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A multi-centre study of *ACE* and the risk of late-onset Alzheimer's disease

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Abstract

A key pathological feature of late-onset Alzheimer's disease (LOAD) is the abnormal extracellular accumulation of the amyloid beta (Aβ) peptide. Thus altered Aβ degradation could be a major contributor to the development of LOAD. Variants in the gene encoding the Aβ-degrading enzyme, angiotensin-1 converting enzyme (ACE) therefore represent plausible candidates for association with LOAD pathology and risk. Following Alzgene meta-analyses of all published case-control studies, the *ACE* variants rs4291 and rs1800764 showed significant association with LOAD risk. Furthermore *ACE* haplotypes are associated with both plasma ACE levels and LOAD risk. We tested three *ACE* variants (rs4291, rs4343 and rs1800764) for association with LOAD in ten Caucasian case-control populations (n=8,212). No association was found using multiple logistic models (all p>0.09). We found no population heterogeneity (all p>0.38) or evidence for association with LOAD risk following meta-analysis of the ten populations for rs4343 (OR=1.00), rs4291 (OR=0.97) or rs1800764 (OR=0.99). Although we found no haplotypic association in our complete dataset (p=0.51), a significant global haplotypic p-value was observed in one population (p=0.007) due to an association of the H3 haplotype (OR=0.72, p=0.02) and a trend towards an association of H4 (OR=1.38, p=0.09) and H7 (OR=2.07, p=0.08) although these did not survive Bonferroni correction. Previously reported associations of *ACE* variants with LOAD will be

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diminished following this study. At best, *ACE* variants have modest effect sizes, which are likely part of a complex interaction between genetic, phenotypic and pharmacological effects that would be undetected in traditional case-control studies.

Keywords

Alzheimer Disease; Late Onset; Angiotensin-1 Converting Enzyme; Haplotype; Heterogeneity; Meta-Analysis

Introduction

Late-onset Alzheimer's disease (LOAD; MIM 104300) is the most common form of dementia accounting for almost two-thirds of all dementia cases. Its key pathological features include the formation of intracellular neurofibrillary tangles comprised of microtubule-associated tau, abnormal extracellular accumulations of amyloid beta (Aβ) peptide in the form of characteristic senile plaques and in most LOAD cases, deposition of intracerebrovascular Aβ in the form of cerebral amyloid angiopathy [1, 2]. It is increasingly recognised that altered degradation and clearance of Aβ is likely to be of importance in the development and progression of LOAD [3] and that this may be contributed to by both environmental and genetic factors. Cumulative evidence from *in vitro*, *in vivo* and *ex vivo* studies now strongly support the role of ACE (EC 3.4.15.1), a zinc metalloprotease widely expressed in the brain, as an Aβ degrading enzyme (reviewed in [4]). Taken together with the observation that increased ACE levels and activity are observed in LOAD brains (reviewed in [5]) and are associated with increased plasma levels of Aβ [6] and reduced levels of Aβ in CSF [7, 8], these all point to the likely involvement of ACE in Aβ-related pathology in AD. This is further supported by evidence that variation in the gene encoding ACE (*ACE*; OMIM 106180), may play a role in LOAD pathology and modify LOAD risk. For example, the insertion/deletion (indel) of a 287bp *Alu* repeat in intron 16 (rs1799752 *Alu* I/D) of *ACE*, and perhaps the most widely studied for LOAD association, is predicted to explain 29–47% of the variation in plasma ACE levels [9–11]. In their meta-analysis of 39 case-control series, comprising 6,037 LOAD cases and 12,099 controls, Lehmann et al reported that homozygotes for the *Alu* deletion were at reduced risk of LOAD (p=0.0004), while heterozygotes were at increased risk [12], thus supporting a genetic association of *ACE* with LOAD. The fact that the indel does not account for all of the observed variation in ACE levels suggests that other functional ACE variants may be present and in turn associated with ACE levels and/or LOAD risk.

