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Multiple Loci Influencing Hippocampal Degeneration Identified by Genome Scan

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Abstract

Objective—Large genome-wide association studies (GWAS) have identified many novel genes influencing Alzheimer disease (AD) risk, but most of the genetic variance remains unexplained. We conducted a two-stage GWAS for AD-related quantitative measures of hippocampal volume (HV), total cerebral volume (TCV), and white matter hyperintensities (WMH).

Methods—Brain MRI measures of HV, TCV and WMH were obtained from 981 Caucasian and 419 African American AD cases and their cognitively normal siblings in the MIRAGE Study, and from 168 AD cases, 336 individuals with mild cognitive impairment and 188 controls in the ADNI Study. A GWAS for each trait was conducted in the two Caucasian datasets in stage 1. Results from the two datasets were combined by meta analysis. In stage 2, one SNP from each region that was nominally significant in each dataset ($p<0.05$) and strongly associated in both datasets ($p<1.0\times10^{-5}$) was evaluated in the African American dataset.

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Results—Twenty-two markers (14 for HV, 3 for TCV, and 5 for WMH) from distinct regions met criteria for evaluation in stage 2. Novel genome-wide significant associations ($p<5.0\times10^{-8}$) were attained for HV with SNPs in the *APOE, F5/SELP, LHFP* and *GCFC2* gene regions. All of these associations were supported by evidence in each dataset. Associations with different SNPs in the same gene ($p<1\times10^{-5}$ in Caucasians and $p<2.2\times10^{-4}$ in African Americans) were also observed for *PICALM* with HV, *SYNPR* with TCV and *TTC27* with WMH.

Interpretation—Our study demonstrates the efficacy of endophenotypes for broadening our understanding of the genetic basis of AD.

INTRODUCTION

Difficulties in the search for susceptibility genes for Alzheimer disease (AD) have been attributed to the etiological heterogeneity of the clinically defined disease phenotype.[1] Genome-wide association studies (GWAS) using very large samples have increased the number of robust associations to ten genes including APOE,[1–3] however these loci account for no more than 35% of the inherited risk of AD.[3] Heritable AD-related endophenotypes obtained by magnetic resonance imaging (MRI) provide in vivo measures of neurodegenerative and cerebrovascular brain injury and can serve as intermediate phenotypes for genetic studies of AD.[4] The heritability for hippocampal volume (HV) and white matter hyperintensities (WMH) are 0.40 and 0.73, respectively, among elderly male twins,[5,6] and MRI measures of cerebrovascular disease and neurodegeneration are highly heritable in AD families.[4]

Candidate gene studies have revealed genetic associations with several AD-related MRI traits. Cortical structural changes are associated with specific *APOE* genotypes among non-demented elderly, as ε4 carriers have demonstrably smaller hippocampal volumes than non-ε4 carriers.[7] One study observed a correspondence of SNPs and haplotypes from the two AD-associated regions of *SORL1* with MRI and neuropathological measures of WMH and HV.[8] Association of HV with several variants and haplotypes in the *TTR* gene has also been reported.[9] Genome-wide association studies of structural and volumetric changes[10–13] and using a voxel-based approach[14] confirmed SNPs in the *APOE* and *TOMM40* genes as markers strongly associated with multiple brain regions including the amygdala and hippocampus, and yielded promising findings ($p<10^{-6}$) with several other genes (reviewed in [15]).

In this paper, we report results from a GWAS for three AD-related MRI measures in a multi ethnic sample.

METHODS

Subjects

One group of subjects are participants of the Multi Institutional Research in Alzheimer's Genetic Epidemiology (MIRAGE) Study, a family-based genetic epidemiological study of AD described in detail elsewhere.[16] A second sample was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) Study. Brain imaging, biological samples, and clinical assessments were longitudinally collected for healthy controls and patients with mild cognitive decline (MCI) and AD. Details regarding subject ascertainment and evaluation have been previously described (http://www.adni-info.org).[17] Study protocols were approved by Institutional Review Boards at each recruitment site. Characteristics of the ADNI and MIRAGE subjects included in this study are summarized in Table 1.

MRI traits

In the MIRAGE sample, MR images of the brain were obtained with 1.5 Tesla magnetic field strength scanners using a standard protocol of 3D T1-weighted high resolution sequence, a double spin-echo sequence and a FLAIR sequence. Semi-quantitative measures of bilateral medial temporal (hippocampal) volume (HV), total cerebral volume (TCV) and white matter hyperintensities (WMH) were obtained from these scans and using methods previously described which account for total intracranial volume.[18] These measures were designed to be simple to use and have been shown to linearly correlate with image quantification.[19] MR scans were evaluated by a single rater blinded to age, gender, and affection status to reduce inter-rater variability common to semi-quantitative methods. TCV and WMH were rated on a scale of 0 to 100. HV was rated on an ordinal scale of 0 to 4 according to previously described methods.[20] TCV and HV were scored as damage expressed as a percentage of the overall brain volume. WMH scores were log10 transformed.

