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Association Studies Between Risk for Late-Onset Alzheimer's Disease and Variants in Insulin Degrading Enzyme

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Linkage studies have suggested there is a susceptibility gene for late onset Alzheimer's disease (LOAD) in a broad region of chromosome 10. A strong positional and biological candidate is the gene encoding the insulin-degrading enzyme (IDE), a protease involved in the catabolism of Aβ. However, previous association studies have produced inconsistent results. To systematically evaluate the role of variation in *IDE* in the risk for LOAD, we genotyped 18 SNPs spanning a 276 kb region in and around *IDE*, including three "tagging" SNPs identified in an earlier study. We used four case-control series with a total of 1,217 cases and 1,257 controls. One SNP (IDE_7) showed association in two samples (*P*-value = 0.0066, and *P* = 0.026, respectively), but this result was not replicated in the other two series. None of the other SNPs showed association with LOAD in any of the tested samples. Haplotypes, constructed from the three tagging SNPs, showed no globally significant association. In the UK2 series, the CTA haplotype was over-represented in cases (*P* = 0.046), and in the combined data set, the CCG haplotype was more frequent in controls (*P* = 0.015). However, these weak associations observed in our series were in the opposite direction to the results in previous studies. Although our results are not universally negative, we were unable to replicate the results of previous studies and conclude that common variants or haplotypes of these variants in *IDE* are not major risk factors for LOAD.

KEY WORDS: LOAD; IDE; SNP; haplotype; association studies

INTRODUCTION

Type II diabetes mellitus has previously been suggested as a risk factor for late-onset Alzheimer's disease (LOAD), because of their frequent co-occurrence [Ott et al., 1999; Arvanitakis et al., 2004]. A recent study [Watson et al., 2003] has shown that boosting insulin levels in human subjects increases β-amyloid levels in cerebrospinal fluid, suggesting that chronically high insulin levels may accelerate the accumulation of β-amyloid in the brain as amyloid plaques, one of the hallmarks of Alzheimer's disease. It has been proposed that there might be competition between insulin and β-amyloid for degradation by the protease insulin-degrading enzyme (IDE). IDE degrades both extracellular amyloid β-protein (Aβ) [Qiu et al., 1998; Vekrellis et al., 2000] and the intracellular domain of APP, which is released from the precursor by γ-secretase [Edbauer et al., 2002]. Animal models have also shown that lack of IDE increases Aβ deposition in mouse brain [Farris et al., 2003; Miller et al., 2003]. Besides insulin and Aβ, IDE also hydrolyzes glucagon, atrial natriuretic factor (ANP), transforming growth factor α (TNFα), β-endorphin, and amylin [Duckworth et al., 1998]. The *IDE* gene resides at 115 cM, within a region of chromosome 10q linked to increased risk for LOAD in several studies [Bertram et al., 2000; Ertekin-Taner et al., 2000; Myers et al., 2000; Li et al., 2002; Blacker et al., 2003]. A fifth study reported linkage to the same region with elevated plasma Aβ levels, suggesting that the LOAD risk locus may influence risk for AD by modulating Aβ levels [Ertekin-Taner et al., 2000].

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Thus, *IDE* is a strong functional and positional candidate gene for LOAD.

Several groups, including ourselves, have screened variants in *IDE* for association with AD [Abraham et al., 2001; Boussaha et al., 2002; Edland et al., 2003; Prince et al., 2003; Bian et al., 2004; Ertekin-Taner et al., 2004; Sakai et al., 2004]. The results remain controversial, because some groups have reported association with specific haplotypes and the clinical diagnosis of LOAD or LOAD correlated phenotypes while others have failed to observe any association.

In an effort to resolve this controversy we have undertaken a comprehensive examination of 18 single nucleotide polymorphisms (SNPs) in and around *IDE* in multiple, large datasets. To attempt to replicate the findings of Prince et al. [2003], we included three SNPs that tagged haplotypes they had observed to be over- or underrepresented in LOAD cases versus controls.

