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Association of *TTR* polymorphisms with hippocampal atrophy in Alzheimer disease families

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Abstract

In vitro and animal model studies suggest that transthyretin (TTR) inhibits the production of the amyloid β protein, a major contributor to Alzheimer disease (AD) pathogenesis. We evaluated the association of 16 *TTR* single nucleotide polymorphisms (SNPs) with AD risk in 158 African American and 469 Caucasian discordant sibships from the MIRAGE Study. There was no evidence for association of TTR with AD in either population sample. To examine the possibility that *TTR* SNPs affect specific components of the AD process, we tested association of these SNPs with four measures of neurodegeneration and cerebrovascular disease defined by magnetic resonance imaging (MRI) in a subset of 48 African American and 265 Caucasian sibships. Five of seven common SNPs and several haplotypes were significantly associated with hippocampal atrophy in the Caucasian sample. Two of these SNPs also showed marginal evidence for association in the African American sample. Results for the other MRI traits were unremarkable. This study highlights the potential value of neuroimaging endophenotypes as a tool for finding genes influencing AD pathogenesis.

Keywords: Alzheimer disease; Transthyretin; Hippocampal atrophy; Magnetic resonance imaging; Genetic association

1. Introduction

Amyloid plaques and neurofibrillary tangles in the brain, along with possible contribution of cerebrovascular damage, are hypothesized to be the major contributors to Alzheimer disease (AD) pathogenesis (Van Broeck et al., 2008). The common late-onset form of AD is highly heritable. The ε4 allele of the apolipoprotein E (*APOE*) gene has been established as a major risk factor for AD in diverse populations (Farrer et al., 1997). The exact role of APOE in AD is unclear, but evidence suggests that the APOE-mediated removal of degraded toxic amyloid-β (Aβ) protein is depressed among individuals with ε4 (Rebeck et al., 2006).

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Only a few other genes have been robustly associated with AD and all have much lower estimates of attributable risk than *APOE* (Bertram et al., 2007). Some of these genes (e.g. *SORL1*) probably act through multiple pathways, affecting both Aβ processing and cerebrovascular changes leading to AD-related damage (Cuenco et al., 2008a).

Transthyretin (TTR) is a symmetrical tetramer composed of four identical 127 amino acid subunits. It is a normal protein made by the liver, spleen and brain, previously called prealbumin (Hamilton and Benson, 2001). It is involved in the transport in the blood, brain and central nervous system of retinol, and thyroxine (Chanoine et al., 1992; Monaco, 2000). Schwarzman et al., 1994 reported that the amyloid beta protein binds to TTR in stable complexes, which may be important in preventing or inhibiting aggregation and formation of amyloid deposits. Kinetic modeling studies suggest that TTR binds preferentially to aggregated rather than monomeric Aβ (Liu and Murphy, 2006). TTR is also involved in Aβ proteolysis (Costa et al., 2008). *Caenorhabditis elegans* bioengineered to express human TTR have diminished abnormalities when Aβ protein expression is induced (Link, 1995). TTR prevents the accumulation of the Aβ protein in cultured vascular smooth muscle cells (Mazur-Kolecka et al., 1995). A role for TTR in AD pathogenesis is also supported by mouse models (Wati et al., 2008). In an elegant set of experiments, Stein et al. (2004) showed that TTR expression is markedly elevated in early life in TG2576 transgenic mice in hippocampus and cortex before the development of Aβ pathology. Furthermore, delivery of anti-TTR antibodies in these mice led to dramatically accelerated Aβ deposits, tau protein fibrils, neuronal loss and apoptosis. These findings suggest that the high levels of Aβ found in TG2576 mice may cause an adaptive increased expression of TTR. Expression of the TTR gene has been reported to be up-regulated by an "enriched" environment in transgenic APPswe/PS1ΔE9 mice (Lazarov et al., 2005). In one study, APP23 mice which have cognitive deficits mirroring human AD symptoms were bred with mice over-expressing human TTR (hTTR). The resultant APP23/hTTR+ progeny had significantly better cognitive performance and less evidence of Aβ in the hippocampus than APP23/hTTR− mice (Buxbaum et al., 2008). Consistent with a protective role for TTR, TTR+/− mice that also contain the APP swe/PS1ΔE9 have accelerated deposits of Aβ compared to TTR+/+ mice (Choi et al., 2007). TTR may also be involved in AD pathogenesis through a vascular mechanism. Liz et al. (2007) showed that apolipoprotein A-I (ApoA-I), a constituent of HDL, when cleaved by TTR results in reduced cholesterol efflux and increased formation of amyloid fibrils.

