RESEARCH ARTICLE

Genetic Association of the APP Binding Protein 2 Gene (APBB2) with Late Onset Alzheimer Disease

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Alzheimer disease (AD) is a complex neurodegenerative disorder predisposed by multiple genetic factors. Mutations in amyloid β precursor protein (APP) are known to be associated with autosomal dominant, early onset familial AD and possibly also late onset AD (LOAD). A number of genes encoding proteins capable of binding to APP have been identified, but their contribution to AD pathobiology remains unclear. Conceivably, mutations in these genes may play a role in affecting AD susceptibility, which appears to be substantiated by some genetic studies. Here we report results of the first genetic association study with APBB2, an APP binding protein (also known as FE65L), and LOAD, in three independently collected case–control series totaling approximately 2,000 samples. Two SNPs were significantly associated with LOAD in two sample series and in meta-analyses of all three sample sets (for rs13133980: odds ratio $[OR]_{hom} = 1.36$ [95% CI: 1.05–1.75], $OR_{het} = 1.32$ [95% CI: 1.04–1.67], minor allele frequency = 43%, P = 0.041; and for hCV1558625: $OR_{hom} = 1.37$ [95% CI: 1.06–1.77], $OR_{het} = 1.02$ [95% CI: 0.82–1.26], minor allele frequency = 48%, P = 0.026). One of these SNPs, located in a region conserved between the human and mouse genome, showed a significant interaction with age of disease onset. For this marker, the association with LOAD was most pronounced in subjects with disease onset before 75 years of age ($OR_{hom} = 2.43$ [95% CI: 1.61–3.67]; $OR_{het} = 2.15$ [95% CI: 1.46–3.17]; P = 0.00006) in the combined sample set. Our data raise the possibility that genetic variations in APBB2 may affect LOAD susceptibility. Hum Mutat 25:270–277, 2005. © **2005 Wiley-Liss, Inc.**

KEY WORDS: Alzheimer disease; AD; LOAD; association; APP; APBB2; SNP

DATABASES:
APBB2 – OMIM: 602710, 606626 (LOAD); Genbank: NT_006238.10 (Chr. 4 genomic), NM_173075.2 (mRNA)
APP – OMIM: 104760, 104300 (AD); Genbank: NT_004511 (Chr. 21 genomic), NM_000484, NM_201413, NM_201414 (mRNA)

INTRODUCTION

Amyloid β precursor protein (APP; MIM# 104760) is known to be a central player in Alzheimer disease (AD; MIM# 104300), an age-related neurodegenerative disease, characterized pathologically by neurofibrillary tangles and amyloid plaques and clinically by progressive impairment of mental functioning. A central role for APP in AD is supported by the observations that the major proteinaceous components of plaques are amyloid β peptides [Glenner and Wong, 1984], which are produced by proteolytic cleavage of APP, and that several mutations in *APP* are linked to autosomal dominant, early onset familial AD [Goate et al., 1991]. Processing of APP to generate Aβ is mediated by β- and γ-secretases. Mutations in presenilins (*PSEN1*; MIM# 104311; *PSEN2*; MIM# 600759), a component of γ-secretase, are also associated with early onset familial AD [Levy-Lahad et al., 1995a, b; Rogaev et al., 1995; Sherrington et al., 1995]. Activity of these secretases is tightly regulated and can be modulated by various molecules including APP binding proteins (see Guenette et al. [1999]).

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A number of APP binding proteins have been identified, mostly through the yeast two-hybrid system using various segments of APP as bait. However, their functions have not yet been well characterized, and whether they are relevant to AD pathogenesis remains largely unclear. In an effort to identify genetic mutations predisposing to late onset AD (LOAD; MIM# 606626), such genes are strong functional candidates. Prior studies have reported that *APP* polymorphisms may be associated with LOAD [Athan et al., 2002; Olson et al., 2001; Wavrant-De Vrieze et al., 1999] and that an intronic polymorphism in the APP binding protein FE65 (alternative name: *APBB1*; MIM# 602709) is associated with LOAD [Hu et al., 1998]. However, it should be noted that unlike *apolipoprotein E* (*APOE;* MIM# 107741) [Roses, 1998; Strittmatter et al., 1993], the association of neither *APP* nor *FE65* polymorphisms with LOAD has been consistently replicated [Bertram et al., 2000; Cousin et al., 2003; Guenette et al., 2000; Lambert et al., 2000; Papassotiropoulos et al., 2000; Prince et al., 2001].