The *APOE* ε4 allele (107741) remains the most widely studied and accepted susceptibility gene for LOAD since its first report as a candidate gene almost 20 years ago [13, 14]. The remaining genetic component of AD risk may involve many genes, each with individually small-to-moderate effect sizes that interact to produce greater effects on disease susceptibility and/or disease modification. However, detection and confirmation of the involvement of genes with these effect sizes requires very large sample sizes. By example, over the last two decades 664 different genes and almost 3,000 variants have been investigated as susceptibility factors for LOAD risk [15] and until recently, the majority of these studies have been relatively underpowered, often resulting in inconclusive or inconsistent results for the majority of putative candidate genes. AlzGene (www.Alzgene.org) [15] was designed and established to resolve this problem to some extent by regularly performing meta-analyses of published data as it emerged to continually compile a list of "Top LOAD genes" that show the strongest associations in LOAD. A relatively constant member of this list has been *ACE* for which two (rs4291 and rs1800764) of the six variants studied show significant association with LOAD risk following the

AlzGene meta-analyses based on total sample sizes of n=10,588 and n=4,756, respectively. Notably, rs1800764 has also been associated with elevated CSF Aβ42/Aβ40 ratio [7].

Despite the large number of reported independent genetic associations between *ACE* variants and LOAD in the last decade (22 out of 55 populations published to-date; for details see AlzGene), few studies utilised more comprehensive haplotype approaches [7, 8, 16–19]. Keavney and colleagues identified seven haplotypes in a Caucasian British population derived from data from ten polymorphisms spanning 26kb of *ACE*. From these haplotypes, they constructed a cladogram that contained three main branches (clades A, B and C), which accounted for 90% of the observed haplotypes. Clade A has since been associated with low plasma ACE levels and increased risk of LOAD [16], clades B and C with higher ACE levels [19–21] and clade C with increased risk for LOAD in families [18]. Kehoe and colleagues also analysed seven variants within *ACE* (rs4363, rs4362, rs4343, rs4331, rs4309, rs4291, rs1800764) that formed ten haplotypes with an LD structure that enabled the selection of three 'tagging' variants (rs4291, rs4343 and rs1800764) [8]. The most frequent haplotype (H1) contained the previously reported AD-associated ('risk') *ACE* indel I allele [22] while the H2 haplotype contained the ('protective') D allele [8]. Some indication that the indel was not the only functional *ACE* variant involved in LOAD pathogenesis came from H5 (also containing the I allele) which was also associated with a reduced risk of LOAD ref [8].

In line with previous haplotype and cladistic approaches described we have used the three tagging variants rs4291, rs4343 and rs1800764 to investigate the association of *ACE* with LOAD in our large multi-centre cohort comprising ten case-control series, nine of which 3,930 LOAD cases and 4,282 controls. This represents the largest study to-date to investigate the effects of *ACE* haplotypes in LOAD.

Materials and Methods

European Patient Samples

Informed consent was obtained from all subjects included in this study, which was approved by the local Ethics Committee. This European Caucasian cohort combined three case control sample collections; 1) the Alzheimer's Research Trust (ART) Collaborative Sample Collection (1,197 LOAD patients and 886 controls) supplied from six ART network centres across the UK, 2) the Medical Research Council (MRC) Collaborative Sample Collection collected from both community and hospital settings in the UK (816 LOAD patients and 959 controls) and 3) a Swedish sample collection (156 LOAD patients and 59 controls). It must be noted that nine of the ten case-control series used in this cohort do not overlap with those previously published in case-control association studies of *ACE* variants. The remaining series (Oxford) has previously been reported with respect to the *ACE* indel (rs1799752) but not for the three tagging SNPs investigated here. A summary of patient details from each centre is shown in Table 1.

US Patient samples

A total of 4,139 Caucasian samples were obtained with written consent from the Mayo Clinic, USA. These samples included 592 autopsy-confirmed AD patients and 374 autopsy-confirmed controls (AUT), 1,169 clinically diagnosed LOAD patients and 976 controls from Mayo Clinic Jacksonville (JS) and Mayo Clinic, Rochester (RS). None of the samples used in this cohort overlap with those previously published in case-control association studies of *ACE* variants. Further information regarding these samples can be found in Table 1.