Brain MRI scans (1.5 Tesla) were obtained from ADNI subjects as described elsewhere.[21] Available longitudinal scans were also analyzed (supplementary Table 1). TCV and HV were measured at the baseline visit and 12 months later using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/), an open-source program that converts MRI data into volumetric measures.[11] WMH volume was measured at 0, 6, 12, 18, 24 and 36 months. In order to obtain a normal distribution for analysis, an adjustment value of 0.5 was added and then log10 transformed.

Genotyping, Quality Control Procedures, and Imputation

ADNI participants and approximately two-thirds of the MIRAGE subjects were genotyped on the Illumina Infinium Human 610-Quad BeadChip. The remaining MIRAGE subjects were genotyped with the Illumina Infinium HUmanCNV370-Duo BeadChip. Genotyping for the *APOE* ε2/ε3/ε4 alleles was performed in the MIRAGE dataset using a Roche diagnostics LightCycler® 480 instrument (Roche). APOE genotypes in the ADNI cohort were obtained by pyrosequencing or restriction fragment length polymorphism analysis.

Prior to analysis, SNPs with a call rate less than 98%, with a minor allele frequency (MAF) less than 0.05 or not in Hardy-Weinberg equilibrium ($p<10^{-6}$) among unaffected, unrelated individuals were excluded. We excluded individual samples with SNP call rates below 98% among the remaining SNPs, or whose gender as determined by analysis of X-chromosome data (performed using PLINK[22]) was inconsistent with the reported gender. Per-SNP and per-subject call rates were determined within subsets of MIRAGE individuals genotyped on each chip, as well as among all MIRAGE subjects. Individuals and SNPs in MIRAGE were filtered separately based on quality measurements, and then population stratification was checked in each MIRAGE dataset using SNPs that were common across chips. PLINK was used to identify cryptic relatedness within each dataset. All relationships were examined using PREST and corrected if possible when necessary.[23] Subjects whose relationship could not be resolved were dropped from further analysis. Population of origin was confirmed using STRUCTURE in the MIRAGE dataset and EIGENSTRAT in the ADNI dataset.[24,25] Principal component (PC) analysis implemented in EIGENSTRAT was used to evaluate population substructure within each dataset.

Imputation of genotypes for autosomal SNPs was performed using the Markov Chain Haplotyping (MaCH) software based on the HapMap 2 and 3 reference SNP panels for Caucasians and Yorubans (http://hapmap.ncbi.nlm.nih.gov/).[26] We excluded SNPs with low MAF (<1%), not in HWE ($p <10^{-6}$), and with potential for undetected strand flips (C/G and A/T coding) to ensure consistency of allele frequencies between the test and reference

haplotypes and to improve the quality of imputation. Within each dataset, only SNPs imputed with $r^2 \geq 0.80$ were included in analysis.

GWAS Design and Statistical Methods

The GWAS was carried out in two stages. The MIRAGE Caucasian families and Caucasian subjects in ADNI were included in the first stage and the MIRAGE African American families were added in Stage 2. SNPs were excluded from analyses if the MAF was below 5%. Imputed SNPs were tested for association with each MRI trait using generalized estimating equations assuming an additive genetic model. A quantitative estimate between 0 and 2 representing the dose of the minor allele was assigned to the SNP variable. Regression models also included terms for age at the time of the MRI scan, sex and the first three principal components. The models were not adjusted for disease status so that association signals were not diluted by the correlation of disease status with MRI measures. In the ADNI dataset, hippocampal and cerebral volume measures were also adjusted for total intracranial volume to account for differences in head size. Multiple values for MRI measures in ADNI were treated as sequential repeated measures. Generalized estimating equations implemented in the GEEPACK package within the R statistical programming language (version 12.2.1)[27] were used to account for the MIRAGE family-based design and repeated measures in the ADNI dataset. SNP association results obtained from the ADNI and MIRAGE datasets were combined by meta-analysis using the z-score method in METAL.[28]

In the Stage 1 analysis, SNPs attaining a meta-analysis p-value $<1\times10^{-5}$ and at least nominal significance ($p<0.05$) in both Caucasian datasets were identified as threshold SNPs. In instances where multiple correlated SNPs ($r^2>0.8$) met threshold criteria, the most significant SNP was included in Stage 2. For SNPs located within a gene, all SNPs within the coding, intronic and promoter regions were included in follow up investigation in the African American sample. Otherwise, if a threshold SNP was located within 100 kb of a gene, then the follow-up region was extended from the SNP position to and including the nearest gene. Primary association tests were performed for threshold SNPs in the African American families with the same model used for the Caucasian families. Secondary analyses were carried out for all other SNPs in the defined regions to allow for lack of polymorphism in the threshold SNPs and different haplotype structures between Caucasians and African Americans. Results from stages 1 and 2 were combined using METAL.