MATERIALS AND METHODS

Clinical Samples

We used four Caucasian case-control series in this study: three clinical samples and an autopsy series. The Washington University (WashU) series was collected through the Washington University Alzheimer's Disease Research Center (ADRC) patient registry. Samples from the School of Medicine, Cardiff University, and King's College, London were collected as part of the MRC Late Onset AD Genetic Resource and combined in the UK1 sample. The third series was collected through the ADRC of the University of California, San Diego (UCSD). Cases in these series have a clinical diagnosis of dementia of the Alzheimer's type (probable/definite) according to NINCDS-ADRDA [McKhann et al., 1984] or similar criteria with a minimum age at onset of 60 years. Non-demented controls were screened for dementia and sampled from the same source populations as their matched cases. The UK2 series consists of cases and controls from North East England; cases were recruited from the Institute for Ageing and Health (IAH) case register and subsequently confirmed upon postmortem neuropathological examination. Age matched controls were neurologically normal and showed only age-associated pathology at autopsy. All four series show an expected age- and *APOE ε4*-genotype distribution. Characteristics of all of these samples are summarized in Table I. To determine whether our control samples were well matched to their respective case samples we tested for population structure as described in Li et al. [2004].

SNP Selection and Genotyping

From the Prince et al. [2003] study, we chose three intronic SNPs that tag the five most common haplotypes (>5% frequency) in the 276 kb genomic region spanning from the 3′-end of *IDE* through the 5′ end of *IDE* to the 3′-end of hematopoetically expressed homeobox gene (*HHEX*), including Kinesin-like 1 (*KNSL1*): *IDE_7* (rs2251101), *IDE_14* (rs1832196) and *HHEX_23* (rs1544210). We also genotyped a promoter polymorphism: *IDE1* from Abraham et al. [2001] (rs3758505), which is −1,002 bp upstream of ATG. The 5′–3′ orientation of *IDE* is inverted compared to the other genes in the region. Genotyping was performed using Pyrosequencing technology™. Sequences for the PCR and SNP primers for these four SNPs are available from the authors by request.

Fourteen intronic SNPs, located between the 3′-end of *IDE* and the 3′-end *HHEX* (Fig. 1) were chosen from the Celera human genome database and genotyped in the WashU sample. *IDE_7*, *IDE_14*, and *HHEX_23* were also genotyped in the WashU, UCSD, and Cardiff series using allele-specific real time PCR [Germer et al., 2000]. All markers and locations are listed in Table II.

APOE Genotyping

APOE genotyping was performed following the protocol on the Pyrosequencing™ website (http://www.pyrosequencing.com/pages/assay_register_clin_gen.html).

Statistical Analysis

Chi-square tests were used to determine whether the observed genotypic frequencies deviated from Hardy–Weinberg equilibrium in case and control samples. Logistic regression analysis was used to calculate the allelic and genotypic association between each SNP and LOAD. Odds ratios and 95% confidence intervals were calculated for allelic associations and haplotypes. Fisher's exact test (2-tail) was used to test for association in samples stratified by *APOE ε4* genotype. Linkage disequilibrium (LD) between markers was computed using the program COCAPHASE from the UNPHASED software suite [Dudbridge, 2003]. Significant associations detected in SNPs C1-C14 were subject to multiple test correction via the method of Nyholt [2004]. SNPs *IDE1*, *IDE_7*, *IDE_14*, and *HHEX_23* were used to test a prior hypothesis and were therefore not corrected for multiple testing.

Using the 18 SNPs individually, we performed a case-only analysis on age-of-onset using data from all populations (except for UK2, where the phenotype was unavailable). Note that not all SNPs were genotyped in all samples (see Tables II, IIIA,B). Analyses were performed using the ANOVA procedure in SAS and APOE (coded as presence/absence of an E4 allele) was included. We also performed age-of-onset analyses on the *HHEX23-IDE14-IDE7* haplotype with the QTPHASE program from the UNPHASED software package.