LOAD Diagnosis

The majority of samples were diagnosed possible or probable AD (n=3,215) or control (n=3,968) using NINCDS-ADRDA criteria [23]. The remaining samples were histopathologically confirmed as definite AD (n=1,091) or control (n=476) using NINCDS-ADRDA (AUT) or CERAD criteria (ART) [24]. All patients with evidence of an autosomal dominant AD trait, where a first-degree relative had been diagnosed with AD or where there was evidence for other causes of dementia were excluded.

Genotyping

Genotyping data from the ART samples was obtained using fluorescently-labelled (VIC or FAM) allele-specific TaqMan probes that were designed by ABI; all assays performed by Geneservice (Cambridge, UK). In addition to assay controls incorporated by Geneservice, 15% of the samples assayed were sequenced for genotype at source, 10% of samples were assayed in duplicate for quality assurance. Data were only accepted when there was 100% concordance between duplicates. All genotype plots were subjected to quality control upon receipt and assays were only accepted when call rates were above 95%. A detailed description of the ascertainment and assessment of the MRC sample collection has been reported previously [25]. The data from the Swedish samples was generated using the Dynamic Allele-Specific Hybridization (DASH) method as described elsewhere [8]. The Mayo Clinic samples were genotyped using TaqMan® SNP Genotyping Assays in an ABI PRISM® 7900HT Sequence Detection System with 384-Well Block Module from Applied Biosystems. All variants passed the $p>0.01$ cut-off for deviation from Hardy-Weinberg equilibrium as suggested by Wigginton et al. when investigating >100 samples [26].

Single variant analysis

Odds ratios and 95% CI were calculated by binary logistic regression (allelic dose model) using the --logistic command in PLINK software [27]. The covariates age-at-onset (where unknown, age-at-death minus the 8 year average disease duration was used), carriage of the *APOE* ε4 allele and sex were added into the model using the --covar command. The total dataset was also tested for association by binary logistic regression under dominant and recessive models adjusted for covariates using StatsDirect v2.5.8. For meta-analyses, summary ORs, 95% CI and Breslow-Day tests were calculated under the DerSimonian and Laird (1986) random-effects model using StatsDirect v2.5.8 software.

Haplotype association

Haplotype frequencies were estimated using the expectation-maximization approach implemented in the haplo.em function of Haplo.stats v1.2.2 [28] using R programming software. Global haplotype association and individual haplotype score tests adjusted for *APOE* ε4 dose, sex and age-at-diagnosis were performed using the haplo.score function of Haplo.stats v1.2.2.

Results

We tested three *ACE* variants for association with LOAD in our seven European and three North American case-control series (series details are shown in Table 1). Genotype and allele counts for each series are shown in Table 2. We first tested for association with LOAD in each case-control series by logistic regression using an additive/allelic dosage model correcting for sex, age-at-diagnosis and possession of at least one copy of the *APOE* ε4 allele as covariates (Table 2). None of the variants were associated with LOAD risk in any series (all $p>0.09$). We also tested for association the three variants in all ten series pooled (n=8,212) but again found no association (all $p>0.18$). Since some genetic variants may

exert dominant or recessive effects we also performed logistic regression for the total dataset using these models but found no association with LOAD risk (all p>0.36). We also tested for association of all three variants with LOAD in individuals not possessing the *APOE* ε4 allele in all series (1,495 LOAD, 3,175 controls) but found no association (all p>0.13; data not shown). In order to determine whether the lack of observation could be attributed to population heterogeneity, we performed meta-analyses of each variant testing for association in the combined data from all ten series using a DerSimonion-Laird random effects model to estimate a pooled odds ratio and testing for heterogeneity between series using the Breslow-Day test (Fig. 1). We found no evidence for population heterogeneity (all p>0.38) or for association of rs4343 (OR=1.00, p=0.90), rs4291 (OR=0.97, p=0.47) or rs1800764 (OR=0.99, p=0.72) with LOAD risk.