RESULTS

Genotypes were evaluated for 2,131,250 imputed SNPs that passed MAF and imputation quality thresholds in the ADNI (n=692) and MIRAGE Caucasian (n=991) datasets. We observed only modest levels of genomic control inflation for any of the traits in either Caucasian dataset (maximum $\lambda = 1.045$ for HV in ADNI), suggesting that the GWAS results were not impacted by population stratification (supplementary Fig 1). Meta-analysis of results from the two discovery cohorts revealed genome-wide significant association between HV and SNPs spanning the *F5* and *SELP* genes (best result with *F5* SNP rs3917836, $p=5.53\times10^{-9}$), and with several variants in the *APOE* region (best result with *APOE*, $p=5.23\times10^{-31}$) (Fig 1, supplementary Fig 2).

A total of 99 SNPs in 14 distinct regions met threshold criteria for association with HV (supplementary Table 2). Threshold SNPs in *F5, GCFC2,* and *LHFP* showed nominal evidence of association in the African American sample, and were genome-wide significant in the combined (stages 1+2) sample (Table 2). The threshold SNP in *COL18A1* (rs2838923) was not significantly associated with HV in the African Americans, but the effect direction was the same as in Caucasians and the association with this SNP was more

significant after combining the results from both populations ($p=7.94\times10^{-7}$). *APOE* ε4 was associated with HV in African Americans ($p=2.75\times10^{-4}$) leading to an even more significant result in the combined sample ($p=1.58\times10^{-33}$). Other notable results in the African Americans were observed with non-threshold SNPs in *SELP* (rs3917854, $p=5.70\times10^{-6}$), *NKAIN2* (rs7773205, $p=2.14\times10^{-4}$) and *PICALM* (rs17148741, $p=9.4\times10^{-5}$). *SELP* SNP rs3917854 is 8,072 base pairs from the genome-wide significant SNP in *F5.* There was also strong evidence of association with HV in Caucasians with SNPs in intergenic regions on chromosomes 1 (rs2942354, $p=4.71\times10^{-7}$) and 9 (rs11139399, $p=6.67\times10^{-7}$).

Further scrutiny of the results in the four regions showing genome-wide significance with HV revealed strong corroborative evidence with other SNPs in *F5/SELP, LHFP,* and in the *APOE* region (Fig 1). Numerous SNPs within a 40 kb region spanning the proximal portion of *F5* and distal portion of *SELP* were also significant. These 19 SNPs are in very high linkage disequilibrium ($r^2>0.95$) in both Caucasians and African Americans with consistent effect directions, and are all intronic except for rs1018828 ($p=1.49\times10^{-9}$) within the ~2,500 base pair intergenic region (Fig 2). Six *LHFP* SNPs spanning a 30 kb region in intron 2 approached or exceeded genome-wide significance. The evidence for association of HV with *GCFC2* is derived from a solitary imputed SNP (rs2298948). The imputation quality of rs2298948 was high ($r>0.99$), the minor allele was relatively common in both Caucasians (average MAF=0.31) and African Americans (MAF=0.08), and there was evidence for association in all three datasets. Rs2298948 was moderately correlated with one adjacent SNP (rs7560262, $r^2=0.43$) and less correlated with all other SNPs in this region ($r^2<0.4$).

APOE ε4 was also very strongly associated with TCV in each of the Caucasian datasets yielding a genome-wide significant result in the combined groups ($p=4.25\times10^{-10}$), but not in the African American dataset (Table 2). In fact, the pattern of effect was opposite in the African American sample. SNPs in two regions associated with TCV and five regions associated with WMH met threshold criteria for further analysis (supplementary Table 2). None of the threshold SNPs for either trait attained genome-wide significance in the combined sample (Table 2). However, results for alternate SNPs in the African American sample gave strong support for association of TCV with *SYNPR* (rs935793, $p=7.12\times10^{-5}$) and for association of WMH with *TTC27* (rs3769573, $p=2.18\times10^{-4}$).

DISCUSSION

Our two-stage genome-wide association study identified highly significant associations between several loci and three brain MRI AD-related traits in two Caucasian samples and one African American sample containing AD, cognitively impaired, and cognitively healthy subjects. Genome-wide significant results were obtained for HV with SNPs in four gene regions including *APOE, F5/SELP, LHFP* and *GCFC2.* All of these associations were supported by evidence in each dataset. However, the *GCFC2* finding is less certain because it is based on evidence with only a single SNP. Noteworthy associations ($p<1\times10^{-6}$ in Caucasians and $p\leq2.18\times10^{-4}$ in African Americans) were also observed with different SNPs in the same gene for *SYNPR* with TCV and for *TTC27* with WMH. With the exception of *APOE,* there are no reports of association of any of these genes with AD in any studies including very large GWAS.[2,3]

All of the genome-wide significant results were found for HV only. Hippocampal atrophy is well-known to occur early in the disease and is correlated with impaired memory function as well as neurofibrillary tangle density in the hippocampus.[29,30] Previously, we demonstrated in the MIRAGE study that hippocampal atrophy is highly heritable,[4] may be a marker of subclinical disease,[18] and is associated with particular 3-SNP haplotypes in *SORL1* and