APBB2 (MIM# 602710) was originally identified as *hFE65L* or human *FE65*-like protein through its binding to the cytoplasmic domain of APP [Guenette et al., 1996]. Unlike FE65, APBB2 does not appear to be involved in APP-dependent transcriptional activation. Rather, it has been implicated in regulating APP processing [Chang et al., 2003; Guenette et al., 1999]. Overexpression of *APBB2* increases maturation and secretion of APP and the production of amyloid β peptide, and lowers the response to apoptotic stimuli [Cao et al., 2000]. Thus, *APBB2* is a strong functional candidate gene for AD. Here we test the hypothesis that there are common SNPs in the *APBB2* gene that are associated with LOAD. For this purpose, we developed SNP assays for *APBB2* and genotyped three independently collected LOAD case–control series. This led us to identify two common *APBB2* SNPs that are significantly associated with LOAD.

MATERIALS AND METHODS

Clinical Samples

This study included a total of 970 LOAD cases and 1,102 controls from three case–control series collected at Washington University in St. Louis (WashU), University of California at San Diego (UCSD), and from a combined effort by the Cardiff University, Wales College of Medicine and King's College, London (UK), respectively (Table 1). The UK sample was used as the exploratory sample set. Significant markers were followed up in the WashU and UCSD samples. All individuals were of Caucasian origin. Cases from these sample sets have a clinical diagnosis of dementia of the Alzheimer type according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [McKhann et al., 1984] or similar criteria with an age of disease onset of 65 years or more. All three series show the expected association with the *APOE4* genotype. Analysis using the program STRUCTURE [Pritchard et al., 2000] revealed that there was no evidence of population stratification among the three case–control series [Li et al., 2004].

Genotyping

SNPs were chosen from dbSNP (www.ncbi.nlm.nih.gov/projects/SNP/) and the Celera human genome SNP database (http://myscience.appliedbiosystems.com/navigation/ssoLogin.jsp) for assay design (Table 2). Genotyping of SNPs was done by allele-specific real-time PCR with individual samples [Germer et al., 2000]. A mixture of cases and controls were always run on the same plate. Overall, our genotyping accuracy was better than 99%, as determined by internal comparisons of differentially designed assays for the same marker and comparisons for the same marker and samples across our groups.

Statistical Analysis

For quality control of genotyping results, we determined whether observed genotype frequencies deviated from Hardy Weinberg equilibrium (HWE) for both the case and control samples. Assays with significant deviation from HWE ($P < 0.05$) were then closely reexamined for genotyping quality. HWE tests in cases and controls were also used to detect genetic association [Nielsen et al., 1998]. The Wald test from a logistic regression model was used to assess association between a marker and disease status, as well as for interaction between the marker and age of disease onset, gender, and *APOE4* status. In order to determine if younger or older individuals contribute to an age-at-onset effect, we dichotomized the continuous trait, age of disease onset in cases and age of exam in controls into two groups, 75 years of age or older and younger than 75 years of age to test for homogeneity of odds ratios (ORs) in both age groups. Unless otherwise specified, the logistic regression models were carried out by assigning codes of 0, 1, and 2 for the major allele homozygote, heterozygote, and minor allele homozygote genotypes, respectively, resulting in a test for an additive effect on the log ORs (or a multiplicative effect on the ORs). Tables 2 and 3 also display the P-value from a two degrees of freedom logistic regression model, testing whether any of the three genotypes were associated with disease status. Genotypic odds ratios were calculated using the homozygotes that carry the least risk as the reference. In rs13133980, the reference genotype is the minor allele homozygote 11 in Table 3. Thus the OR values Het (OR_{het}) and Hom (OR_{hom}) in Table 3 indicate the odds of heterozygote vs. that of minor allele homozygote and the odds of major allele homozygote vs. that of minor allele homozygote, respectively. In hCV1558625, the reference genotype (with least risk) is the major allele homozygote 22 in Table 4. Unless otherwise specified, ORs are unadjusted for other variables. The meta-analysis of the combined sample sets was performed with logistic regression, as described above, but a term was added to the model for sample-set, to control for potential confounding effects due to differences among sample-sets. The Friedman's

