a simpler variable, thereby pooling individuals with widely varying degrees of infection or different ARV regimens into the same group. Finally, we have acknowledged that the very small sample size likely impacted our ability to detect true associations. HAND is a heterogeneous syndrome likely to have a varied pathogenesis influenced by myriad immunologic, virologic, neurotrophic, and other factors (Kraft-Terry et al., 2009). As such, a sample size considerably larger than the current one would be required to determine the extent to which functional genetic variants in such genes affects risk for HAND. We are optimistic that as more HIV cohorts undergo genome-wide genotyping, larger studies will be possible. Alternatively, a more robust biological phenotype for HAND could be the focus of future studies. While none have been identified thus far that demonstrate adequate reliability for diagnostic purposes, efforts are underway to identify CSF, plasma, and neuroimaging, biomarkers of HAND. In addition, genetic association studies that focus on neuropathological phenotypes may be useful in identifying which neuropathological changes, if any, underlie HAND. Only one such study has been reported to date, with negative findings (Diaz-Arrastia et al., 2004). However, with improved imaging and quantification methods coupled with the identification of additional neuropathological markers putatively associated with HAND(Everall et al., 1999; Moore et al., 2006), the likelihood of positive genetic associations will likely be enhanced.

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References

AAN. Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. Report of a Working Group of the American Academy of Neurology AIDS Task Force. Neurology. 1991; 41(6):778–85. [PubMed: 2046917]

Achim CL, Wiley CA. Inflammation in AIDS and the role of the macrophage in brain pathology. Curr Opin Neurol. 1996; 9(3):221–5. [PubMed: 8839616]

An P, Winkler CA. Host genes associated with HIV/AIDS: advances in gene discovery. Trends Genet. 2010; 26(3):119–31. [PubMed: 20149939]

Ances BM, Ellis RJ. Dementia and neurocognitive disorders due to HIV-1 infection. Semin Neurol. 2007; 27(1):86–92. [PubMed: 17226745]

Andres MA, Feger U, Nath A, Munsaka S, Jiang CS, Chang L. APOE epsilon 4 allele and CSF APOE on cognition in HIV-infected subjects. J Neuroimmune Pharmacol. 2011; 6(3):389–98. [PubMed: 21184197]

Anthony IC, Bell JE. The Neuropathology of HIV/AIDS. Int Rev Psychiatry. 2008; 20(1):15–24. [PubMed: 18240059]

Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, Gisslen M, Grant I, Heaton RK, Joseph J, Marder K, Marra CM, McArthur JC, Nunn M, Price RW, Pulliam L, Robertson KR, Sacktor N, Valcour V, Wojna VE. Updated research nosology for HIV-associated neurocognitive disorders. Neurology. 2007; 69(18):1789–99. [PubMed: 17914061]

Aouizerat BE, Pearce CL, Miaskowski C. The search for host genetic factors of HIV/AIDS pathogenesis in the post-genome era: progress to date and new avenues for discovery. Curr HIV/AIDS Rep. 2011; 8(1):38–44. [PubMed: 21221856]

Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. BMC Bioinformatics. 2010; 11:134. [PubMed: 20233392]

Becker JT, Lopez OL, Dew MA, Aizenstein HJ. Prevalence of cognitive disorders differs as a function of age in HIV virus infection. Aids. 2004; 18(Suppl 1):S11–8. [PubMed: 15075493]

Belbin O, Carrasquillo MM, Crump M, Culley OJ, Hunter TA, Ma L, Bisceglio G, Zou F, Allen M, Dickson DW, Graff-Radford NR, Petersen RC, Morgan K, Younkin SG. Investigation of 15 of the top candidate genes for late-onset Alzheimer's disease. Hum Genet. 2011; 129(3):273–82. [PubMed: 21132329]

Benos DJ, Hahn BH, Bubien JK, Ghosh SK, Mashburn NA, Chaikin MA, Shaw GM, Benveniste EN. Envelope glycoprotein gp120 of human immunodeficiency virus type 1 alters ion transport in astrocytes: implications for AIDS dementia complex. Proc Natl Acad Sci U S A. 1994; 91(2):494–8. [PubMed: 8290553]

Bol SM, Booiman T, van Manen D, Bunnik EM, van Sighem AI, Sieberer M, Boeser-Nunnink B, de Wolf F, Schuitemaker H, Portegies P, Kootstra NA, van 't Wout AB. Single Nucleotide Polymorphism in Gene Encoding Transcription Factor Prep1 Is Associated with HIV-1-Associated Dementia. PLoS One. 2012; 7(2):e30990. [PubMed: 22347417]

Borjabad A, Brooks AI, Volsky DJ. Gene expression profiles of HIV-1-infected glia and brain: toward better understanding of the role of astrocytes in HIV-1-associated neurocognitive disorders. J Neuroimmune Pharmacol. 2010; 5(1):44–62. [PubMed: 19697136]

Boven LA, Middel J, Verhoef J, De Groot CJ, Nottet HS. Monocyte infiltration is highly associated with loss of the tight junction protein zonula occludens in HIV-1-associated dementia. Neuropathol Appl Neurobiol. 2000; 26(4):356–60. [PubMed: 10931369]

Boven LA, van der Bruggen T, van Asbeck BS, Marx JJ, Nottet HS. Potential role of CCR5 polymorphism in the development of AIDS dementia complex. FEMS Immunol Med Microbiol. 1999; 26(3–4):243–7. [PubMed: 10575135]

Braskie MN, Jahanshad N, Stein JL, Barysheva M, McMahon KL, de Zubicaray GI, Martin NG, Wright MJ, Ringman JM, Toga AW, Thompson PM. Common Alzheimer's disease risk variant

within the CLU gene affects white matter microstructure in young adults. J Neurosci. 2011; 31(18):6764–70. [PubMed: 21543606]

Browning SR, Browning BL. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. Am J Hum Genet. 2007; 81(5):1084–97. [PubMed: 17924348]

Burt TD, Agan BK, Marconi VC, He W, Kulkarni H, Mold JE, Cavrois M, Huang Y, Mahley RW, Dolan MJ, McCune JM, Ahuja SK. Apolipoprotein (apo) E4 enhances HIV-1 cell entry in vitro, and the APOE epsilon4/epsilon4 genotype accelerates HIV disease progression. Proc Natl Acad Sci U S A. 2008; 105(25):8718–23. [PubMed: 18562290]