TTR in Caucasians.[8,9] In this much larger dataset, in Caucasians there was only weak evidence of association of HV with individual *SORL1* and *TTR* SNPs (most significant p-values ~ 0.01). However, in African Americans, five *SORL1* SNPs were associated with HV at $p<1.8\times10^{-3}$ (most significant SNP: rs4420280, $p=1.70\times10^{-5}$). A prior GWAS of HV in the ADNI study identified sub-genome-wide significant ($1\times10^{-7}<p<1\times10^{-5}$) associations with SNPs in *TOMM40, CAND1, MAG12, ARSB, PRUNE2* and *EFNA5*, but that study lacked a replication sample.[12] Of these loci, only *TOMM40* was significant in our meta analysis of the ADNI and MIRAGE datasets. It is important to note that *F5/SELP, LHFP* and *GCFC2*, were weakly associated with AD risk in a large GWAS including 7–8 fold more subjects in the discovery sample than in this study (most significant results: *F5,* rs6035, p=0.15; *SELP,* rs3917687, p=0.053; *LHFP,* rs7333587, p=0.022; *GCFC2,* rs17741889, p=0.032).[3] Two recent large GWAS of HV in adults of European ancestry identified genome-wide significant association with intergenic SNP rs7294919 located between *HRK* and *FBXW8* in chromosome 12q24.[31,32] One of these studies comprising prospectively followed population-based cohorts also reported genome-wide significant results with chromosome 12q14 SNPs rs17178006 in *MSRB3* and rs6581612 in *WIF1*.[31] These findings suggest that genes influencing hippocampal changes concomitant with AD are different from those associated with aging per se.

Extremely strong evidence of association ($p=1.58\times10^{-33}$) was identified between HV and *APOE* ε4, a well-established AD risk factor and known determinant of the rate of HV.[33,34] The ε4 allele was also very strongly associated with TCV in Caucasians ($p=4.25\times10^{-10}$), a measure that is highly correlated with HV in Caucasians (ADNI: r=0.54, MIRAGE: r=0.64), but not with TCV in African Americans (r=0.16). Highly significant results with adjacent SNPs are unlikely to be evidence of independent contributions from genes other than *APOE* because of very strong linkage disequilibrium in this region.[35] Of note, there was very little evidence of association between *APOE* ε4 and WMH suggesting that *APOE* contributes more substantially to AD-related neuronal loss than to cerebrovascular mechanisms associated with AD. This idea is consistent with the preponderance of evidence suggesting that apoE4 has a direct role in production or clearance of amyloid β (Aβ), potentiating Aβ-induced lysosomal leakage and/or activating the endoplasmic reticulum stress response, leading to increased apoptosis.[36]

We also observed genome-wide significant association of HV with 24 SNPs spanning the proximal portion of *F5* and the distal portion of *SELP.* Support for the most significant SNP in this region (rs6703865 in *F5*) was derived primarily from the Caucasian datasets ($p=6.58\times10^{-9}$), whereas the most significant SNP in the African dataset (rs3917854, $p=5.70\times10^{-6}$) is located 8,072 base pairs away in *SELP.* In light of the high LD among the top-ranked SNPs in this region in both Caucasians and African Americans (Fig 2), it is not possible to ascertain whether the functional variant underlying the association peak is in *F5* or *SELP,* or perhaps there are functional variants in both genes.

F5 encodes Factor V which is an essential cofactor of the blood coagulation cascade and functions to allow Factor Xa to activate thrombin. Recently, Sherva et al. observed significant association of *F5* SNP rs2213865 with rate of cognitive decline among 331 AD cases in ADNI ($p=3.02\times10^{-7}$),[37] but this SNP is located more than 30 kb from the margin of and in a different haplotype block from our association peak (Figure 2). Factor V Leiden, a G169A mutation (rs6025), prevents efficient activation of factor V leading to overproduction of thrombin and excess clotting. This mutation is relatively uncommon in the general population (4% in Caucasians and < 1% in African Americans). In the Rotterdam Study, carriers of this mutation had a significantly increased risk of vascular dementia (OR=4.28, 95% CI=1.26–14.5) and an elevated risk of AD (OR=2.15, 95%

CI=0.82–5.63).[38] Rs6025, located approximately 10 kb from the edge of our association peak for HV, was not associated with HV in our sample (Fig 2).

P-selectin encoded by *SELP*, is a 140 KDa granule membrane protein that mediates the interaction of activated endothelial cells or platelets with leukocytes. Stellos et al. observed significantly higher baseline blood levels of P-selectin in AD patients with fast cognitive decline compared to AD with slow cognitive decline during a 1-year follow-up period.[39] In another 2-year follow-up study of 72 AD patients and 6 controls, P-selectin levels were decreased in AD and lowest in AD patients with the highest cognitive decline.[40] These findings suggest that P-selectin may induce alterations of endothelial regulation and thereby influence AD-related neurodegenerative processes.