RESULTS

We conducted an intensive screen for association of LOAD with SNPs in and around *IDE* stretching over a 276 kb region of chromosome 10, a region previously linked to LOAD. Fourteen markers (Table II: C1–C14) covering the region from the 3′-end

TABLE I. Characteristics of the Samples

	Combined		WashU		UK1		UCSD		UK2	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Subjects	1,217	1,257	420	388	376	384	246	360	175	125
Female/male	778/436	811/441	262/158	231/157	287/89	289/95	112/134	230/130	117/55[a]	61/59[a]
AAO (± SD)	77.1 (7.0)	na	76.2 (6.8)	na	75.8 (7.1)	na	72.1 (6.3)	na	na	na
AAE/AAD (± SD)	na	77.6 (7.3)	na	77.7 (7.4)	na	76.5 (6.3)	na	78.6 (7.6)	81.5 (6.6)	78.2 (8.6)
APOE4+ (%)	35.9	12.7	33.2	11.8	37.2	13.4	38.5	12.5	35.8	13.6

na, not available; AAO, age of onset; AAE/AAD, age at exam or age at death.
[a]Some of the numbers do not add up because of missing data.

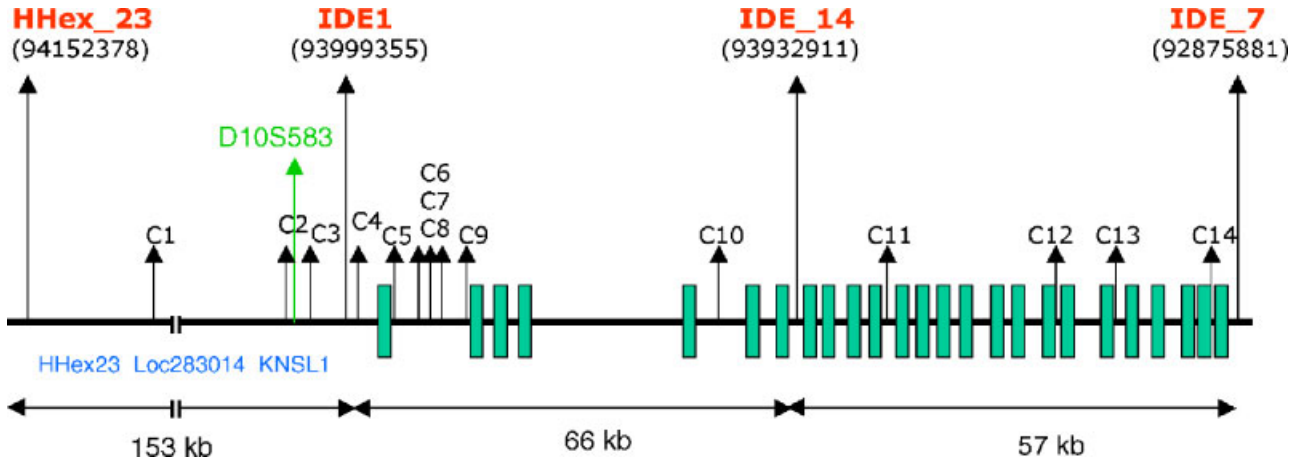

Fig. 1. Schematic map of *IDE*. The locations for SNPs C1-C14 are described in Table II. The 5′–3′ orientation of *IDE* is inverted compared to the other genes in the region, *IDE_7* is at the 3′ end and *IDE1* is at the 5′ end of the gene. The numbers in brackets below *HHEX_23*, *IDE1*, *IDE_7*, and *IDE_14* are the chromosome 10 position (bp) in the NCBI data base. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of *IDE*, *KNSL1*, to the 3′ end of *HHEX* (Fig. 1) were genotyped in the WashU case-control series (390 cases, 354 controls). All SNPs were in Hardy–Weinberg equilibrium in the whole population. *IDE_7*, *IDE_14*, and *HHEX_23* were genotyped in some individuals using two methods for quality control. The discrepancies were 2 of 446 for *IDE_7*, 4 of 443 for *IDE_14* and 5 of 455 for *HHEX_23*.

Analyses using the program *STRUCTURE* [Pritchard et al., 2000] and a selection of SNPs throughout the genome did not reveal any evidence for population stratification among the clinical case-control series (data not shown). Insufficient numbers of markers have been genotyped in the autopsy series to allow similar analyses.