TABLE 1. Clinical Sample Summary

Sample set	Country of origin	AOO (±SD)	AAE (±SD)	ApoE4+ (Case/ctrls)	Male (Case/ctrls)	Female (Case/ctrls)
UK	UK	76.7 (6.3)	76.6 (6.2)	(36.4/13.1)%	(78/101)	(282/295)
WashU	USA	76.2 (6.9)	77.5 (7.4)	(37.0/11.9)%[b]	(140/135)	(249/219)
UCSD	USA	73.3 (5.5)[a]	78.8 (7.2)	(33.8/11.9)%[c]	(119/126)	(102/226)

[a]15 cases missing AOO data.
[b]One control missing ApoE genotype.
[c]Two cases and two controls missing ApoE genotypes.
AOO, age of disease onset for cases; AAE, age at exam for controls; Case/ctrls, Cases/controls.

TABLE 2. Information of Markers Tested for Association

Marker ID[a]	Alleles (1/2)[b]	Name (genomic)[c]	Name (cDNA)[d]
rs10938438	..TACTC[C/T]GCCCA..	g.615577T>C	
hCV317172	..TGACT[A/G]AAATG..	g.628305G>A	
rs7680971	..TTAAT[G/T]GTGAC..	g.648147T>G	
rs6839964	..GCCTT[A/G]AATCT..	g.676908G>A	
rs12509038	..CCATA[G/T]GAGTC..	g.682703T>G	
rs13133980	..ATCCT[C/G]GAAGA..	g.704912C>G	
rs4861358	..TGCCT[C/T]GGTTC..	g.717865T>C	c.536T>C
hCV1558549	..CACCT[A/G]TGTGT..	g.742879A>G	
rs12641331	..GGGTA[C/T]GAACC..	g.766606C>T	
rs4861368	..CCATC[A/T]CTGAA..	g.795588A>T	
hCV11298078	..CCATG[C/T]TTGGC..	g.808419C>T	
hCV1558615	..AACAC[A/G]GAGAA..	g.822010G>A	
hCV1154464	..AAGCA[C/T]CAAGG..	g.826435C>T	
hCV1558625	..GGAGG[C/T]TGGCA..	g.837510T>C	
rs10002235	..ATCCA[G/T]CCTCT..	g.846949G>T	
rs10031715	..AAATG[C/T]ACCTG..	g.858319C>T	

[a]Marker IDs are either from the dbSNP database or Celera Genome Assembly Release R27.
[b]Alleles are defined in the context of short flanking sequences.
[c]Name (genomic) is based on NT_006238.10.
[d]Name (cDNA) is based on NM_173075.2, SNP position is based on +1 being the A of the ATG start codon.

two-way analysis of variance (ANOVA) by ranks was used to test for association of genotype with age of onset among cases, controlling for sample-set. For single sample sets, association of genotype with age of onset among cases was performed with the Kruskal-Wallis test. For testing the haplotype-LOAD association, marker-marker linkage disequilibrium (LD) measures, D′ and r^2, were first calculated using ldmax (www.sph.umich.edu/csg/abecasis/GOLD/docs/ldmax.html) to identify regions of high LD. Haplotype frequencies were estimated for both the case and control samples. Their association with disease status was then assessed using a score test, with haplotypes coded in an additive fashion [Schaid et al., 2002].