Synaptoporin is a synaptic membrane protein of synaptic vesicles and a member of the synaptophysin family which is involved in uptake, storing, docking and regulating release of neurotransmitters. The synaptoporin gene (*SYNPR)* encodes a highly conserved protein, has two known splicing variants, and is specifically expressed in the brain.[41] Clathrin-mediated endocytosis is the major mechanism of vesicle retrieval after neurotransmitter release in the hippocampus.[42] Notably, both *SYNPR* and *PICALM* (phosphatidylinositol-binding clathrin assembly protein) are involved in clathrin-mediated endocytosis,[41,43] and in our study showed significant association of both loci with MRI measures of cerebral degeneration.

The function of *GCFC2* is relatively unknown, but a haplotype for this GC-rich sequence DNA-binding factor gene has been associated with dyslexia in a set of Finnish families.[44] Similarly, little is known about the Lipoma HMGIC fusion partner gene (*LHFP*) which encodes a tetraspan transmembrane protein, or *TTC27* which is a member of proteins containing a tetratricopeptide repeat domain. Mutations in another *LHFP*-like gene result in deafness in humans and mice.[45,46]

Among the nine novel GWAS genes for AD risk,[2,3] we detected strong evidence of association of HV and *PICALM* in both Caucasians and African Americans, albeit with different top-ranked SNPs in the two populations These SNPs also differ from the top-ranked SNPs in AD GWAS in Caucasians and African Americans.[2,3,47] Discordance in the association patterns between Caucasians and African Americans could be related to population differences in allele frequencies or LD patterns. Alternatively, the AD risk variants in these genes may differ across populations (i.e., allelic heterogeneity) as we observed previously in *SORL1*.[48]

Results of our study should be interpreted cautiously. The MIRAGE and ADNI studies differ in multiple ways. The MIRAGE cohort contains AD subjects and cognitively normal siblings whereas the ADNI cohort includes unrelated subjects, approximately one-half of whom have MCI. Hippocampal and total cerebral volumes in the MIRAGE subjects are semi-quantitative measures whereas more precise quantitative measures were obtained for the ADNI subjects. Thus, effect sizes could not be estimated for the combined group of subjects. In addition, the sample size of the study is relatively small for a GWAS. However, we were able to increase the effective sample by capturing the phenotypic information obtained from ADNI subjects at multiple examinations. We enhanced the power of the sample by using quantitative outcome measures which are more precise and more likely to be directly influenced by specific genes than a complex disease outcome. This is exemplified by the top-ranked findings for *PICALM* which attained p-values of 4.75×10^{-6} and 9.39×10^{-5} in the Caucasian and African American datasets, respectively. By comparison, the top results for the association between *PICALM* and AD in a GWAS of more than 2,000 cases and 5,300 controls had p-values between 0.01 and 0.001.[49] Finally, our study lacks a true replication sample. However, our top results, particularly the genome-

wide significant ones, are supported by evidence in studies with very different designs and genetic background.

Our study demonstrates the efficacy of endophenotypes for broadening our understanding of the genetic basis of AD. It is very likely that the volume and specificity of these associations will increase through future studies using larger samples and focused on additional precise structural and functional MRI measures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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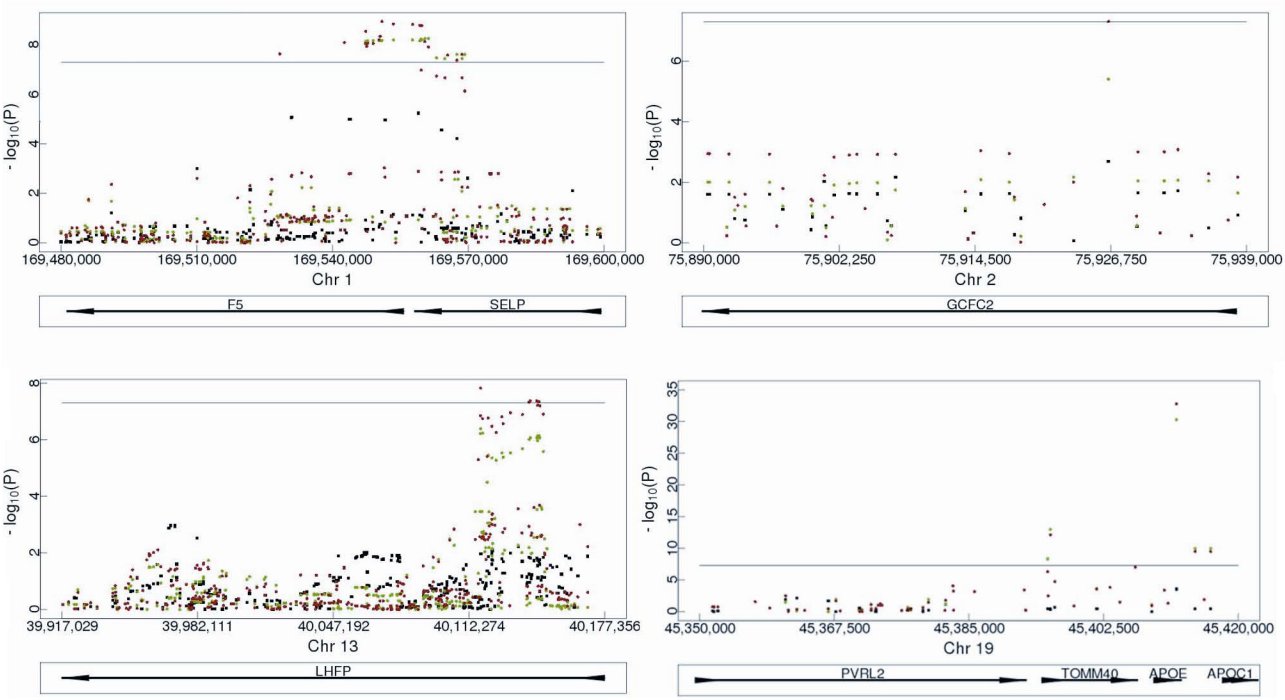

Figure 1.