Chang L, Andres M, Sadino J, Jiang CS, Nakama H, Miller E, Ernst T. Impact of apolipoprotein E epsilon4 and HIV on cognition and brain atrophy: Antagonistic pleiotropy and premature brain aging. Neuroimage. 2011; 58(4):1017–27. [PubMed: 21803164]

Cherner M, Ellis RJ, Lazzaretto D, Young C, Mindt MR, Atkinson JH, Grant I, Heaton RK. Effects of HIV-1 infection and aging on neurobehavioral functioning: preliminary findings. Aids. 2004; 1(18 Suppl):S27–34. [PubMed: 15075495]

Cherner M, Letendre S, Heaton RK, Durelle J, Marquie-Beck J, Gragg B, Grant I. Hepatitis C augments cognitive deficits associated with HIV infection and methamphetamine. Neurology. 2005; 64(8):1343–7. [PubMed: 15851720]

Comalli PE Jr, Wapner S, Werner H. Interfernce effects of Stroop color-word test in childhood, adulthood, and aging. J Genet Psychol. 1962; 100:47–53. [PubMed: 13880724]

Corder EH, Robertson K, Lannfelt L, Bogdanovic N, Eggertsen G, Wilkins J, Hall C. HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. Nat Med. 1998; 4(10):1182–4. [PubMed: 9771753]

Cysique LA, Brew BJ. Neuropsychological functioning and antiretroviral treatment in HIV/AIDS: a review. Neuropsychol Rev. 2009; 19(2):169–85. [PubMed: 19424802]

Cysique LA, Letendre SL, Ake C, Jin H, Franklin DR, Gupta S, Shi C, Yu X, Wu Z, Abramson IS, Grant I, Heaton RK. Incidence and nature of cognitive decline over 1 year among HIV-infected former plasma donors in China. AIDS. 2010; 24(7):983–90. [PubMed: 20299964]

Cysique LA, Maruff P, Brew BJ. Variable benefit in neuropsychological function in HIV-infected HAART-treated patients. Neurology. 2006; 66(9):1447–50. [PubMed: 16682686]

Devlin B, Roeder K. Genomic control for association studies. Biometrics. 1999; 55(4):997–1004. [PubMed: 11315092]

Diaz-Arrastia R, Gong Y, Kelly CJ, Gelman BB. Host genetic polymorphisms in human immunodeficiency virus-related neurologic disease. J Neurovirol. 2004; 1(10 Suppl):67–73. [PubMed: 14982742]

Dore GJ, McDonald A, Li Y, Kaldor JM, Brew BJ. Marked improvement in survival following AIDS dementia complex in the era of highly active antiretroviral therapy. Aids. 2003; 17(10):1539–45. [PubMed: 12824792]

Dunlop O, Goplen AK, Liestol K, Myrvang B, Rootwelt H, Christophersen B, Kvittingen EA, Maehlen J. HIV dementia and apolipoprotein E. Acta Neurol Scand. 1997; 95(5):315–8. [PubMed: 9188909]

Everall I, Salaria S, Roberts E, Corbeil J, Sasik R, Fox H, Grant I, Masliah E. Methamphetamine stimulates interferon inducible genes in HIV infected brain. J Neuroimmunol. 2005a; 170(1–2): 158–71. [PubMed: 16249037]

Everall I, Vaida F, Khanlou N, Lazzaretto D, Achim C, Letendre S, Moore D, Ellis R, Cherner M, Gelman B, Morgello S, Singer E, Grant I, Masliah E, National Neuro ATC. Cliniconeuropathologic correlates of human immunodeficiency virus in the era of antiretroviral therapy. J Neurovirol. 2009; 15(5–6):360–70. [PubMed: 20175693]

Everall IP, Hansen LA, Masliah E. The shifting patterns of HIV encephalitis neuropathology. Neurotox Res. 2005b; 8(1–2):51–61. [PubMed: 16260385]

Everall IP, Heaton RK, Marcotte TD, Ellis RJ, McCutchan JA, Atkinson JH, Grant I, Mallory M, Masliah E. Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. HNRC Group. HIV Neurobehavioral Research Center. Brain Pathol. 1999; 9(2):209–17. [PubMed: 10219738]

Fama R, Rosenbloom MJ, Nichols BN, Pfefferbaum A, Sullivan EV. Working and episodic memory in HIV infection, alcoholism, and their comorbidity: baseline and 1-year follow-up examinations. Alcohol Clin Exp Res. 2009; 33(10):1815–24. [PubMed: 19656122]

Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, Zhang K, Gumbs C, Castagna A, Cossarizza A, Cozzi-Lepri A, De Luca A, Easterbrook P, Francioli P, Mallal S, Martinez-Picado J, Miro JM, Obel N, Smith JP, Wyniger J, Descombes P, Antonarakis SE, Letvin NL, McMichael AJ, Haynes BF, Telenti A, Goldstein DB. A whole-genome association study of major determinants for host control of HIV-1. Science. 2007; 317(5840):944–7. [PubMed: 17641165]

Fox L, Alford M, Achim C, Mallory M, Masliah E. Neurodegeneration of somatostatin-immunoreactive neurons in HIV encephalitis. J Neuropathol Exp Neurol. 1997; 56(4):360–8. [PubMed: 9100666]

Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, Pasternak S, Wheeler DA, Willis TD, Yu F, Yang H, Zeng C, Gao Y, Hu H, Hu W, Li C, Lin W, Liu S, Pan H, Tang X, Wang J, Wang W, Yu J, Zhang B, Zhang Q, Zhao H, Zhao H, Zhou J, Gabriel SB, Barry R, Blumenstiel B, Camargo A, Defelice M, Faggart M, Goyette M, Gupta S, Moore J, Nguyen H, Onofrio RC, Parkin M, Roy J, Stahl E, Winchester E, Ziaugra L, Altshuler D, Shen Y, Yao Z, Huang W, Chu X, He Y, Jin L, Liu Y, Shen Y, Sun W, Wang H, Wang Y, Wang Y, Xiong X, Xu L, Waye MM, Tsui SK, Xue H, Wong JT, Galver LM, Fan JB, Gunderson K, Murray SS, Oliphant AR, Chee MS, Montpetit A, Chagnon F, Ferretti V, Leboeuf M, Olivier JF, Phillips MS, Roumy S, Sallee C, Verner A, Hudson TJ, Kwok PY, Cai D, Koboldt DC, Miller RD, Pawlikowska L, Taillon-Miller P, Xiao M, Tsui LC, Mak W, Song YQ, Tam PK, Nakamura Y, Kawaguchi T, Kitamoto T, Morizono T, Nagashima A, Ohnishi Y, Sekine A, Tanaka T, Tsunoda T, Deloukas P, Bird CP, Delgado M, Dermitzakis ET, Gwilliam R, Hunt S, Morrison J, Powell D, Stranger BE, Whittaker P, Bentley DR, Daly MJ, de Bakker PI, Barrett J, Chretien YR, Maller J, McCarroll S, Patterson N, Pe'er I, Price A, Purcell S, Richter DJ, Sabeti P, Saxena R, Schaffner SF, Sham PC, Varilly P, Altshuler D, Stein LD, Krishnan L, Smith AV, Tello-Ruiz MK, Thorisson GA, Chakravarti A, Chen PE, Cutler DJ, Kashuk CS, Lin S, Abecasis GR, Guan W, Li Y, Munro HM, Qin ZS, Thomas DJ, McVean G, Auton A, Bottolo L, Cardin N, Eyheramendy S, Freeman C, Marchini J, Myers S, Spencer C, Stephens M, Donnelly P, Cardon LR, Clarke G, Evans DM, Morris AP, Weir BS, Tsunoda T, Mullikin JC, Sherry ST, Feolo M, Skol A, Zhang H, Zeng C, Zhao H, Matsuda I, Fukushima Y, Macer DR, Suda E, Rotimi CN, Adebamowo CA, Ajayi I, Aniagwu T, Marshall PA, Nkwodimmah C, Royal CD, Leppert MF, Dixon M, Peiffer A, Qiu R, Kent A, Kato K, Niikawa N, Adewole IF, Knoppers BM, Foster MW, Clayton EW, Watkin J, Gibbs RA, Belmont JW, Muzny D, Nazareth L, Sodergren E, Weinstock GM, Wheeler DA, Yakub I, Gabriel SB, Onofrio RC, Richter DJ, Ziaugra L, Birren BW, Daly MJ, Altshuler D, Wilson RK, Fulton LL, Rogers J, Burton J, Carter NP, Clee CM, Griffiths M, Jones MC, McLay K, Plumb RW, Ross MT, Sims SK, Willey DL, Chen Z, Han H, Kang L, Godbout M, Wallenburg JC, L'Archeveque P, Bellemare G, Saeki K, Wang H, An D, Fu H, Li Q, Wang Z, Wang R, Holden AL, Brooks LD, McEwen JE, Guyer MS, Wang VO, Peterson JL, Shi M, Spiegel J, Sung LM, Zacharia LF, Collins FS, Kennedy K, Jamieson R, Stewart J. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007; 449(7164):851–61. [PubMed: 17943122]

Gaskill PJ, Calderon TM, Luers AJ, Eugenin EA, Javitch JA, Berman JW. Human immunodeficiency virus (HIV) infection of human macrophages is increased by dopamine: a bridge between HIV-associated neurologic disorders and drug abuse. Am J Pathol. 2009; 175(3):1148–59. [PubMed: 19661443]

Gelman BB. The neuropathology of HIV. Handb Clin Neurol. 2007; 85:301–17. [PubMed: 18808989]

Gelman BB, Soukup VM, Schuenke KW, Keherly MJ, Holzer C 3rd, Richey FJ, Lahart CJ. Acquired neuronal channelopathies in HIV-associated dementia. Journal of Neuroimmunology. 2004; 157(1–2):111–9. [PubMed: 15579287]

Gonzalez E, Rovin BH, Sen L, Cooke G, Dhanda R, Mummidi S, Kulkarni H, Bamshad MJ, Telles V, Anderson SA, Walter EA, Stephan KT, Deucher M, Mangano A, Bologna R, Ahuja SS, Dolan MJ, Ahuja SK. HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. Proc Natl Acad Sci U S A. 2002; 99(21):13795–800. [PubMed: 12374865]

Gonzalez R, Cherner M. Co-factors in HIV neurobehavioural disturbances: substance abuse, hepatitis C and aging. Int Rev Psychiatry. 2008; 20(1):49–60. [PubMed: 18240062]

Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, Marcotte TD, Atkinson JH, Rivera-Mindt M, Vigil OR, Taylor MJ, Collier AC, Marra CM, Gelman BB, McArthur JC, Morgello S, Simpson DM, McCutchan JA, Abramson I, Gamst A, Fennema-Notestine C, Jernigan TL, Wong J, Grant I. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. Neurology. 2010; 75(23):2087–96. [PubMed: 21135382]

Heaton RK, Franklin DR, Ellis RJ, McCutchan JA, Letendre SL, Leblanc S, Corkran SH, Duarte NA, Clifford DB, Woods SP, Collier AC, Marra CM, Morgello S, Mindt MR, Taylor MJ, Marcotte TD, Atkinson JH, Wolfson T, Gelman BB, McArthur JC, Simpson DM, Abramson I, Gamst A, Fennema-Notestine C, Jernigan TL, Wong J, Grant I. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. J Neurovirol. 2011; 17(1):3–16. [PubMed: 21174240]

Hinkin CH, Castellon SA, Levine AJ, Barclay TR, Singer EJ. Neurocognition in individuals co-infected with HIV and hepatitis C. J Addict Dis. 2008; 27(2):11–7. [PubMed: 18681187]

Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, Leow AD, Dinov ID, Toga AW, Saykin AJ, Shen L, Foroud T, Pankratz N, Huentelman MJ, Craig DW, Gerber JD, Allen AN, Corneveaux JJ, Stephan DA, DeCarli CS, DeChairo BM, Potkin SG, Jack CR Jr, Weiner MW, Raji CA, Lopez OL, Becker JT, Carmichael OT, Thompson PM. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proc Natl Acad Sci U S A. 2010; 107(18):8404–9. [PubMed: 20404173]

Jaen A, Esteve A, Miro JM, Tural C, Montoliu A, Ferrer E, Riera M, Segura F, Force L, Sued O, Vilaro J, Garcia I, Masabeu A, Altes J, Coltet B, Podzamczer D, Murillas J, Navarro G, Gatell JM, Casabona J. Determinants of HIV progression and assessment of the optimal time to initiate highly active antiretroviral therapy: PISCIS Cohort (Spain). J Acquir Immune Defic Syndr. 2008; 47(2): 212–20. [PubMed: 18297762]