TTR concentrations are significantly lower in cerebrospinal fluid (CSF) of AD patients than in elderly controls with a similar age distribution (Serot et al., 1997). The presence of TTR concurrent with Aβ in CSF leads to Aβ sequestration by TTR and a reduction in amyloid plaque formation (Schwarzman et al., 1994). Craft et al. (2007) reported that CSF TTR levels vary with APOE genotype, with APOE ε4 carriers having lower TTR CSF levels, which might result in greater Aβ burden. One genetic study that focused on *TTR* did not find association of AD with variants identified by single strand conformation polymorphism (SSCP) analysis in 19 early onset or 47 sporadic AD patients (Palha et al., 1996). However, SSCP analysis has limited sensitivity for identifying single base changes (Sheffield et al., 1993). In fact, a recent study indicated that specific post-translational (thiol) modifications to TTR in CSF differentiate AD subjects from controls with a sensitivity of >90% and specificity of >70% (Biroccio et al., 2006).

In light of evidence supporting a role for TTR in AD pathogenesis via Aβ formation and cholesterol transport mechanisms and lack of notable findings with *TTR* in multiple AD genome wide association studies (Coon et al., 2007; Li et al., 2008), we investigated the association of *TTR* single nucleotide polymorphisms (SNPs) with AD and measures of neurodegeneration and cerebrovascular disease defined by magnetic resonance imaging in Caucasian and African American families participating in the MIRAGE Study.

2. Materials and methods

2.1. Study population

Caucasian and African American AD patients and their unaffected siblings who were recruited between 1996 and 2006 from 17 clinical sites located in North America and Western Europe for the MIRAGE Study, a family-based genetic epidemiological study of AD, were included in this study (Green et al., 2002). Subjects were considered affected if they met NINCDS/ADRDA standards for probable or definite AD (McKhann et al., 1984). Siblings were classified as non-demented based on a Telephone Interview of Cognitive Status score (TICS) ≥86 (Roccaforte et al., 1992). TICS scores between 83 and 86 were considered borderline and required additional documentation about cognitive functioning from informants to confirm the assignment as unaffected. Age of AD onset was defined as earliest age of proxy's report of symptoms. Participants self-reported their ethnicity as Caucasian or African American. Institutional Review Boards from each study site reviewed and approved all protocols.

2.2. SNPs and genotyping

Genomic DNA was extracted from peripheral blood lymphocytes. SNP genotyping was carried out using assays and protocols developed by Applied Biosystems, Inc. on an ABI 7900 platform. Seven SNPs located inside the *TTR* gene and its promoter region (Table 1) were selected based on minor allele frequency, linkage disequilibrium patterns of the locus, and availability of SNP assays. Nine additional SNPs (including several obtained from Applied Biosystems designated with the prefix hCV) with unconfirmed reports in the Genbank database as polymorphic for an amino acid substitution were also tested. All of the unconfirmed SNPs

Table 1
TTR SNP characteristics.

Marker number	dbSNP ID	Physical map location (bp)[a]	SNP function	Alleles	Minor allele (frequency) Caucasians	Minor allele (frequency) African Americans
1	rs3764479	27,423,823	Promoter	C/T	C (0.37)	C (0.29)
2	rs3764478	27,424,481	Promoter	A/C	A (0.13)	A (0.10)
3	rs723744	27,426,474	Intronic	A/C	A (0.38)	A (0.42)
4	rs1800458	27,426,863	S26G	A/G	A (0.10)	A (0.02)
5	rs1080094	27,427,793	Intronic	A/G	G (0.43)	A (0.16)
6	rs3764476	27,430,458	Intronic	A/C	A (0.37)	A (0.34)
7	rs3794884	27,430,969	Intronic	A/C	C (0.38)	C (0.37)

[a] Locations are based on National Center for Biotechnology Information genome build 36.3.

(hCV27864114, hCV11628247, hCV11628241, rs1803084, hCV27536470, hCV27529287, rs1804117, hCV27864134, and hCV602164) proved to be monomorphic in this sample and were excluded from further study. None of the remaining SNPs demonstrated departure from Hardy–Weinberg equilibrium in controls within each ethnic group.