Plots of gene regions yielding genome-wide significant associations with hippocampal volume. Genome-wide significance is indicated by the horizontal line corresponding to a p-value of 5×10^{-8}. P-values for each SNP are shown for the African American dataset (black dots), two Caucasian datasets (green dots), and all datasets combined (red dots). Gene location and transcription direction are shown below each plot by an arrow. SNP and gene locations were obtained from NCBI builds dbSNP135 and 37.3, respectively.

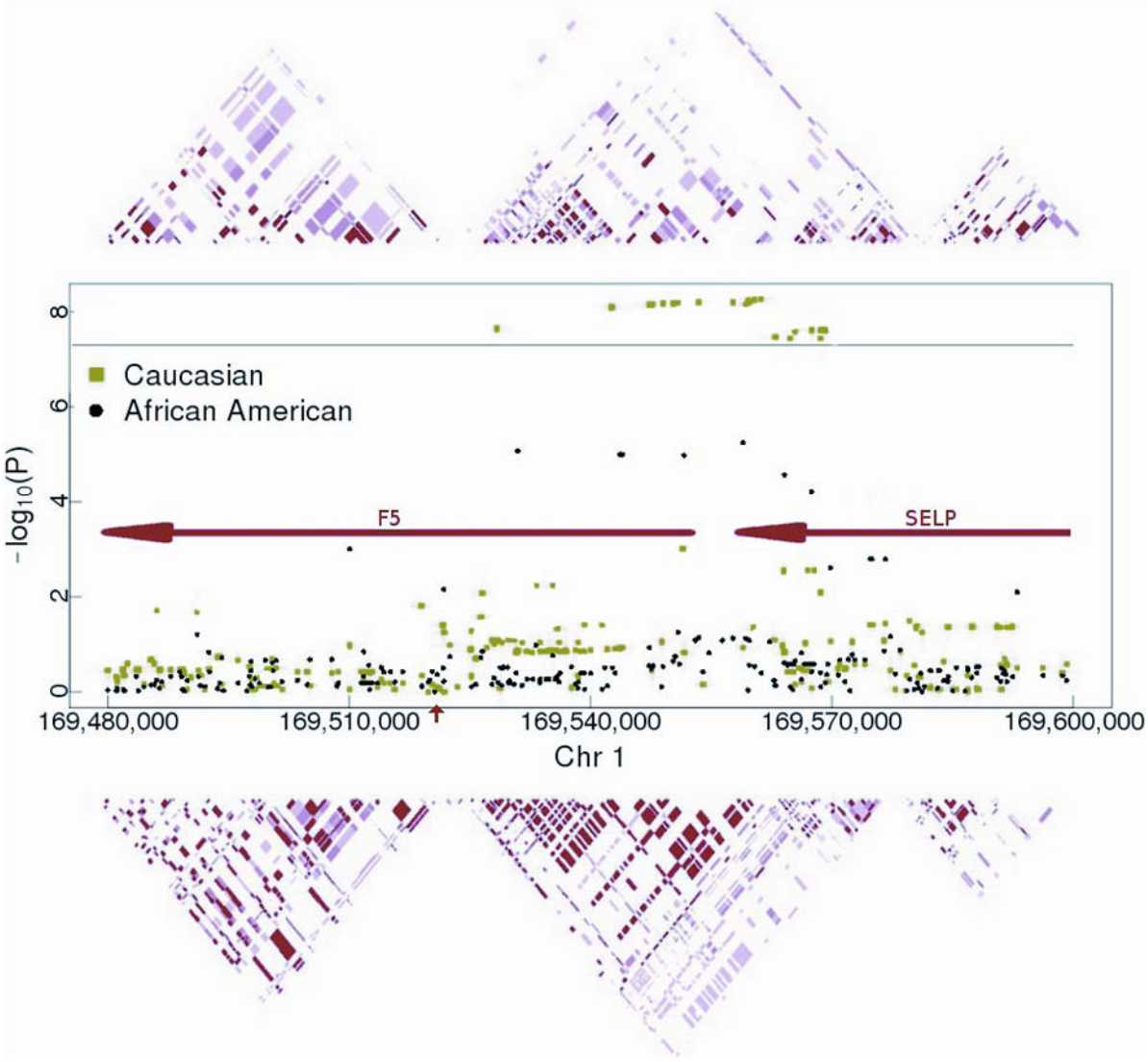

Figure 2.