None of the IDE SNPs showed significant association (*P*-value <0.05) (Table II). Linkage disequilibrium (LD) between the marker pairs was assessed and the results are summarized in Figure 2. For *IDE_7*, *IDE_14*, and *HHEX_23*, we estimated similar D′ and r^2 values to those seen in previous studies [Prince et al., 2003; Ertekin-Taner et al., 2004]. Because previous studies had reported association with *IDE* haplotypes but not individual SNPs, we then analyzed three marker sliding window haplotypes across the entire gene using the COCAPHASE program for these 14 SNPs plus *IDE_7*, *IDE_14*, and *HHEX_23*, but none of the haplotypes showed a global *P*-value <0.05 (data not shown).

The haplotype tagging SNPs from Prince et al. [2003], *IDE_7*, *IDE_14*, and *HHEX_23*, were genotyped in four different case-control series, WashU (420 cases, 388 controls), UK1 (376 cases, 384 controls), UCSD (246 cases, 360 controls), and UK2 (175 cases, 125 controls), in a total of 1,217 cases and 1,257 controls. In the UK1 and the UK2 series, *IDE_7* gave a significant allelic *P*-value of 0.0066 (OR 1.37, 1.1–1.71) and 0.026 (OR 1.51, 1.05–2.17), respectively, but this result was not replicated in the WashU or the UCSD series. *STRUCTURE* analysis using the UK1 and the UCSD samples did not show evidence for population stratification between these samples using 261 markers genome wide. Combining all samples (1,190 cases and 1,236 controls) yielded an allelic p-value of 0.014

TABLE II. Markers in and Around *IDE* and Their Allele Frequencies

Marker		Celera R27 bp position	NCBI rs number	NCBI bp position	Cases N (freq)	Controls N (freq)	Odds ratio (95% CI)	*P*-value
IDE_7		87952724	2251101	93875881				
hCV25653847	C14	87955779	4646958	93878936	66 (0.09)	63 (0.09)	0.96 (0.67–1.37)	0.811
hCV12116624	C13	87965584	1887922	93888742	139 (0.18)	121 (0.17)	1.05 (0.81–1.37)	0.713
hCV22271787	C12	87971331	4646957	93894489	308 (0.37)	252 (0.34)	1.14 (0.94–1.39)	0.192
hCV1819814	C11	87998635	7084090	93921795	139 (0.18)	120 (0.17)	1.06 (0.81–1.39)	0.659
IDE_14		88009750	1832196	93932911				
hCV1819799	C10	88012596			166 (0.20)	142 (0.19)	1.06 (0.84–1.35)	0.607
hCV11194366	C9	88046690			197 (0.24)	181 (0.24)	0.98 (0.78–1.22)	0.841
hCV115183	C8	88059144			209 (0.27)	190 (0.27)	1 (0.80–1.25)	0.995
hCV11194331	C7	88061129			145 (0.19)	130 (0.18)	1.01 (0.79–1.30)	0.916
hCV12116611	C6	88061653	1999764	93984716	63 (0.08)	61 (0.09)	0.93 (0.65–1.35)	0.716
hCV11194313	C5	88068650	7100623	93991733	146 (0.19)	120 (0.17)	1.12 (0.86-1.47)	0.402
hCV22272896	C4	88075450			145 (0.19)	129 (0.19)	1.01 (0.79–1.30)	0.926
IDE1		88076273	3758505	93999355				
hCV22273765	C3	88108739			319 (0.38)	278 (0.37)	1.05 (0.86–1.28)	0.657
hCV1819830	C2	88136908			285 (0.37)	253 (0.37)	1.04 (0.84–1.28)	0.742
hCV116179	C1	88170979	7918084	94094044	372 (0.44)	323 (0.43)	1.07 (0.87–1.30)	0.532
HHEX_23		88229203	1544210	94152378				

The locations of the SNPs on chromosome 10 in the Celera map (R27) and in the NCBI map (Build 34.3, when available) are shown. The SNPs were genotyped in the WashU sample. The case and controls counts and frequencies of the minor allele, the odds ratios and the *P*-values are listed. The results for *IDE1*, *IDE_7*, *IDE_14*, and *HHEX_23* are shown in Table IIIA,B.