In an attempt to replicate previous findings that the two most common haplotypes (H1 and H2) are significantly associated with opposing risk for LOAD [8], we constructed haplotypes using the three tagging variants. As shown in Table 3, the haplotype frequencies were comparable across all series and are consistent with previous studies [8]. In order to limit the number of tests used, global association of the six haplotypes in each series were tested and individual haplotypes only tested for association for populations with a global p-value <0.05. A significant global haplotypic p-value was observed in the MRC sample only (p=0.007), largely to the protective association of H3 (OR=0.72, p=0.02) and the trend towards a risky association of H4 (OR=1.38, p=0.09) and H7 (OR=2.07, p=0.08), associations that were not previously observed by Kehoe and colleagues [8]. The global haplotypic p-value for our complete dataset (n=7,557) was not significant (p=0.51) and the individual haplotypic ORs observed in our complete dataset for the two most common haplotypes gave weaker, ORs compared to those observed by Kehoe et al in their complete dataset (H1; OR=1.0 vs OR=1.2, H2; OR=0.96 vs 0.80 [8]). Therefore, these data do not replicate the previously reported association of the *Alu* indel [12], which tags H1.

Discussion

We have conducted a large case-control study of three haplotype tagging variants in the LOAD candidate gene, *ACE*, that has previously been associated with LOAD risk [8, 29–34]. Meta-analyses of ten case-control series totalling 3,930 LOAD and 4,282 controls showed no population heterogeneity (all p>0.38) or evidence for association of rs4343 (OR=1.00 p=0.90), rs4291 (OR=0.97 p=0.47) or rs1800764 (OR=0.99 p=0.72) with LOAD risk.

We also tested for association of six *ACE* haplotypes with LOAD but found no evidence for association in the total dataset (global p=0.51). However, we did observe a significant global haplotypic p-value of 0.007 in one of the series (the MRC population - 1,430 LOAD patients and 1,611 controls) from the UK, which was largely due to a novel protective association of H3, the haplotype containing the major allele (G-A-T) at all three variants (9% LOAD, 11% Controls, OR=0.72, p=0.02). However, this association was not observed in any of the other case-control populations (all p>0.07) or in the pooled dataset (p=0.58). Indeed the directionality of ORs observed in all other populations was opposite to that seen for H3 (OR=1.03–1.25) but none produced significant findings. The modest p-value (p=0.03) for association of H3 with LOAD, which would not survive Bonferroni correction for the six haplotypes studied (p<0.008) along with the lack of association of H3 in the other nine populations studied here or in previously published studies suggests that the significance of this association should be treated with caution.

The lack of association in any of our ten series for the two *ACE* variants that have been significantly associated with LOAD following AlzGene meta-analyses is perhaps not

surprising. None of the eight Caucasian populations used previously to study rs4291 and included in AlzGene showed significant association for AD and only one [35] of the seven Caucasian populations used to study rs1800764 showed significant association (OR=0.86 95% CI 0.74–0.99). Despite this, AlzGene reported significant association for both rs4291 (OR=0.87, 95%CI 0.80–0.95) and rs1800764 (OR=0.84, 95%CI 0.77.0.92) prior to this study. This is largely due to the fact that there was a consistently similar directionality of the ORs in the majority of the Caucasian populations previously used to study rs4291 and rs1800764 and the resulting increase in sample size achieved by analyzing the studies together provides sufficient power to detect association. When the present data is eventually incorporated into Alzgene these overall associations will likely diminish further towards the null. However, the fact that we also observed same direction ORs in seven out of ten series for rs4291 and six out of ten series for rs1800764, raises the possibility that a true association of modest effect size (OR~0.90) is present, but which requires even larger studies to gain sufficient power for detection.

It is possible that the initial association may have, by chance, been the result of an over-estimation of the effect size of these variants that has since diminished in subsequent follow-up case-control studies. For example, in the case of rs4291, the initial study reported ORs ranging from 0.76–0.84 in four Caucasian populations each consisting of ~400 subjects [8]. In comparison, the four subsequent Caucasian studies (all of equal or larger size than the initial study populations) reported ORs ranging from 0.76–1.00 [31, 32, 36, 37] and here we report ORs ranging from 0.80–1.28 in ten populations of equal or larger size than the initial study thus further diminishing the effect size. The same appears true for rs1800764 where the initial study reported ORs ranging from 0.74–0.84 [8], compared to ORs ranging from 0.80–1.09 in subsequent follow-up studies [31, 38, 39] and in the ORs 0.83–1.07 reported here. This further supports the need for multiple, large follow-up studies and meta-analyses of all data to reduce the likelihood of an over-estimation of the effect size.