Jernigan TL, Gamst AC, Archibald SL, Fennema-Notestine C, Mindt MR, Marcotte TD, Heaton RK, Ellis RJ, Grant I. Effects of methamphetamine dependence and HIV infection on cerebral morphology. Am J Psychiatry. 2005; 162(8):1461–72. [PubMed: 16055767]

Khanlou N, Moore DJ, Chana G, Cherner M, Lazzaretto D, Dawes S, Grant I, Masliah E, Everall IP. Increased frequency of alpha-synuclein in the substantia nigra in human immunodeficiency virus infection. J Neurovirol. 2009; 15(2):131–8. [PubMed: 19115126]

Kraft-Terry SD, Buch SJ, Fox HS, Gendelman HE. A coat of many colors: neuroimmune crosstalk in human immunodeficiency virus infection. Neuron. 2009; 64(1):133–45. [PubMed: 19840555]

Laumet G, Chouraki V, Grenier-Boley B, Legry V, Heath S, Zelenika D, Fievet N, Hannequin D, Delepine M, Pasquier F, Hanon O, Brice A, Epelbaum J, Berr C, Dartigues JF, Tzourio C, Campion D, Lathrop M, Bertram L, Amouyel P, Lambert JC. Systematic analysis of candidate genes for Alzheimer's disease in a French, genome-wide association study. J Alzheimers Dis. 2010; 20(4):1181–8. [PubMed: 20413850]

Letendre S, Marquie-Beck J, Capparelli E, Best B, Clifford D, Collier AC, Gelman BB, McArthur JC, McCutchan JA, Morgello S, Simpson D, Grant I, Ellis RJ. Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system. Arch Neurol. 2008; 65(1):65–70. [PubMed: 18195140]

Levine AJ, Hardy DJ, Miller E, Castellon SA, Longshore D, Hinkin CH. The effect of recent stimulant use on sustained attention in HIV-infected adults. J Clin Exp Neuropsychol. 2006; 28(1):29–42. [PubMed: 16448974]

Levine AJ, Singer EJ, Shapshak P. The Role of Host Genetics in the Susceptibility for HIV-associated Neurocognitive Disorders. AIDS Behav. 2009a; 13(1):118–32. [PubMed: 18264751]

Levine AJ, Singer EJ, Sinsheimer JS, Hinkin CH, Papp J, Dandekar S, Giovanelli A, Shapshak P. CCL3 genotype and current depression increase risk of HIV-associated dementia. Neurobehav HIV Med. 2009b; 1:1–7. [PubMed: 20725607]

Levine AJ, Singer EJ, Sinsheimer JS, Hinkin CH, Papp J, Dandekar S, Giovanelli A, Shapshak P. CCL3 genotype and current depression increase risk of HIV-associated dementia. Neurobehavioral HIV Medicine. 2009; 1:1–7. [PubMed: 20725607]

Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34(8):816–34. [PubMed: 21058334]

Lin K, Taylor MJ, Heaton R, Franklin D, Jernigan T, Fennema-Notestine C, McCutchan A, Atkinson JH, Ellis RJ, McArthur J, Morgello S, Simpson D, Collier AC, Marra C, Gelman B, Clifford D, Grant I. Effects of traumatic brain injury on cognitive functioning and cerebral metabolites in HIV-infected individuals. Journal of Clinical and Experimental Neuropsychology. 2011; 33(3): 326–34. [PubMed: 21229435]

Martin-Thormeyer EM, Paul RH. Drug abuse and hepatitis C infection as comorbid features of HIV associated neurocognitive disorder: neurocognitive and neuroimaging features. Neuropsychol Rev. 2009; 19(2):215–31. [PubMed: 19468837]

Masliah E, DeTeresa RM, Mallory ME, Hansen LA. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. Aids. 2000; 14(1):69–74. [PubMed: 10714569]

McArthur JC. HIV dementia: an evolving disease. J Neuroimmunol. 2004; 157(1–2):3–10. [PubMed: 15579274]

Miller EN, Selnes OA, McArthur JC, Satz P, Becker JT, Cohen BA, Sheridan K, Machado AM, Van Gorp WG, Visscher B. Neuropsychological performance in HIV-1-infected homosexual men: The Multicenter AIDS Cohort Study (MACS). Neurology. 1990; 40(2):197–203. [PubMed: 2405289]

Moore DJ, Masliah E, Rippeth JD, Gonzalez R, Carey CL, Cherner M, Ellis RJ, Achim CL, Marcotte TD, Heaton RK, Grant I. Cortical and subcortical neurodegeneration is associated with HIV neurocognitive impairment. Aids. 2006; 20(6):879–87. [PubMed: 16549972]

Nath A, Hauser KF, Wojna V, Booze RM, Maragos W, Prendergast M, Cass W, Turchan JT. Molecular basis for interactions of HIV and drugs of abuse. J Acquir Immune Defic Syndr. 2002; 31(Suppl 2):S62–9. [PubMed: 12394784]

Navia BA, Jordan BD, Price RW. The AIDS dementia complex: I. Clinical features. Ann Neurol. 1986; 19(6):517–24. [PubMed: 3729308]

Noorbakhsh F, Ramachandran R, Barsby N, Ellestad KK, LeBlanc A, Dickie P, Baker G, Hollenberg MD, Cohen EA, Power C. MicroRNA profiling reveals new aspects of HIV neurodegeneration: caspase-6 regulates astrocyte survival. FASEB Journal. 2010; 24(6):1799–812. [PubMed: 20097875]

Parsons TD, Tucker KA, Hall CD, Robertson WT, Eron JJ, Fried MW, Robertson KR. Neurocognitive functioning and HAART in HIV and hepatitis C virus co-infection. Aids. 2006; 20(12):1591–5. [PubMed: 16868439]

Pemberton LA, Brew BJ. Cerebrospinal fluid S-100beta and its relationship with AIDS dementia complex. J Clin Virol. 2001; 22(3):249–53. [PubMed: 11564589]

Pemberton LA, Stone E, Price P, van Bockxmeer F, Brew BJ. The relationship between ApoE, TNFA, IL1a, IL1b and IL12b genes and HIV-1-associated dementia. HIV Med. 2008; 9(8):677–80. [PubMed: 18631256]