Regional plot showing association results for hippocampal atrophy in the *F5/SELP* region in Caucasians (green dots) and African Americans (black dots). Red horizontal arrows show the approximate location of the *F5* and *SELP* loci. Genome-wide significance is indicated by the horizontal line corresponding to a p-value of 5×10^{-8}. Location of the Factor V Leiden mutation (rs6025) is indicated by a red vertical arrow. Linkage disequilibrium (LD) in this region is shown for African Americans above and for Caucasians below the Manhattan plot. The measure of LD (r^2) among all possible pairs of SNPs is shown graphically according to the shade of red where white represents very low r^2 and dark red represents very high r^2.

Table 1

Characteristics of study cohorts.

Characteristic	ADNI			MIRAGE Caucasian		MIRAGE African American	
	AD	MCI	CON	AD	CON	AD	CON
Sample size	168	336	188	454	537	188	231
Percentage Female	0.536	0.643	0.559	0.602	0.604	0.670	0.707
Age at baseline MRI (mean ±SD)	75.4 +−7.6	75.2 +−7.1	75 +−4.9	73.2 +−8.3	69 +−8.7	74.7 +−9.4	68.4 +−10.2
APOE ε4 allele frequency	0.420	0.342	0.144	0.291	0.194	0.335	0.205

AD=Alzheimer disease, MCI=mild cognitive impairment, CON= cognitively healthy control

Table 2

Top association findings for hippocampal volume, total cerebral volume and white matter hyperintensities in Caucasians and African Americans

					Effect Allele Frequency			p-value					
Chr.	**Chromosome Position***	**SNP**	**Allele**	**Gene**	**ADNI**	**MIRAGE Caucasian**	**MIRAGE African American**	**ADNI**	**MIRAGE Caucasian**	**Meta Analysis (Caucasian)**	**MIRAGE African American**	**Meta Analysis (all datasets)**	**Direction of Effect†**
Hippocampalvolume													
1	53,581,669	rs3820201	A	SLC1A7	0.63	0.62	0.25	7.47E-04	1.10E-03	2.79E-06	1.63E-01	1.18E-06	---
	53,590,870	rs1769309	A		0.37	0.36	0.15	1.12E-01	2.65E-01	5.35E-02	1.41E-03	2.49E-03	---
1	82,282,061	rs12141366	C	LPHN2	0.29	0.30	0.17	3.58E-01	8.30E-01	5.80E-01	7.83E-03	5.97E-01	+--
	82,551,516	rs10874285	C		0.06	0.07	0.10	2.17E-02	1.35E-05	4.44E-06	2.98E-01	1.34E-04	++-
1	**169,550,962**	**rs6703865**	**A**	**F5/SELP**	**0.09**	**0.08**	**0.17**	**1.59E-06**	**8.95E-04**	**6.58E-09**	**5.57E-02**	**1.14E-09**	---
	169,559,034	rs3917854	C		0.71	0.72	0.91	1.52E-02	6.92E-01	1.16E-01	5.70E-06	1.29E-03	+-+
1	212,708,370	rs2942354	A	ATF3	0.43	0.42	0.22	7.16E-04	1.53E-04	4.71E-07	3.19E-01	2.14E-05	++-
	212,736,470	rs12729140	C		0.13	0.15	0.10	5.20E-01	2.38E-01	7.73E-01	5.43E-03	1.77E-01	-++
2	**75,926,564**	**rs2298948**	**C**	**GCFC2**	**0.33**	**0.29**	**0.08**	**4.25E-04**	**2.83E-03**	**3.87E-06**	**2.05E-03**	**4.89E-08**	+++
3	120,833,992	rs16832111	C	STXBP5L	0.85	0.84	0.92	9.23E-01	4.22E-01	6.50E-01	1.99E-03	1.04E-01	+--
	121,121,957	rs9878946	C		0.94	0.94	0.84	2.26E-03	6.44E-04	5.52E-06	7.40E-01	1.61E-05	+++
6	124,637,676	rs9491114	C	NKAIN2	0.92	0.91	0.83	2.27E-05	2.54E-02	3.12E-06	8.55E-01	1.28E-05	---
	124,853,366	rs7773205	G		1.00	NA	0.89	1.46E-01	NA	1.46E-01	2.14E-04	5.85E-01	-?+
8	100,611,402	rs16897488	C	VPS13B	0.96	0.95	0.92	3.80E-01	7.04E-01	3.62E-01	1.03E-02	6.57E-02	---
	100,835,181	rs959695	C		0.16	0.18	NA	1.92E-05	1.19E-02	1.09E-06	NA	1.09E-06	++?
	100,846,839	rs12545602	A		0.14	0.16	0.12	8.48E-05	1.66E-02	5.70E-06	6.69E-01	6.06E-05	--+
9	84,281,311	rs10119841	C	TLE1	0.29	0.30	0.12	8.89E-01	1.58E-03	4.88E-02	3.89E-03	4.92E-01	+-+
	84,372,740	rs11139399	C		0.42	0.41	0.67	1.84E-06	3.66E-02	6.67E-07	4.46E-01	1.09E-06	---
11	85,654,586	rs596864	A	PICALM	0.40	0.44	0.28	4.65E-03	2.01E-04	4.75E-06	6.01E-01	1.00E-05	---
	85,765,790	rs17148741	C		0.97	0.98	0.86	2.93E-01	3.12E-01	1.45E-01	9.39E-05	8.55E-01	++-
13	**40,118,067**	**rs9315702**	**A**	**LHFP**	**0.44**	**0.44**	**0.22**	**8.81E-04**	**9.87E-05**	**4.08E-07**	**1.07E-02**	**1.52E-08**	---
	40,135,975	rs7996238	A		0.52	0.52	0.32	1.81E-01	6.80E-03	5.34E-03	6.11E-03	2.78E-04	---
18	3,998,438	rs7505600	C	DLGAP1	0.47	0.46	0.53	7.67E-01	4.96E-01	8.23E-01	3.72E-03	1.81E-01	-++
	4,022,526	rs4798157	A		0.20	0.18	0.37	1.81E-02	3.41E-05	6.68E-06	8.05E-01	2.21E-05	---