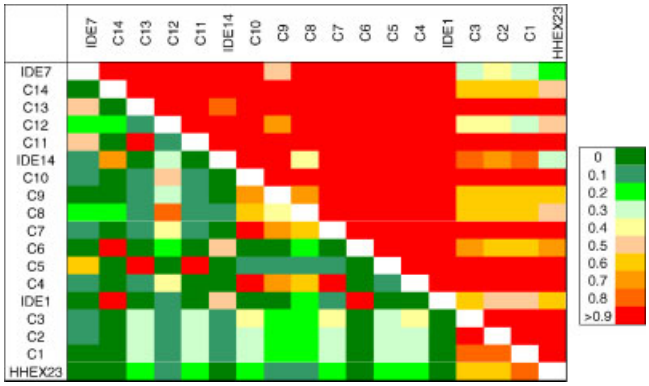

Fig. 2. Linkage disequilibrium between markers in and around *IDE*. The table for the figure was computed with COCAPHASE; EM algorithm was used to estimate frequencies. The upper-right half shows the D′ values, the lower-left half the r^2 values. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(OR 1.17, 1.03–1.33) for *IDE_7* (Table IIIB). *IDE_14* and *HHEX_23* did not show significant differences in the allelic distributions between cases and controls in any single series or in the combined dataset in our study (Table IIIB).

IDE1 was genotyped in two smaller sample sets: WashU (235 cases, 228 controls) and UK1 (317 cases, 324 controls). This SNP did not show significant association with LOAD either (Table IIIA), a result consistent with those from the Abraham et al. [2001] study, which did not find any association with SNPs in *IDE*, including *IDE1* and LOAD. We also stratified each analyses by the presence or absence of *APOE ε4* alleles; none were significant (data not shown). This is in contrast to the Edland et al. [2003] study, in which *IDE1* was associated with AD within the *ε4*-negative subjects (*P*-value $\leq$0.02). Age-of-onset analysis with single SNPs did not show a significant *P*-value.

In an effort to replicate the results of Prince et al., we then used the tagging SNPs (*IDE_7*, *IDE_14*, and *HHEX_23*) to generate haplotypes. The haplotypic association produced non-significant global *P*-values in all four case control series. We also did the age-of-onset analysis with these haplotypes, but these were not significant. In the UK2 series, the CTA haplotype was significantly over-represented in cases (OR = 2.78, $P = 0.046$, 1.09–7.1) (Table IVA) and in the combined data set the CCG haplotype was over-represented in the controls (OR = 0.75, $P = 0.015$, 0.61–0.93) (Table IVB). However, both of these results are in the opposite direction to the results of previous studies: the CTA haplotype corresponds to H5 haplotype in other studies, which showed higher frequencies in the controls of set B and E [Prince et al., 2003] and reduced $A\beta_{42}$ levels in the LOAD families [Ertekin-Taner et al., 2003]. It was also associated with lower CSF-Tau in two sets and later age-of-onset in three sets [Prince et al., 2003]. The CCG haplotype corresponds to the H8 haplotype in the Prince et al. [2003] and in the Ertekin-Taner et al. [2004] studies. In the Prince study this haplotype was associated with higher frequency in the cases but also higher MMSE scores. In the study of Ertekin-Taner et al. [2004] it was associated with increased risk for AD in one of their series and elevated $A\beta_{42}$ levels in the LOAD families.

We also used cladistic analyses to investigate the evolutionary relationships between haplotypes and test for association with LOAD [Templeton, 1995; Templeton et al., 2005]. While these analyses yielded no significant associations with LOAD, the cladograms showed evidence of homoplasy throughout the region (for more information about methods and results see supplementary data).

DISCUSSION

The *IDE* gene resides at 115 cM within a region of chromosome 10 that has been linked to an increased risk for LOAD, LOAD age of onset, and elevated plasma $A\beta_{42}$ levels [Bertram et al., 2000; Ertekin-Taner et al., 2000; Myers et al., 2000; Li et al., 2002; Blacker et al., 2003]. The reported linkage regions cover a broad region of the long arm of chromosome 10. While Myers et al. [2000], Ertekin-Taner et al. [2000], and Blacker et al. [2003] reported linkage peaks around ~80–100 cM, Li et al. [2002] and Bertram et al. [2000] reported linkage peaks between 115 and 127cM, which would correspond well with the *IDE* region. Subsequent linkage studies using plasma Aβ levels have also suggested that there may be two linkage signals on chromosome 10, one near 80 cM and a second peak around 140 cM [Ertekin-Taner et al., 2003]. Thus, it remains unclear whether there is more than one gene on the long arm of chromosome 10 involved in the development of LOAD and/or elevated plasma Aβ levels.