Failure to detect association could also be explained by these variants merely tagging one or more truly functional variants. In such instances the corresponding degree of linkage disequilibrium between these variants could differ between series leading to weaker and/or opposing effects. However, given the Caucasian background of all these series and the lack of significant population heterogeneity or association with LOAD risk for any variant in any individual population, this possibility seems unlikely.

If *ACE* variants modify LOAD risk this effect may be dependent on interactions with other environmental/phenotypic background. For example, ACE mediates hypertensive effects by its function on angiotensin I to convert it to the vasoactive angiotensin II [4]. Thus the co-occurrence of hypertension in sub-groups of AD patients and controls and this being treated to varying extents by drugs that target the pathway in which ACE is functional is likely to be a confounding variable in studies for *ACE*. Indeed studies of AD brain tissue have shown that while *ACE* genotypes did not influence levels or activity of brain ACE [40], rs4343 and rs1800764 have been associated with soluble Aβ levels [41] and exposure of neuronal SH-SY5Y cell lines to oligomeric Aβ1–42 for 24 hours resulted in significant increases in ACE protein level and activity [41]. These collectively suggest that along with previous evidence of elevated ACE activity in AD brain [40, 42] that there could be phenotypic-specific post-translational changes to ACE that contribute to AD pathogenesis. Further information supporting this are the findings of Ellul and colleagues [43]. They noted in a longitudinally assessed clinical cohort, that drugs affecting the Renin Angiotensin System, in which ACE is very important, can slow the rates of deterioration of AD and could serve as a confounder in clinical outcome measurement in clinical trials. The possibility of phenotype-specific pharmacogenetic considerations are also reinforced by findings from a recent GWAS [44] of two young-onset hypertension populations totalling 1,023 subjects that reported eight *ACE*

variants were significantly associated with ACE activity and rs4343 showing the strongest association ($p=3.0\times10^{-25}$). In the same study an association between blood type and ACE activity in an independent young-onset hypertension family study (n=428) was reported and showed a potential differential blood pressure response to anti-hypertensive treatment (ACE inhibitors) in subjects dependent on *ACE* genotype. This latter observation is particularly important in view of findings from a number of observational studies where anti-hypertensive treatments such as inhibitors of ACE activity (i.e. ACE-inhibitors) or of angiotensin II function (i.e. angiotensin II receptor antagonists – ARAs) appeared to be protective against the development of and/or progression of LOAD and Mild Cognitive Impairment (MCI) [45–51].

These data do not replicate previously reported haplotype associations with LOAD risk in a large case-control series of 3,930 LOAD patients and 4,282 controls. However, it is clear that when considered alongside data from other disciplines, these findings contribute just one important part of what appears to be a highly complex interaction between *ACE* genetics, phenotype and pharmacological effects in AD and which traditional case-control studies are not equipped to unpick. Larger studies which would include richer phenotypic data that would allow for more accurate adjustment for confounders and where possible incorporation of additional measurements specific to candidate gene function (e.g. qPCR, protein based measurements, etc), would likely increase the chances of unpicking the real genetic involvement of many current candidate genes.

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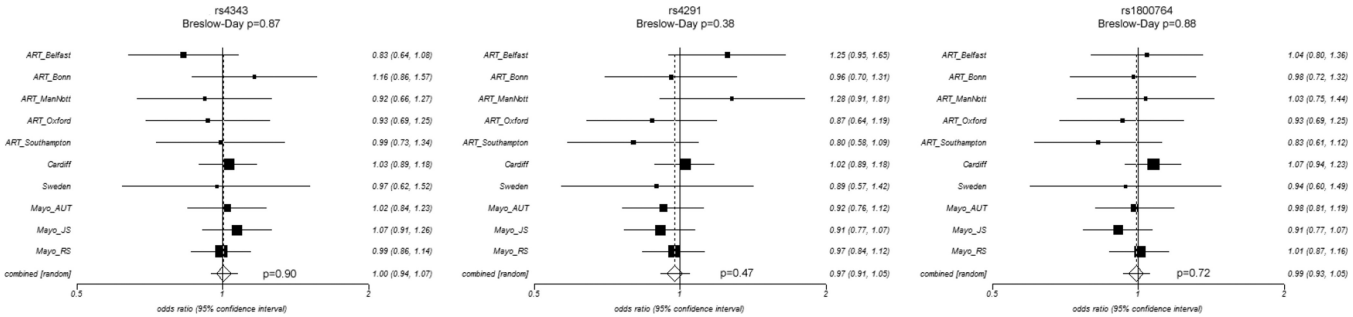