Pomara N, Belzer KD, Silva R, Cooper TB, Sidtis JJ. The apolipoprotein E epsilon4 allele and memory performance in HIV-1 seropositive subjects: differences at baseline but not after acute oral lorazepam challenge. Psychopharmacology. 2008; 201(1):125–35. [PubMed: 18668226]

Posthuma D, Neale MC, Boomsma DI, de Geus EJ. Are smarter brains running faster? Heritability of alpha peak frequency, IQ, and their interrelation. Behavior Genetics. 2001; 31(6):567–79. [PubMed: 11838534]

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38(8): 904–9. [PubMed: 16862161]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559–75. [PubMed: 17701901]

Quasney MW, Zhang Q, Sargent S, Mynatt M, Glass J, McArthur J. Increased frequency of the tumor necrosis factor-alpha-308 A allele in adults with human immunodeficiency virus dementia. Ann Neurol. 2001; 50(2):157–62. [PubMed: 11506397]

Qureshi AI, Hanson DL, Jones JL, Janssen RS. Estimation of the temporal probability of human immunodeficiency virus (HIV) dementia after risk stratification for HIV-infected persons. Neurology. 1998; 50(2):392–7. [PubMed: 9484360]

Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Applied Psychological Measurement. 1977; 1(1):385–401.

Reitan R. Validity of the trail making test as an indicator of organic brain damage. Percept Mot Skills. 1958; 8:271.

Rippeth JD, Heaton RK, Carey CL, Marcotte TD, Moore DJ, Gonzalez R, Wolfson T, Grant I. Methamphetamine dependence increases risk of neuropsychological impairment in HIV infected persons. J Int Neuropsychol Soc. 2004; 10(1):1–14. [PubMed: 14751002]

Roberts ES, Masliah E, Fox HS. CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE). J Neuropathol Exp Neurol. 2004; 63(12):1255–64. [PubMed: 15624762]

Robertson KR, Smurzynski M, Parsons TD, Wu K, Bosch RJ, Wu J, McArthur JC, Collier AC, Evans SR, Ellis RJ. The prevalence and incidence of neurocognitive impairment in the HAART era. AIDS. 2007; 21(14):1915–21. [PubMed: 17721099]

Rothlind JC, Greenfield TM, Bruce AV, Meyerhoff DJ, Flenniken DL, Lindgren JA, Weiner MW. Heavy alcohol consumption in individuals with HIV infection: effects on neuropsychological performance. Journal of the International Neuropsychological Society. 2005; 11(1):70–83. [PubMed: 15686610]

Sassoon SA, Fama R, Rosenbloom MJ, O'Reilly A, Pfefferbaum A, Sullivan EV. Component cognitive and motor processes of the digit symbol test: differential deficits in alcoholism, HIV infection, and their comorbidity. Alcohol Clin Exp Res. 2007; 31(8):1315–24. [PubMed: 17550370]

Selnes OA, Jacobson L, Machado AM, Becker JT, Wesch J, Miller EN, Visscher B, McArthur JC. Normative data for a brief neuropsychological screening battery. Multicenter AIDS Cohort Study. Percept Mot Skills. 1991; 73(2):539–50. [PubMed: 1766784]

Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carassquillo MM, Lambert JC, Harold D, Schrijvers EM, Ramirez-Lorca R, Debette S, Longstreth WT Jr, Janssens AC, Pankratz VS, Dartigues JF, Hollingworth P, Aspelund T, Hernandez I, Beiser A, Kuller LH, Koudstaal PJ, Dickson DW, Tzourio C, Abraham R, Antunez C, Du Y, Rotter JI, Aulchenko YS, Harris TB, Petersen RC, Berr C, Owen MJ, Lopez-Arrieta J, Varadarajan BN, Becker JT, Rivadeneira F, Nalls MA, Graff-Radford NR, Campion D, Auerbach S, Rice K, Hofman A, Jonsson PV, Schmidt H, Lathrop M, Mosley TH, Au R, Psaty BM, Uitterlinden AG, Farrer LA, Lumley T, Ruiz A, Williams J, Amouyel P, Younkin SG, Wolf PA, Launer LJ, Lopez OL, van Duijn CM, Breteler MM. Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA. 2010; 303(18):1832–40. [PubMed: 20460622]

Singh KK, Barroga CF, Hughes MD, Chen J, Raskino C, McKinney RE, Spector SA. Genetic influence of CCR5, CCR2, and SDF1 variants on human immunodeficiency virus 1 (HIV-1)-related disease progression and neurological impairment, in children with symptomatic HIV-1 infection. J Infect Dis. 2003; 188(10):1461–72. [PubMed: 14624371]

Singh KK, Ellis RJ, Marquie-Beck J, Letendre S, Heaton RK, Grant I, Spector SA. CCR2 polymorphisms affect neuropsychological impairment in HIV-1-infected adults. J Neuroimmunol. 2004; 157(1–2):185–92. [PubMed: 15579296]

Smith, A. Symbol digit modalities test. Los Angeles: Western Psychological Service; 1982.

Spector SA, Singh KK, Gupta S, Cystique LA, Jin H, Letendre S, Schrier R, Wu Z, Hong KX, Yu X, Shi C, Heaton RK. APOE epsilon4 and MBL-2 O/O genotypes are associated with neurocognitive impairment in HIV-infected plasma donors. AIDS. 2010; 24(10):1471–9. [PubMed: 20442634]

Stins JF, van Baal GC, Polderman TJ, Verhulst FC, Boomsma DI. Heritability of Stroop and flanker performance in 12-year old children. BMC Neurosci. 2004; 5:49. [PubMed: 15579206]

Swan GE, Carmelli D. Evidence for genetic mediation of executive control: a study of aging male twins. Journals of Gerontology Series B-Psychological Sciences & Social Sciences. 2002; 57(2): 133–43.