The position of *IDE* and its biology make it a strong candidate for LOAD and for plasma Aβ levels. We found statistically significant associations between risk for LOAD and *IDE_7* in the UK1 and the UK2 series but this result did not replicate in our two other sample sets. When we combined the results of all samples in our study in a meta-analysis the *P*-value for *IDE_7* was still significant.

Given that IDE degrades a number of peptides [Duckworth et al., 1998], the effect of IDE expression or activity on Aβ degradation may be influenced by the levels of these peptides. For example, it is known that insulin and Aβ compete for degradation in vitro [Qiu et al., 1998; Vekrellis et al., 2000]. Furthermore, two recent studies showed significant association between *IDE_7* (rs2251101) or a marker (rs1887922) in high LD with *IDE_7* and plasma insulin levels or type II diabetes [Karamohamed et al., 2003; Gu et al., 2004]. Given these observations, insulin levels and type II diabetes status

TABLE IIIA. Genotypic and Allelic Distribution of *IDE1*

			Genotypes			Alleles				
SNP	Sample	N	AA	AC	CC	A	C	Allelic *P*-value	OR	95% CI
IDE1	UK1									
	Cases	317	268 (0.85)	49 (0.15)	0	585 (0.92)	49 (0.08)			
	Controls	324	272 (0.84)	49 (0.15)	3 (0.01)	593 (0.92)	55 (0.08)	0.62	1.11	0.74–1.66
	WashU									
	Cases	235	203 (0.86)	30 (0.13)	2 (0.01)	436 (0.93)	34 (0.07)			
	Controls	228	201 (0.87)	23 (0.11)	4 (0.02)	425 (0.93)	31 (0.07)	0.8	0.935	0.57–1.55
	All									
	Cases	552	471 (0.85)	79 (0.14)	2 (0.004)	1021 (0.92)	83 (0.08)			
	Controls	552	473 (0.86)	72 (0.13)	7 (0.01)	1018 (0.92)	86 (0.08)	0.81	1.04	0.76–1.42

TABLE IIIB. Genotypic and Allelic Distribution of *IDE_7*, *IDE_14*, and *HHEX_23*