Figure 1. Forest plots for meta-analysis for each variant

ORs (boxes) and 95%CI (whiskers) are plotted for each series and shown on the right of each plot. Combined OR is the overall OR calculated by the meta-analysis using a random effects model. P-values from Breslow-Day tests of heterogeneity and meta-analysis are provided.

Table 1

Details of samples used in this study.

Ethnicity	Sample Collection	Series	N			Mean Age		% Female		%E4+	
			LOAD	CTRL	Total	LOAD	CTRL	LOAD	CTRL	LOAD	CTRL
Caucasian European	ART	Belfast	238	236	474	78.3	76.1	0.66	0.65	0.58	0.28
		Bonn	175	198	373	75.9	70.9	0.76	0.49	0.55	0.27
		Nottingham/Manchester	381	102	483	71.0	72.6	0.54	0.34	0.62	0.23
		Oxford	173	205	378	72.7	77.3	0.56	0.57	0.64	0.25
		Southampton	230	145	375	80.6	75.8	0.58	0.49	0.47*	0.26
	MRC	UK	816	959	1,775	81.5	76.6	0.72	0.64	0.63	0.24
	Sweden	Sweden	156	59	215	77.4	73.9	0.70	0.41	0.68	0.31
Caucasian North American	Mayo Clinic	Autopsy-confirmed	592	374	966	81.2	75.7	0.60	0.43	0.60	0.23
		Jacksonville	608	602	1210	78.1	78.0	0.62	0.60	0.62	0.26
		Rochester	561	1,402	1,963	79.5	78.4	0.62	0.54	0.56	0.25
Caucasian	**All**	**Total**	**3,930**	**4,282**	**8,212**	**78.6**	**76.9**	**0.64**	**0.56**	**0.61**	**0.25**

* *APOE* E4 status was unknown for 50% of LOAD samples from Southampton

The number of LOAD patients (LOAD) and controls (CON), mean age-at-diagnosis, percentage that are female and percentage that possess at least one copy of the *APOE* ε4 allele are given for each individual and pooled series. Mean age is given as age-at-diagnosis/entry with the standard deviation (SD) from the mean in parentheses.