Taylor J. Heritability of Wisconsin Card Sorting Test (WCST) and Stroop Color-Word Test performance in normal individuals: implications for the search for endophenotypes. Twin Res Hum Genet. 2007; 10(6):829–34. [PubMed: 18179394]

Tozzi V, Balestra P, Lorenzini P, Bellagamba R, Galgani S, Corpolongo A, Vlassi C, Larussa D, Zaccarelli M, Noto P, Visco-Comandini U, Giulianelli M, Ippolito G, Antinori A, Narciso P. Prevalence and risk factors for human immunodeficiency virus-associated neurocognitive impairment, 1996 to 2002: results from an urban observational cohort. J Neurovirol. 2005; 11(3): 265–73. [PubMed: 16036806]

Valcour V, Shikuma C, Shiramizu B, Watters M, Poff P, Selnes OA, Grove J, Liu Y, Abdul-Majid KB, Gartner S, Sacktor N. Age, apolipoprotein E4, and the risk of HIV dementia: the Hawaii Aging with HIV Cohort. J Neuroimmunol. 2004a; 157(1–2):197–202. [PubMed: 15579298]

Valcour V, Shikuma C, Shiramizu B, Williams A, Watters M, Poff P, Grove J, Selnes O, Sacktor N. Diabetes, insulin resistance, and dementia among HIV-1-infected patients. J Acquir Immune Defic Syndr. 2005; 38(1):31–6. [PubMed: 15608521]

Valcour V, Shikuma C, Watters M, Sacktor N. Cognitive impairment in older HIV-1-seropositive individuals: prevalence and potential mechanisms. Aids. 2004b; 18(Suppl 1):S79–86. [PubMed: 15075502]

van Rij RP, Portegies P, Hallaby T, Lange JM, Visser J, de Roda Husman AM, van 't Wout AB, Schuitemaker H. Reduced prevalence of the CCR5 delta32 heterozygous genotype in human immunodeficiency virus-infected individuals with AIDS dementia complex. J Infect Dis. 1999; 180(3):854–7. [PubMed: 10438379]

Wojna V, Skolasky RL, Hechavarria R, Mayo R, Selnes O, McArthur JC, Melendez LM, Maldonado E, Zorrilla CD, Garcia H, Kraiselburd E, Nath A. Prevalence of human immunodeficiency virus-associated cognitive impairment in a group of Hispanic women at risk for neurological impairment. J Neurovirol. 2006; 12(5):356–64. [PubMed: 17065128]

Woods SP, Moore DJ, Weber E, Grant I. Cognitive neuropsychology of HIV-associated neurocognitive disorders. Neuropsychol Rev. 2009; 19(2):152–68. [PubMed: 19462243]

Woods SP, Rippeth JD, Frol AB, Levy JK, Ryan E, Soukup VM, Hinkin CH, Lazzaretto D, Cherner M, Marcotte TD, Gelman BB, Morgello S, Singer EJ, Grant I, Heaton RK. Interrater reliability of clinical ratings and neurocognitive diagnoses in HIV. J Clin Exp Neuropsychol. 2004; 26(6): 759–78. [PubMed: 15370374]

Zheng J, Gendelman HE. The HIV-1 associated dementia complex: a metabolic encephalopathy fueled by viral replication in mononuclear phagocytes. Curr Opin Neurol. 1997; 10(4):319–25. [PubMed: 9266156]

Zhou L, Rua R, Ng T, Vongrad V, Ho YS, Geczy C, Hsu K, Brew BJ, Saksena NK. Evidence for predilection of macrophage infiltration patterns in the deeper midline and mesial temporal structures of the brain uniquely in patients with HIV-associated dementia. BMC Infect Dis. 2009; 9:192. [PubMed: 19951441]

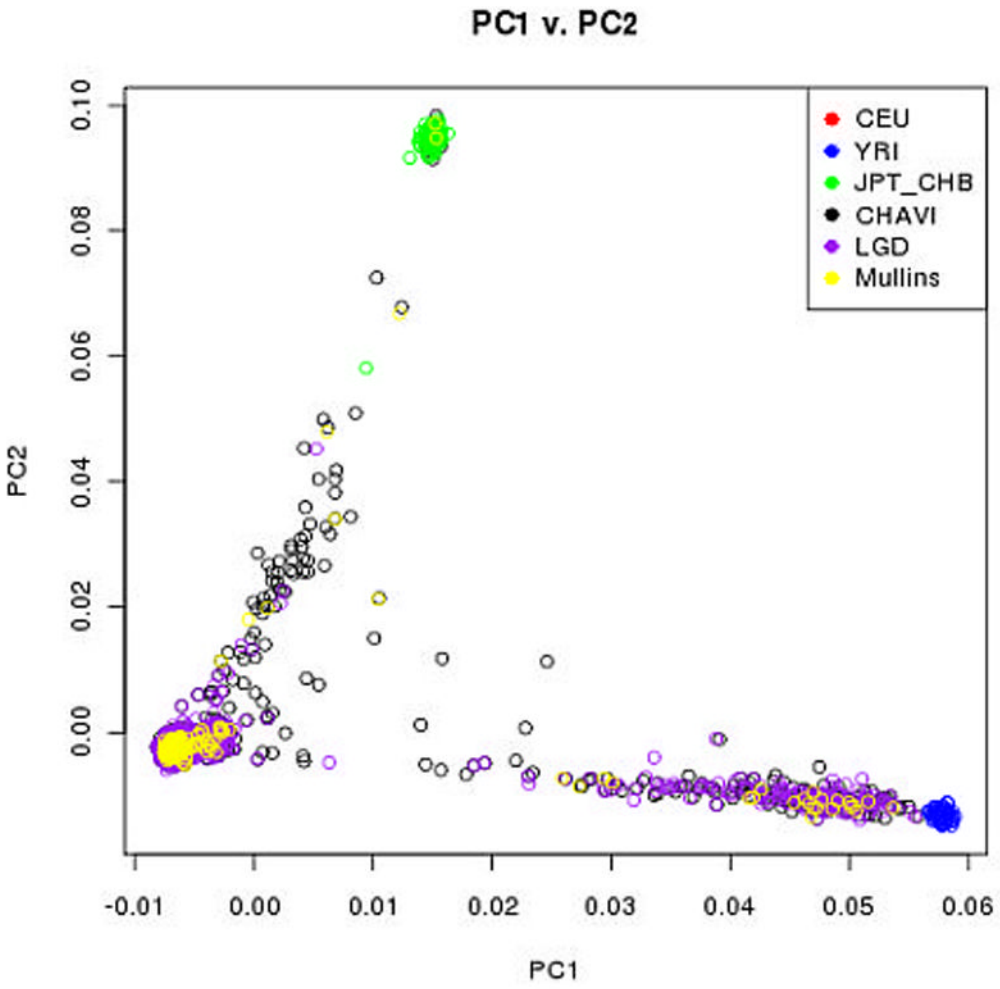

Figure 1.