					Effect Allele Frequency			p-value					
Chr.	**Chromosome Position***	**SNP**	**Allele**	**Gene**	**ADNI**	**MIRAGE Caucasian**	**MIRAGE African American**	**ADNI**	**MIRAGE Caucasian**	**Meta Analysis (Caucasian)**	**MIRAGE African American**	**Meta Analysis (all datasets)**	**Direction of Effect†**
19	**45,395,618**	**Isoforms**	***ε4***	**APOE**	**0.14**	**0.19**	**0.20**	**5.30E-28**	**4.62E-07**	**5.23E-31**	**2.75E-04**	**1.58E-33**	+++
21	46,839,903	rs2838917	C	COL18A1	0.74	0.71	0.43	1.81E-01	8.09E-01	3.95E-01	1.85E-02	8.86E-02	-+-
	46,846,943	rs2838923	A		0.71	0.73	0.30	2.69E-03	1.05E-04	1.51E-06	1.93E-01	7.94E-07	---
	Total cerebral volume												
3	63,432,679	rs935793	A	SYNPR	0.77	0.80	0.79	1.28E-02	5.51E-01	1.33E-01	7.12E-05	2.11E-01	-++
	63,508,819	rs11708252	C		0.92	0.93	0.97	5.50E-05	1.74E-02	4.04E-06	5.16E-01	1.19E-03	--+
3	193,018,777	rs12630096	A	ATP13A5	0.73	0.73	0.74	6.64E-02	4.67E-01	6.19E-02	8.51E-03	8.89E-01	++-
	193,063,372	rs3732523	A		0.51	0.51	0.52	4.45E-04	6.57E-03	9.20E-06	8.54E-01	3.01E-04	+++
19	**45,377,097**	**isoforms**	***ε4***	**APOE**	**0.14**	**0.19**	**0.20**	**1.71E-06**	**5.96E-05**	**4.25E-10**	**2.96E-01**	**1.20E-07**	**+++**
	White matter hyperintensities												
1	64,853,699	rs10789157	C	CACHD1	0.59	0.59	0.39	9.62E-02	4.69E-01	7.12E-02	5.79E-03	3.89E-01	--+
	64,862,972	rs6695900	C		0.23	0.26	0.16	4.34E-04	5.89E-03	9.93E-06	6.83E-01	4.60E-05	++-
2	32,865,520	rs1031261	C	TTC27	0.13	0.11	0.11	4.45E-03	3.95E-05	6.73E-06	1.33E-01	2.07E-06	---
	32,981,964	rs3769573	C		0.55	0.60	0.69	3.74E-01	5.89E-02	8.83E-02	2.18E-04	5.69E-03	---
2	172,127,919	rs4667682	C	METTL8	0.84	0.82	0.93	3.36E-03	1.08E-04	8.15E-06	3.22E-01	5.41E-06	---
	172,240,162	rs16859370	A		0.83	0.84	0.90	8.00E-01	4.68E-01	5.63E-01	4.83E-02	2.46E-01	+++
20	22,697,161	rs10485635	A	KRT18P3	0.74	0.71	0.94	2.38E-03	5.97E-01	1.72E-02	1.18E-02	1.37E-01	-++
	22,708,493	rs6036195	G		0.42	0.41	0.13	5.56E-05	4.28E-02	6.63E-06	2.41E-01	8.82E-05	++-
20	40,056,636	rs761024	G	CHD6	0.66	0.66	0.20	1.45E-01	6.24E-01	1.31E-01	6.57E-03	2.28E-02	---
	40,064,558	rs13037749	A		0.94	0.92	NA	2.86E-04	7.97E-03	8.04E-06	NA	8.04E-06	++?

*positions according to NCBI build GrCh37.p5

†Effect direction is listed for each of ADNI Caucasian, MIRAGE Caucasian, and MIRAGE African American.

Genome-wide significant results ($p<5\times10^{-8}$) are shown in bold.