			Genotypes			Alleles				
SNP	Sample	N	AA	AG	GG	A	G	Allelic *P*-value	OR	95% CI
IDE_7	WashU									
	Cases	413	220 (0.53)	159 (0.38)	34 (0.09)	599 (0.73)	227 (0.27)			
	Controls	380	191 (0.50)	162 (0.43)	27 (0.07)	544 (0.72)	216 (0.28)	0.68	1.05	0.84–1.31
	UK1									
	Cases	375	210 (0.56)	143 (0.38)	22 (0.06)	563 (0.75)	187 (0.25)			
	Controls	383	190 (0.50)	147 (0.38)	46 (0.12)	527 (0.69)	239 (0.31)	**0.0066**	**1.37**	**1.09–1.71**
	UCSD									
	Cases	237	117 (0.49)	108 (0.46)	12 (0.05)	342 (0.72)	132 (0.28)			
	Controls	356	188 (0.53)	137 (0.38)	31 (0.09)	513 (0.72)	199 (0.28)	0.97	1	0.78–1.30
	UK2									
	Cases	165	89 (0.54)	64 (0.39)	12 (0.07)	242 (0.73)	88 (0.27)			
	Controls	117	48 (0.41)	55 (0.47)	14 (0.12)	151 (0.65)	83 (0.35)	**0.026**	**1.51**	**1.05–2.17**
	All									
	Cases	1190	636 (0.53)	474 (0.40)	80 (0.07)	1746 (0.73)	634 (0.27)			
	Controls	1236	617 (0.50)	501 (0.40)	118 (0.10)	1735 (0.70)	737 (0.30)	**0.014**	**1.17**	**1.03–1.33**
			CC	CT	TT	C	T			
IDE_14	WashU									
	Cases	413	292 (0.71)	115 (0.28)	6 (0.01)	699 (0.85)	127 (0.15)			
	Controls	381	286 (0.75)	85 (0.22)	10 (0.03)	657 (0.86)	105 (0.14)	0.37	0.88	0.67–1.16
	UK1									
	Cases	376	272 (0.73)	99 (0.26)	5 (0.01)	643 (0.86)	109 (0.14)			
	Controls	384	278 (0.72)	100 (0.26)	6 (0.02)	656 (0.85)	112 (0.15)	0.96	1.0	0.76–1.34
	UCSD									
	Cases	233	176 (0.76)	53 (0.23)	4 (0.02)	405 (0.87)	61 (0.13)			
	Controls	355	265 (0.75)	82 (0.23)	8 (0.02)	612 (0.86)	98 (0.14)	0.73	1.06	0.75–1.5
	UK2									
	Cases	162	123 (0.76)	34 (0.21)	5 (0.03)	280 (0.86)	44 (0.14)			
	Controls	110	91 (0.83)	18 (0.16)	1 (0.01)	200 (0.91)	20 (0.09)	0.11	0.64	0.36–1.11
	All									
	Cases	1184	863 (0.73)	301 (0.25)	20 (0.02)	2027 (0.86)	341 (0.14)			
	Controls	1230	920 (0.75)	285 (0.23)	25 (0.02)	2125 (0.86)	335 (0.14)	0.43	0.94	0.8–1.1
			CC	CT	TT	C	T			
HHEX_23	WashU									
	Cases	415	117 (0.28)	204 (0.49)	94 (0.23)	438 (0.53)	392 (0.47)			
	Controls	385	109 (0.28)	185 (0.48)	91 (0.24)	403 (0.52)	367 (0.48)	0.86	1.02	0.84–1.24
	UK1									
	Cases	373	102 (0.27)	190 (0.51)	81 (0.22)	394 (0.53)	352 (0.47)			
	Controls	383	113 (0.30)	178 (0.46)	92 (0.24)	404 (0.53)	362 (0.47)	0.98	1	0.82–1.23
	UCSD									
	Cases	226	50 (0.22)	132 (0.58)	44 (0.19)	232 (0.51)	220 (0.49)			
	Controls	359	93 (0.26)	182 (0.51)	84 (0.23)	368 (0.51)	350 (0.49)	0.98	1	0.79–1.27
	UK2									
	Cases	159	36 (0.23)	84 (0.53)	39 (0.24)	156 (0.49)	162 (0.51)			
	Controls	112	33 (0.30)	52 (0.46)	27 (0.24)	118 (0.53)	106 (0.47)	0.41	0.87	0.61–1.22
	All									
	Cases	1173	305 (0.26)	610 (0.52)	258 (0.22)	1220 (0.52)	1126 (0.48)			
	Controls	1239	348 (0.28)	597 (0.48)	294 (0.24)	1293 (0.52)	1185 (0.48)	0.90	0.99	0.89–1.11

TABLE IVA. Haplotypes in the UK2 Sample

Haplotype	Corresponding haplotype*	Cases	Freq	Controls	Freq	*P*-value	OR	95% CI
CCA	H1(+H9)	100	0.34	72	0.35	0.76	1	
CCG	H8	25	0.083	29	0.14	0.052	0.61	0.33–1.1
CTA	H5(+H10)	24	0.08	6	0.03	**0.046**	**2.78**	**1.09–7.1**
TCA	H2(+H7)	76	0.25	46	0.21	0.24	1.25	0.77–2.02
TCG	H3(+H6)	56	0.18	44	0.21	0.31	0.93	0.57–1.53
TTA	H4	19	0.06	13	0.06	0.44	1.08	0.5–2.33

Analysis for Markers *HHEX_23*, *IDE_14* and *IDE_7*, UK sample only, reference haplotype is CCA, global *P*-value = 0.12.
*Corresponding haplotypes in Prince et al. [2003].