First two principal components (PC) from an engine-analysis of the IBS matrix, based on samples from HapMap (www.hapmap.org). CEU is a sample of Northern European origin, YRI are Yorubans from Nigeria, JPT_CHB are samples of Japanese and Han Chinese, respectively. In our PC analysis, data for CEU are completely obscured by data from CHAVI, LGD and Mullins, in the bottom let corner of the plot Samples of Northern European (NE) ancestry were defined as samples with PC1<0.01 and PC2<0.01. Samples of African (A) ancestry were defined as samples with PC1>0.025.

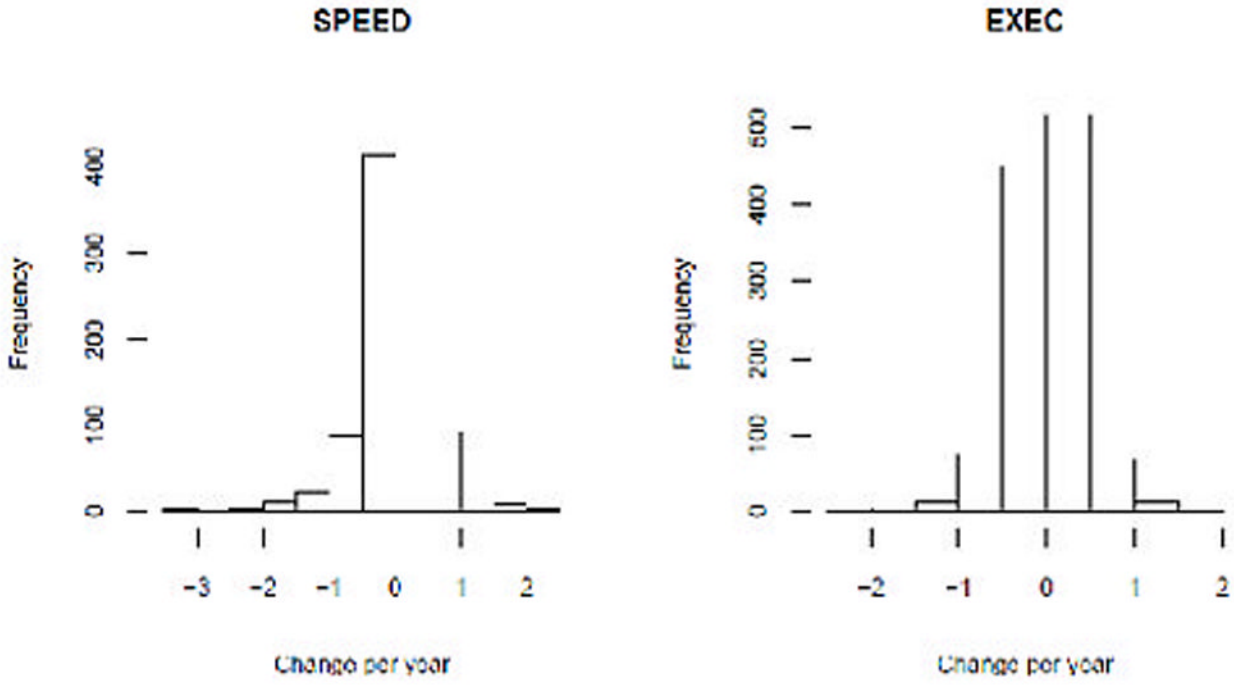

Figure 2.
Distribution of subject-specific slopes for SPEED and EXEC. The x-axis represents the change in SPEED or EXEC per year.

Table 1

Sample Characteristics (N = 1287)

Characteristic	Summary Statistics	
Mean (SD) age (years)*	45.9(9.8)	
Education	Number of Years	Percent
	<12	1.53%
	12	10.7%
	13–15	31.8%
	16	25.3%
	17+	30.6%
Mean (SD) years of HIV infection*	11.8(7.7)	
Mean (SD) Nadir CD4+ T-cell/mm^3	183.5(166)	
Percent with moderate to severe substance abuse**	First visit: 5.4%	
	Final visit: 2.3%	

*At final visit

**Cocaine and methamphetamine only

Table 2

Neuropsychological Measures

Neuropsychological Measures	Cognitive Domain	Heritability (%)
Rey Auditory Verbal Learning Test (Rey, 1941)	Verbal Learning & Memory	56 * (Swan et al., 1999)
Rey-Osterrieth Complex Figure Test (Osterrieth, 1944; Rey, 1964)	Visual Learning & Memory	--
Stroop Color Naming (Comalli et al., 1962)	Processing Speed	53–70 (Stins et al., 2004; Taylor, 2007)
Symbol Digit Modalities (Smith, 1982)	Processing Speed	76 * (Posthuma et al., 2001)
Grooved Pegboard (Klove and Matthews, 1966)	Motor	--
Stroop Interference(Comall I et al., 1962) **	Executive Functioning	57–74 (Stins et al., 2004; Taylor, 2007)
Trail Making Test – Part B (Reitan, 1958)	Executive Functioning	34–68 (Swan and Carmelli, 2002)
CalCAP (Miller, 1990)	Sustained Attention	49–89 * (Fan et al., 2001)

* statistic is based on a similar but not identical test

** heritability based on Interference trial score rather than Stroop effect

Table 3

Summary of association results. Lambda (λ) is the estimate of the genomic control parameter.

Phenotype	# SNPs Imputed in all data sets (λ)	# SNPs Genotyped in all data sets (λ)	Total SNPs* (λ)	Minimum meta-analysis p-value	# SNPs in meta-analysis with $p<10^{-5}$	# SNPs in meta-analysis with $p<10^{-6}$ (see also Table 7)
SPEED	1,450,605 (1.006)	206,004 (0.988)	2,496,111 (1.005)	1.15×10^{-7}	69	10
EXEC	1,450,605 (1.006)	206,172 (0.982)	2,496,307 (1.006)	1.35×10^{-6}	27	0
HAD	1,446,376 (1.066)	206,949 (0.899)	2,492,131 (1.032)	2.25×10^{-6}	9	0
NCI	1,448,490 (1.009)	206,949 (0.926)	2,494,696 (0.999)	8.35×10^{-7}	29	1

*The total number of SNPs is more than the sum of imputed and genotyped SNPs, as there are SNPs imputed in one data set and genotyped in the other that are not tallied in the first two columns.