2.3. Magnetic resonance imaging

A detailed description of the MIRAGE magnetic resonance imaging (MRI) methods and traits has been published elsewhere (Cuenco et al., 2008a,b). Briefly, beginning in 2002 MRIs were obtained from subjects using 1.5 T magnetic field strength scanners. Scanner sequences were standardized to account for site-to-site difference in machines and software. Four semi-quantitative measures of generalized cerebral atrophy (CA), bilateral medial temporal atrophy (MTA), white matter hyperintensity (WMH), and cerebrovascular disease (CVR) were derived from these scans by a single rater (C.D.) blinded to affection status and demographics of the subject. MTA was measured by combining the widths of the choroidal fissure, temporal horn of the lateral ventricle, and height of the hippocampus. CVR is a composite measure of cerebrovascular disease based on the amount of WMH and presence of cerebral infarcts. WMH, CVR and CA were scored between 0 (absent) and 100 (severe). MTA was rated on an ordinal scale of 0–4 (Scheltens et al., 1992). Each of these measures has previously been reported to be correlated with AD and have moderate/high heritability (Lunetta et al., 2007).

2.4. Statistical analysis

Distributions of CA, MTA, WMH, and CVR were assessed for normality. Log transformations were applied to MRI traits with exponential distributions. Family-based tests of association of AD as an outcome and the MRI traits were conducted using an additive genetic model as implemented in PBAT version 3.5 software (Horvath et al., 2004; Lange et al., 2004) and assuming the null hypothesis of no linkage and no association. Analyses of MRI traits included covariates adjusting for age at MRI exam and disease duration (i.e., zero if unaffected, number of years since onset of symptoms otherwise). The addition of *APOE* genotype and sex to the model had negligible effect on association tests, and were excluded in further analyses. The linkage disequilibrium (LD) structure of the *TTR* SNPs was examined using the default settings in Haploview (http://www.broad.mit.edu/mpg/haploview/index.php) which create 95% confidence bounds on D' and r^2 to define SNP pairs in strong LD. Secondary analyses of haplotypes were performed using sliding windows of three contiguous SNPs for traits showing association with at least one SNP. These analyses allowed us to explore whether the observed SNP associations may be due to untyped SNPs that are better tagged by haplotypes. All analyses were carried out separately for the groups of African American and Caucasian families.

3. Results

A total of 627 sibships (158 African American, 469 Caucasian) consisting of at least 1 AD affected and 1 unaffected sibling were genotyped for *TTR* SNPs. A higher proportion of Caucasian participants were males relative to African American participants. Approximately 30% of the African American families and 56% of the Caucasian families had MRI trait data. The age and gender characteristics of the MRI samples were similar to those in the total samples (Table 2). AD cases were significantly older by 3.7 years than the unaffected siblings at the age of the MRI scan in both the African American ($p = 0.026$) and Caucasian datasets ($p < 0.0001$).

There was no evidence of association of AD with any of the *TTR* SNPs in either ethnic group in the total sample or subset of families with MRI data (Table 3). Significant association was observed in the Caucasian families for WMH with rs1800458 and for MTA with five of the seven polymorphic SNPs tested. The three most significantly associated SNPs (i.e., rs3764479, rs3764476, and rs3794884) are in strong LD ($r^2 \geq 0.94$, see Fig. 1). In the African American families, rs3764478 was significantly associated with the two measures of cerebrovascular disease, but these results should be considered tentative since only six families were informative for this SNP. No other significant associations were observed with the MRI traits in this sample, although the results for MTA with the two SNPs in the distal portion of the gene (rs3764476 and rs3794884) were nearly significant.

Table 2
Sample characteristics.

Characteristic	Total sample		MRI sample	
	African American	Caucasian	African American	Caucasian
No. families with ≥1 discordant sibling pair	158	469	48	265
No. AD cases	168	491	49	274
No. unaffected siblings	175	611	66	332
Percent males				
AD cases	27.4	41.1	32.6	44.2
Unaffected siblings	36.6	41.1	34.8	41.0
Mean age (±S.D.)[a]				
AD cases	70.6 (8.9)	68.2 (8.5)	76.0 (8.3)	73.0 (8.6)
Unaffected siblings	71.7 (10.3)	69.7 (8.8)	72.3 (8.9)	69.3 (8.5)

[a] Age = age at onset or exam in total sample, age at MRI scan in MRI sample.