TABLE IVB. Haplotypes in the Combined Data Set

Haplotype	Corresp. haplotype*	Cases	Freq	Control	Freq	*P*-value	OR	95% CI
CCA	H1(+H9)	771	0.34	784	0.33	0.40	1	
CCG	H8	196	0.086	265	0.11	**0.015**	**0.75**	**0.61–0.93**
CTA	H5(+H10)	220	0.1	212	0.09	0.36	1.06	0.85–1.3
TCA	H2(+H7)	564	0.25	587	0.24	0.46	0.98	0.84–1.14
TCG	H3(+H6)	416	0.18	456	0.19	0.32	0.93	0.79–1.1
TTA	H4	114	0.05	120	0.05	0.66	0.97	0.73–1.27

Analysis for Markers *HHEX_23*, *IDE_14* and *IDE_7*, all samples combined, reference haplotype is CCA, global *P*-value = 0.21.
*Corresponding haplotypes in Prince et al. [2003].

could confound or contribute to the inconsistent results observed in the LOAD studies. Self-report of diabetes was available in two of our data sets; in the WashU and the UK1 samples the rate of type II diabetes was lower (10% and 8.5% vs. 16%) than the population norm but equal between cases and controls. In the UK1 data set we tested for confounding effects of a possible association of *IDE_7* with diabetes. We observed no evidence of association between variation in *IDE_7* and diabetes either with LOAD ($\chi^2 = 1.745$, $P = 0.418$) or without ($\chi^2 = 0.649$, $P = 0.723$), indeed, prevalence of case/carer reported diabetes did not differ between cases and controls ($\chi^2 = 0.018$, $P = 0.894$). Logistic regression was used to investigate a potential moderating effect of diabetes on the association between *IDE_7* and LOAD. No interaction was observed between diabetes and *IDE_7* genotype ($P = 0.720$). However, the small number of individuals with *IDE_7* genotype and diabetes status means that the power to detect an interaction was low.

In our study two individual haplotypes were associated with risk for LOAD, although the global *P*-values did not reach significance and the direction of the association was in the opposite direction to the previous positive reports, making interpretation hazardous and implying that the odds ratio for a combined analysis would be very close to unity.

Most of the positive associations reported with the *IDE* SNPs have been examined with quantitative traits that are correlated with LOAD such as MMSE scores, tau levels in CSF, plaque and neurofibrillary tangle density, age of onset or plasma $A\beta_{42}$ levels [Prince et al., 2003; Ertekin-Taner et al., 2004]. Both studies report scattered associations with single SNPs and these quantitative traits; some haplotypes showed significant association with more severe phenotypes while others seem to be associated with less severe phenotypes, suggesting a protective effect. The replication of their case/control and their quantitative trait-haplotype associations failed in half of their series, thereby reflecting the failure to replicate within our study. The overall significance of these findings—inconsistent both across and within studies—remains unclear and contradictory.

To extend on the variants proposed by Prince et al. [2003] we also tested a promoter SNP that was included in a smaller study by Abraham et al. [2001]. We conclude that this SNP and the 14 other SNPs of our study covering *IDE* and two adjacent genes did not show evidence for association with LOAD in the tested sample sets.

The evidence of homoplasy in this gene has some implications regarding the "non-replication" between samples, which is often observed between studies investigating this region. Sites showing homoplasy are not ideal as single-site markers of association because identity by state does not reflect identity by descent [Templeton et al., 2000]. Under such conditions patterns of linkage disequilibrium can be complex, possibly resulting in increased rates of type II error. Thus, the use of polymorphisms exhibiting homoplasy can confound traditional association studies and may contribute to our inability to replicate results between sample populations.

Although *IDE* is an excellent positional and functional candidate gene for LOAD we could not find consistent evidence for association with LOAD with the four SNPs we tested in a large combined sample (1,033 cases, 1,142 controls) or with the 14 markers spanning a region of 276 kb around *IDE*. We cannot exclude the possibility that rare functional variants within *IDE* contribute to LOAD risk but our data are consistent with the conclusion that common variation in *IDE* is not a major risk factor in LOAD.

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