Table 2

Association of *ACE* variants rs4291, rs4343 and rs1800764 with LOAD

	Series	MAF	LOAD			CON			Logistic Regression	
		LOAD:CON	Maj	Het	Min	Maj	Het	Min	OR (95%CI)	p
rs4343 (G:A)	Belfast (474)	0.51:0.56	60	113	65	50	109	77	0.82 (0.63–1.07)	0.14
	Bonn (373)	0.52:0.48	42	84	49	56	93	49	1.17 (0.85–1.62)	0.33
	Notts./Manch. (483)	0.49:0.51	92	197	87	28	42	31	0.90 (0.64–1.25)	0.52
	Oxford (378)	0.47:0.48	50	84	38	57	98	50	0.88 (0.64–1.21)	0.43
	Southampton (375)	0.45:0.45	68	116	46	45	67	32	1.01 (0.72–1.42)	0.93
	MRC (1,775)	0.50:0.49	205	358	200	233	444	216	1.05 (0.90–1.22)	0.51
	Sweden (226)	0.48:0.49	40	81	35	19	22	18	1.22 (0.75–1.97)	0.42
	AUT (966)	0.47:0.46	166	283	129	112	167	85	1.02 (0.84–1.25)	0.88
	JS (1,210)	0.48:0.49	159	302	132	172	295	125	1.05 (0.95–1.16)	0.30
	RS (1,963)	0.48:0.46	144	259	145	336	693	344	1.04 (0.87–1.24)	0.67
	All Additive (8,212)	**0.49:0.49**	**1026**	**1877**	**926**	**1108**	**2030**	**1027**	**0.99 (0.93–1.05)**	**0.75**
	All Dominant								**1.02 (0.91–1.14)**	**0.75**
	All Recessive								**1.02 (0.90–1.14)**	**0.80**
Rs4291 (A:T)	Belfast (474)	0.37:0.32	96	110	32	114	91	28	1.28 (0.96–1.70)	0.09
	Bonn (373)	0.35:0.36	71	81	19	83	87	27	0.84 (0.60–1.20)	0.35
	Notts./Manch. (483)	0.37:0.31	159	158	60	48	44	10	1.31 (0.92–1.87)	0.13
	Oxford (378)	0.35:0.39	71	75	22	75	97	29	0.94 (0.67–1.31)	0.88
	Southampton (375)	0.37:0.42	92	106	32	50	65	28	0.74 (0.52–1.05)	0.09
	MRC (1,775)	0.37:0.36	323	341	117	364	416	118	1.00 (0.85–1.16)	0.91
	Sweden (226)	0.37:0.40	59	78	19	26	19	14	0.77 (0.47–1.23)	0.27
	AUT (966)	0.37:0.38	242	256	85	143	171	57	0.93 (0.76–1.14)	0.50
	JS (1,210)	0.37:0.39	236	292	78	217	294	88	0.96 (0.80–1.16)	0.58
	RS (1,963)	0.35:0.35	241	246	70	586	628	178	0.94 (0.80–1.10)	0.42

	Series	MAF	LOAD			CON			Logistic Regression	
		LOAD:CON	Maj	Het	Min	Maj	Het	Min	OR (95%CI)	p
	All Additive (8,212)	**0.36:0.37**	**1590**	**1743**	**534**	**1706**	**1912**	**577**	**0.99 (0.92–1.05)**	**0.67**
	All Dominant								**0.95 (0.86–1.06)**	**0.36**
	All Recessive								**0.97 (0.84–1.12)**	**0.68**
rs1800764 (T:C)	Belfast (474)	0.41:0.40	82	117	39	92	98	45	1.03 (0.76–1.35)	0.84
	Bonn (373)	0.43:0.44	53	92	29	66	91	41	0.86 (0.61–1.20)	0.37
	Notts./Manch. (483)	0.43:0.42	124	183	70	32	52	16	1.08 (0.76–1.53)	0.66
	Oxford (378)	0.44:0.46	55	84	34	59	104	42	0.96 (0.69–1.33)	0.96
	Southampton (375)	0.47:0.52	60	124	46	39	62	44	0.85 (0.61–1.20)	0.37
	MRC (1,775)	0.44:0.43	262	378	170	316	451	176	1.07 (0.93–1.25)	0.34
	Sweden (226)	0.43:0.45	46	84	25	22	19	16	0.77 (0.48–1.26)	0.30
	AUT (966)	0.47:0.48	169	276	137	104	180	87	1.02 (0.80–1.14)	0.78
	JS (1,210)	0.45:0.47	179	308	113	167	296	130	0.95 (0.80–1.14)	0.57
	RS (1,963)	0.43:0.43	183	261	109	462	655	272	1.00 (0.86–1.15)	0.96
	All Additive (8,212)	**0.44:0.44**	**1213**	**1907**	**772**	**1359**	**2008**	**869**	**1.00 (0.94–1.07)**	**0.95**
	All Dominant								**1.02 (0.92–1.14)**	**0.69**
	All Recessive								**0.97 (0.86–1.10)**	**0.64**

Designated major:minor alleles for each variant are shown in parentheses after the variant name in the first column. The number of samples in each series is shown in parentheses after the series name. The minor allele frequency (MAF) and genotype counts in LOAD and controls (CON) for major allele homozygotes (Maj), heterozygotes (Het) and minor allele homozygotes (Min) are provided for each series. Odds ratios (ORs), 95% confidence intervals (CI) and p-values (p) were calculated for each series using a binary logistic regression additive model. The total pooled data (All) was also tested for association using dominant and recessive models. All logistic regression models included age-at-onset, sex and *APOE* ε4 allele as covariates.