Table 3
Association of *TTR* SNPs with AD risk and MRI traits.

SNP	Alzheimer disease risk				MRI traits				
	Total sample		MRI sample		No. Fams.	*p*-Value			
	No. Fams.	*p*-Value	No. Fams.	*p*-Value		WMH	CVR	CA	MTA
Caucasian families									
rs3764479	122	0.29	65	0.10	71	0.31	0.51	0.20	0.0041
rs3764478	51	0.13	25	0.12	28	0.68	0.88	0.88	0.16
rs723744	190	0.16	123	0.13	129	0.11	0.56	0.23	0.040
rs1800458	50	0.43	32	0.31	37	0.012	0.35	0.46	0.86
rs1080094	195	0.48	127	0.29	128	0.38	0.62	0.10	0.042
rs3764476	198	0.24	125	0.19	129	0.066	0.84	0.20	0.013
rs3794884	199	0.31	123	0.17	127	0.065	0.78	0.15	0.017
African American families									
rs3764479	44	0.51	16	0.33	16	0.86	0.51	0.54	0.32
rs3764478	19	0.73	6	1.00	6	0.031	0.010	0.50	0.69
rs723744	59	0.58	23	0.23	22	0.12	0.27	0.18	0.27
rs1800458	6	0.89	3	0.69	3	0.28	0.17	0.32	0.39
rs1080094	32	0.75	16	0.60	15	0.47	0.58	0.27	0.13
rs3764476	54	0.87	22	1.00	21	0.29	0.61	0.62	0.069
rs3794884	56	0.59	24	0.95	22	0.30	0.46	0.63	0.075

Secondary haplotype analysis for MTA revealed two significant SNP combinations in the Caucasian families (Table 4). The first combination includes rs3764479, 3764478 and rs723744 which span the promoter region and intron 1. The most frequent haplotype (TCC) was associated with increased MTA and the two next most frequent haplotypes (CCC and TCA) were associated with decreased MTA. None of the individual SNPs in the haplotype entirely accounts for the association. The second combination includes rs1800458, rs1080094 and rs3764476 which span exon 2 and part of intron 2. Haplotype GAC was associated with increased MTA and haplotype GAA was associated with decreased MTA. The difference in these haplotypes is in the third position only and suggests that the association is accounted for by intronic SNP rs3764476 and not by rs1800458 which is a non-synonymous coding SNP. Neither of these SNP combinations was significantly associated with MTA in the African American families. However, the significant risk (GAC) and protective (GAA) haplotypes for rs1800458–rs1080094–rs3764476 in Caucasians have the same pattern of association in African Americans. Lack of significance could be associated with reduced sample size or very different haplotype frequencies in African Americans, or both.

4. Discussion

In this dataset of discordant sibs ascertained through an AD proband, *TTR* SNPs were significantly associated with an MRI measure of hippocampal atrophy (MTA) in Caucasian families. Two adjacent intronic SNPs (rs3764476 and rs3794884) also suggested marginal evidence for association in a smaller sample of African American families in the same direction as in the Caucasian families. By comparison, there was no association of *TTR* SNPs and risk of AD in the current set of MIRAGE families or in an enlarged sample of MIRAGE Caucasian or African American families. *TTR* was also not associated with AD in two genome wide association studies (Coon et al., 2007; Li et al., 2008), but few *TTR*