Table 4

SNPs with $p<10^{-6}$

							Meta Analysis			CHAVI				LGD + Mullins			
Phenotype	**Chromosome**	**Base pairs**	**Marker Name**	**Gene†**	**A1***	**A2***	**Total N**	**Z-score**	**P value**	**N**	**Beta****	**P value**	**I/G*****	**N**	**Beta****	**P value**	**I/G*****
NCI	1	83262432	rs12742923		T	C	1287	4.927	8.36E-07	635	1.613	1.08E-02	I	652	2.223	1.05E-05	I
SPEED	2	40249582	rs404005	SLC8A1	T	C	1124	−5.105	3.31E-07	568	−0.124	4.13E-05	I	556	−0.110	1.85E-03	G
SPEED	10	43796113	rs2279553		A	C	1131	5.045	4.54E-07	568	0.608	4.59E-06	I	563	0.494	1.09E-02	I
SPEED	10	43796137	rs2279554		T	C	1131	5.124	2.99E-07	568	0.601	3.83E-06	I	563	0.499	8.74E-03	I
SPEED	10	43812051	rs17154842		A	G	1131	5.149	2.62E-07	568	0.590	3.42E-06	I	563	0.496	8.46E-03	I
SPEED	10	43832559	rs10508879		T	C	1131	−5.292	1.21E-07	568	−0.602	1.27E-06	I	563	−0.496	8.44E-03	I
SPEED	10	43839414	rs17154903		T	C	1131	5.275	1.32E-07	568	0.598	1.32E-06	G	563	0.496	8.80E-03	G
SPEED	10	43844681	rs17154929		T	C	1131	5.301	1.15E-07	568	0.598	1.18E-06	I	563	0.496	8.48E-03	I
SPEED	10	43864581	rs1114215		A	T	1131	−5.285	1.26E-07	568	−0.599	1.14E-06	I	563	−0.492	9.22E-03	I
SPEED	10	43864732	rs1114216		A	C	1131	−5.267	1.39E-07	568	−0.599	1.14E-06	I	563	−0.486	9.94E-03	I
SPEED	13	100798946	rs7996217	NALCN	T	G	1130	4.972	6.62E-07	568	0.081	3.11E-01	I	562	3.351	1.62E-09	G

*A1 is the effect allele, A2 is the alternative allele

**Beta is the effect size. For phenotype NCI Beta is an odds ratio, and reflects the change in odds for having allele A1 vs. A2. For SPEED, Beta is the regression coefficient, and reflects the change in SPEED for each additional copy of allele A1

***Indicates if the marker was imputed (I) or genotyped (G) in the CHAVI or LGD + Mullins samples

†Where no gene is listed, these SNPs likely fall within intergenic regions.

Table 5

Fixed effects results for linear mixed models of SPEED and EXEC

	SPEED			EXEC		
Co-Variate	**Value***	**t-value**	**p-value**	**Value***	**t-value**	**p-value**
Intercept	51.124	63.565	<0.0001	55.424	71.418	<0.0001
Time	−0.125	−2.286	0.0223	0.003	0.074	0.9409
Depression (CES-D)	−0.143	−8.561	<0.0001	−0.100	−6.485	<0.0001
Alcohol use	0.002	2.299	0.0215	−0.002	−1.711	0.087
Substance use	−1.787	−1.315	0.1884	−0.205	−0.166	0.8682
Nadir CD4	0.006	4.674	<0.0001	0.002	1.635	0.102
Hepatitis C	−0.199	−0.164	0.8694	−1.132	−0.988	0.3233
Monotherapy vs. None	−1.253	−3.015	0.0026	0.228	0.595	0.5517
Combination/Potent vs. None	0.051	0.127	0.8988	1.641	4.460	<0.0001

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Table 6

Validation of candidate SNPs associated with HIV-related neurocognitive phenotypes in past studies

Study	Gene/SNP	Disease-related allele or genotype	Cognitive Phenotype	Findings	Other SNPs (genes) examined, with null results
Levine et al., (2009)	MIP1-α (CCL3)/rs1130371	T/T	HIV-associated dementia (AAN)	Compared only homozygous groups. Those homozygous for T allele had two-fold greater risk of dementia	rs1024611 (MCP-1), rs1719130 (CCL5), rs17561 (IL-1α), rs1800872 (IL-10), rs1800629 (TNF-α), rs1801157 (SDF-1)
Quasney et al., (2001)	TNF-α/rs1800629	A	HIV-associated dementia (diagnostic method not clear)	A allele over-represented among those with dementia	None
Spector et al.,(2010)	MBL-2/rs1800450, rs1800451, rs5030737	O/O	Change in status from neurocognitively normal to impaired, as determined by comprehensive neuropsychological testing and cutoff on Global Deficit Score	52% of patients with the O/O genotype declined in cognitive function over 12 months compared with 23% with A/A	rs429358 & rs7412 (ApoE), rs333 (CCR5). CCR5 SNP 59029-G/A; CCR2-180-G/A; rs1801157 (SDF); IL4-589-C/T; rs1024611 (MCP-1); CX3CR1-745-G/A; and CCL3L1 copy number variants
Gonzalez et al., (2002)	MCP-1 (CCL2)/rs1024611	G/G	HIV-associated dementia (criteria unclear – Site anonymous articles)	Homozygosity for G allele associated with 4.7 fold greater risk of dementia	SNPs rs1024611 and another at −2136 defined three haplotypes, designated as AA, GA, and AT, and six haplotype pairs. GA/GA was associated with HAD.
Singh et al., (2003)	SDF-1 (CXCL12)/rs1801157	A/A	Rate of progression to neurocognitive impairment as determined by comprehensive neuropsychological testing and cutoff score on Global Clinical Rating Scale	Homozygosity for A allele associated with faster progression to neurocognitive impairment	rs1799864 (CCR2), rs333 (CCR5), and SNPs on CCR5 located designated as 59029-G/A, 59353-T/C, and 59356-C/T
Bol et al. (2012)	Prep1 rs2839619		HIV-associated dementia (Using various diagnostic systems)	Prep 1 genotype significantly differed between controls and those with HAD	rs1799864 (CCR2), rs333 (CCR5), rs429358 & rs7412 (ApoE), rs1024611 (MCP-1), rs1130371 (MIP-1α), rs1800629 (TNF-α)

Table 7

Impairment status by MBL-2 haplotype

Impairment status	0 copies mutant haplotype	1 copy mutant haplotype	2 copies mutant haplotype
not impaired	9	71	102
Impaired	23	134	246