Table 3

Association of *ACE* haplotypes with LOAD

Series	Haplotype*	Frequency		OR	95%CI	p-value
		LOAD	CTRL			
ART global p-value = 0.27	H1 - AAT	973 (0.46)	810 (0.47)	0.96	0.83 – 1.10	
	H2 - GTC	722 (0.34)	596 (0.34)	1.04	0.90 – 1.21	
	H3 - GAT	236 (0.11)	157 (0.09)	1.25	0.99 – 1.59	
	H4 - GAC	236 (0.11)	270 (0.16)	0.78	0.57 – 1.07	
	H7 - AAC	44 (0.02)	35 (0.02)	0.89	0.52 – 1.52	
	H5 - ATC	30 (0.01)	29 (0.02)	0.76	0.41 – 1.41	
MRC global p-value = 0.007	H1 - AAT	671 (0.47)	760 (0.47)	1.00	0.85 – 1.18	0.99
	H2 - GTC	500 (0.35)	551 (0.34)	1.05	0.88 – 1.25	0.59
	H3 - GAT	129 (0.09)	174 (0.11)	0.72	0.54 – 0.96	0.02
	H4 - GAC	87 (0.06)	83 (0.05)	1.38	0.95 – 1.99	0.09
	H7 - AAC	20 (0.01)	24 (0.02)	2.07	0.92 – 4.63	0.08
	H5 - ATC	23 (0.02)	19 (0.01)	0.88	0.40 – 1.93	0.75
Sweden global p-value = 0.86	H1 - AAT	144 (0.47)	54 (0.47)	1.37	0.57 – 3.28	
	H2 - GTC	115 (0.37)	45 (0.39)	0.70	0.18 – 2.76	
	H3 - GAT	32 (0.10)	9 (0.08)	1.18	0.72 – 1.95	
	H4 - GAC	13 (0.04)	4 (0.03)	1.02	0.15 – 7.01	
	H7 - AAC	6 (0.02)	2 (0.02)	0.81	0.50 – 1.31	
	H5 - ATC	0 (0.00)	0 (0.00)	NA	NA	
Mayo Clinic global p-value = 0.31	H1 - AAT	1478 (0.45)	2043 (0.45)	1.01	0.91 – 1.12	
	H2 - GTC	1140 (0.34)	1593 (0.35)	0.92	0.83 – 1.02	
	H3 - GAT	350 (0.11)	439 (0.10)	1.05	0.88 – 1.24	
	H4 - GAC	212 (0.06)	272 (0.06)	1.03	0.83 – 1.28	
	H7 - AAC	79 (0.02)	83 (0.02)	1.45	1.00 – 2.09	
	H5 - ATC	46 (0.01)	61 (0.01)	1.38	0.85 – 2.21	

Series	Haplotype*	Frequency		OR	95%CI	p-value
		LOAD	CTRL			
All global p-value = 0.51	H1 - AAT	3267 (0.46)	3667 (0.46)	1.00	0.93 – 1.07	
	H2 - GTC	2477 (0.34)	2785 (0.35)	0.97	0.90 – 1.04	
	H3 - GAT	746 (0.11)	779 (0.10)	1.03	0.92 – 1.17	
	H4 - GAC	420 (0.06)	472 (0.06)	1.03	0.88 – 1.20	
	H7 - AAC	152 (0.02)	139 (0.02)	1.28	0.97 – 1.68	
	H5 - ATC	96 (0.01)	114 (0.01)	1.05	0.75 – 1.47	

*Order of variants in haplotype is as follows rs4343, rs4291, rs1800764

Haplotypes are numbered according to their frequency in the Kehoe et al study [8] (only haplotypes with a frequency >1% in this study are shown). Haplotype frequencies are shown for the total dataset and in each of the individual series. A haplotype score test was used to calculate a “global p-value: for the association of the haplotypes in the total dataset and in each of the individual series. ORs, 95% confidence intervals and p-values are shown for the individual haplotypes in the MRC series only due to the significant global p-value.