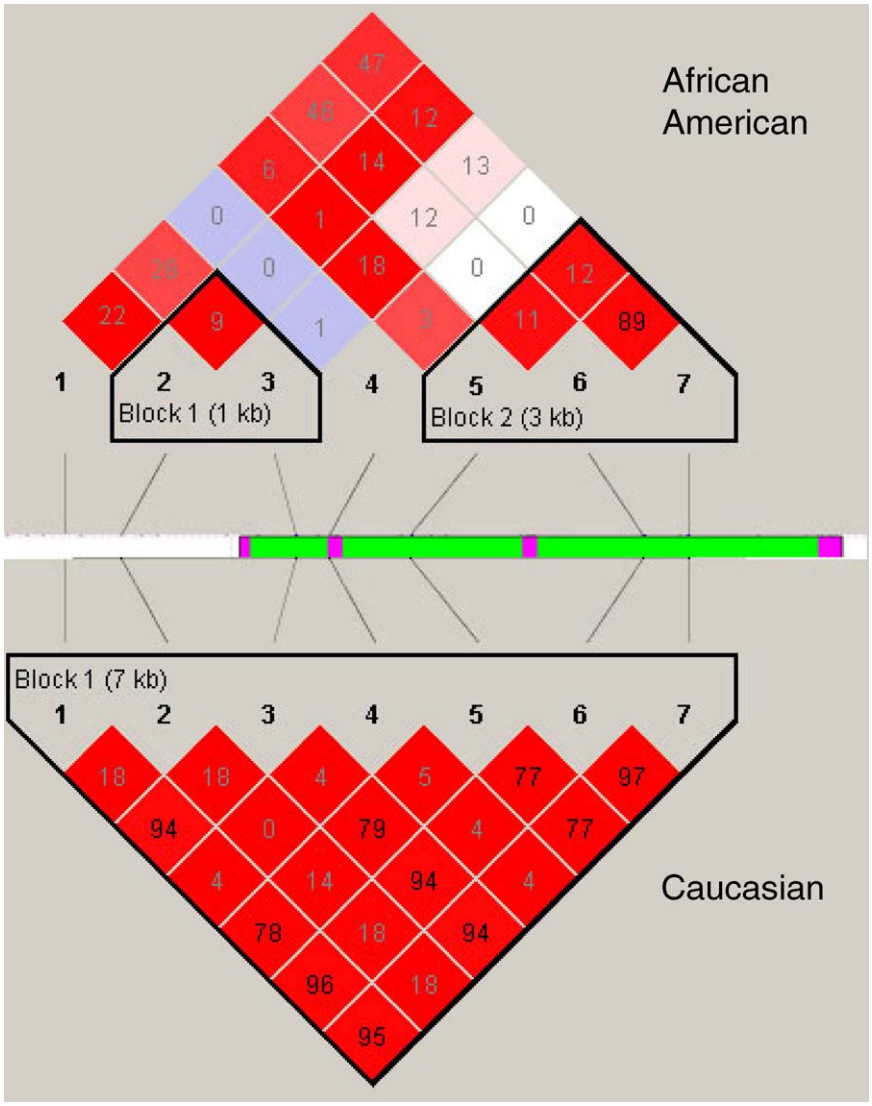

Fig. 1. Locations and linkage disequilibrium (LD) map structure of single nucleotide polymorphisms (SNPs) in the *TTR* gene region. The LD maps for the African American (AA) and Caucasian family samples are shown above and below the gene structure diagram, respectively. Measures of LD among all possible pairs of SNPs (identified by number using the scheme in Table 1) are shown graphically according to the shade of red where white represents very low D' and dark red represents very high D' and numerically denoted by the r^2 values within each square. Indeterminate estimates of LD are denoted by blue shading. The *TTR* gene structure including intergenic regions (white), introns (green) and exons (purple) is shown starting from the 5′ upstream region on the left.

SNPs that passed quality checking were included. To verify the findings in the GWAS by Coon et al. (2007), we imputed genotypes for 23 SNPs in or within 10 kb of *TTR* (including six of seven SNPs in Table 1) in their dataset the Markov Chain Haplotyping software (Li and Abecasis, 2006). None of the results with these imputed SNPs were significantly associated with AD.

Heritable endophenotypes obtained with neuroimaging may help unravel the genetic basis of AD by decomposing the disease into distinct measurable features that individually are more directly related to a pathogenic mechanism influenced by a particular genetic variant (Lunetta et al., 2007), evidenced by the association of SORL1 with specific measures of cognitive decline (Seshadri et al., 2007) and brain abnormalities observed by MRI and at autopsy (Cuenco et al., 2008b). Hippocampal atrophy is well known to occur early in the disease and is correlated with impaired memory function as well as pathology (Deweer et al., 1995; Nagy et al., 1996). Previously, we demonstrated in this sample of families that hippocampal atrophy is highly heritable (Lunetta et al., 2007) and may be a marker of subclinical disease (Cuenco et al., 2008a). Thus, lack of association between TTR and AD could be the result of genetic heterogeneity, mis-classification of asymptomatic genetically prone individuals, or both.

The accumulation of Aβ protein in the cores of neuritic plaques and in the vessel walls in congophilic angiopathy is central to the "amyloid hypothesis" of the disease. The creation of amyloid fibrils, plaques, or the earlier oligomeric forms of Aβ are related to several factors including APP gene dosage, cleavage, proteolysis, clearance from the brain

Table 4
Association of *TTR* haplotypes with MTA.

Haplotype[a]	Caucasian families					African American families				
	Freq.	No. Fams.	*p*-Value	R/P[b]	Global *p*-value	Freq.	No. Fams.	*p*-Value	R/P[b]	Global *p*-Value
1–2–3					0.014					0.35
TCC	0.493	108	0.0014	R		0.447	20	0.17	R	
CCC	0.091	95	0.0027	P		0.065	14	0.29	R	
TAC	0.015	31	0.35	P		0.008	4	0.88	P	
CAC	0.015	22	0.42	P		0.008	4	0.88	P	
TCA	0.095	95	0.0027	P		0.264	23	0.39	R	
CCA	0.214	108	0.31	P		0.158	16	0.25	P	
TAA	0.015	22	0.42	P		0.014	5	0.87	P	
CAA	0.061	41	0.41	P		0.038	6	0.59	P	
4–5–6					0.0059					0.2
GAC	0.406	209	0.0054	R		0.140	16	0.11	R	
AAC	0.051	34	0.44	P		0.003	2	0.54	R	
GGC	0.158	201	0.14	P		0.490	24	0.89	R	
AGC	0.009	26	0.9	P		0.005	3	0.30	P	
GAA	0.100	183	0.021	P		0.028	9	0.20	P	
AAA	0.006	22	0.34	P		0.003	2	0.54	R	
GGA	0.263	190	0.13	R		0.330	21	0.035	P	
AGA	0.006	22	0.34	P		0.005	3	0.30	P	

No. Fams. = number of informative families.
[a] Marker number designations correspond to dbSNP IDs in Table 1.
[b] R: risk, P: protective.

to blood and removal from the blood (St. George-Hyslop et al., 2000). Agents such as TTR which bind to Aβ may be protective by impairing the oligomerization or aggregation of the protein and enhancing its clearance. The Aβ clearance hypothesis posits that transport of the Aβ protein from the brain to the blood helps to diminish its concentration in the brain and impairs its oligomerization and aggregation. Higher levels of TTR in the blood should assist in the clearance process. Hippocampal atrophy may be reduced in those subjects with TTR isoforms most able to bind Aβ and enhance clearance from the brain.

Our results should be interpreted in light of several caveats. The significant association of *TTR* with MTA in Caucasian families may be due to chance given the number of tests performed (7 SNPs × 4 traits = 28 tests). However, the result for rs3764479 remains significant even after applying the Bonferroni correction. Nonetheless, further testing is needed in independent samples. Second, our sample consisting primarily of sibling pairs discordant for AD does not reflect the distribution of hippocampal atrophy in a general elderly population. To address the possibility that the genetic basis of brain changes measured by MRI may differ in persons with and without AD, we included in our analyses covariates for age at the time of MRI scan and duration of illness. Similar, but slightly less significant, results were obtained when adjusting for AD status instead of duration of illness (results not shown), perhaps reflecting the greater sensitivity of a continuous rather than a dichotomous measure of disease progression.

Conversely, our approach may identify genetic associations more reliably with pathology than with symptomatology. Numerous (e.g., Jack et al., 1997), but not all (Sullivan et al., 1995), studies show age-related declines in hippocampal volume and progression of atrophy over time (Jack et al., 1998, 2000). The etiology of these age-related differences is uncertain, but may include incipient AD pathology (Barnes et al., 2006). Given that hippocampal pathology may precede AD diagnosis by years (Price and Morris, 1999), it is likely that the use of a MRI endophenotype may, in fact, identify those at risk for future cognitive impairment who are cognitively normal at the time of imaging. If true, these polymorphisms could be considered "susceptibility" polymorphisms. This study highlights the value of neuroimaging endophenotypes as a tool for finding genes that influence both brain structure and function (Glahn et al., 2007).

Conflicts of interest

The authors do not have conflicts of interest, including financial, personal or relationships with other people or organizations that could inappropriately influence the work. Furthermore, the authors' institutions do not have any contracts relating to this research through which they may gain financially.

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Appendix A. MIRAGE Study Group

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