RESULTS

We examined the association of *APBB2* with LOAD because it is a strong biological candidate gene, relevant to the amyloid hypothesis. According to the RefSeq database, *APBB2* extends over 400 kilobase pairs (kbp) from 40.66 to 41.06 megabase pairs (Mbp) on chromosome 4 (Fig. 1A); dbSNP and the Celera human genome database list over 1,000 SNPs for this gene. Only four of the SNPs represent missense mutations, the majority of the other SNPs are located in noncoding regions, some of which are conserved segments between the human and mouse genomes (HMCS). We determined that three of the four missense SNPs are extremely rare in Caucasians (minor allele frequency [MAF] < 1%), based on the analysis of 64 randomly selected Caucasian DNAs. The fourth SNP (rs4861358) had a MAF of approximately 25%; this SNP is located in the 5′-untranslated region of the *APBB2* transcript NM_173075.2, but results in a missense mutation (Arg179>Gln) from another splice form annotated in the Celera human genome assembly. We used this common missense SNP and another common HMCS SNP to genotype the UK LOAD case–control series (Table 1). The HMCS SNP was selected because it was the only other marker in the *APBB2* gene for which an assay was immediately available for testing. As shown in Table 3, the HMCS SNP, rs13133980, was significantly associated with LOAD (P = 0.03). The marker showed significant interaction with age of disease onset ($P_{Interaction} = 0.019$), but not with ApoE4 status or gender. Its effect was much stronger in individuals with disease onset before 75 years of age vs. 75 years of age or older. No significant association of the missense SNP, rs4861358, with LOAD was observed (case MAF = 22.4% vs. control MAF = 25.1%; P = 0.22). We chose 75 years of age as the cut point a priori rather than in a post hoc bid to maximize the association signal, based on the age at onset effect of *APOE4* that diminishes after 70 years of age [Farrer et al., 1997] and because 75 years of age is close to the mean age at onset (age at exam in controls) of all samples combined.

We next genotyped the significant marker rs13133980 in two other independently collected LOAD case–control series. The association was replicated in the WashU case–control series (Table 3), including the significant interaction with age of disease onset ($P_{Interaction} = 0.032$) and the stronger effect in patients with disease onset before 75 years of age. rs13133980 was not replicated in the UCSD case–control series, but the cases deviated from Hardy-Weinberg equilibrium (HWE; P = 0.0018). The HWE violation was caused by an increase in the heterozygote frequency and was strongest in subjects with disease onset before 75 years of age (HWE; P = 0.00037) and not significant in the later onset cases. The genotype distribution of this SNP conformed to HWE in UCSD controls. Individual reexamination of our genotyping data for all samples did not indicate any genotyping errors. It should also be noted that with the particular genotyping assay we used, errors would more likely lead to dropout of a single allele, leading to a decrease, not an increase, in the heterozygote frequency, as observed for rs13133980. ORs calculated for that marker in the UCSD sample show that OR_{het} (1.71; 95% CI: 1.02–2.85) and OR_{hom} (0.99; 95% CI: 0.56–1.75) do not follow a linear trend. Therefore we tested this marker using a two degrees of freedom genotypic test that does not place a constraint on the pattern of association, and observed a significant association in the UCSD sample (P = 0.014). In a meta-analysis of all three sample series, rs13133980 was significant in the unstratified analysis, either assuming a log-additive model (P = 0.031) or in an unconstrained model (P = 0.041). rs13133980 showed much stronger association with age of disease onset before 75 years of age under both models (P = 0.0001 and P = 0.00006, respectively). Further analysis showed that age of onset differed significantly by rs13133980 genotype among cases in UK, UCSD, WashU (P = 0.044, P = 0.029, P = 0.026, respectively), and the

TABLE 3. Association of rs13133980 with LOAD

		Case[a]						Control[a]									
Stratum	Sample	11	12	22	Sum	MAF	HWE pExact	11	12	22	Sum	MAF	HWE pExact	p (log-additive)	OR Hom (95% Cl)	OR Het (95% Cl)	p (genotypic. 2 df)
ALL	UK	62	170	128	360	0.408	0.66	88	192	116	396	0.465	0.61	0.030	1.57 (1.04:2.36)	1.26 (0.86:1.85)	0.09
	WashU	77	181	128	386	0.434	0.41	83	169	96	348	0.481	0.59	0.037[b]	1.44 (0.96:2.16)	1.15 (0.79:1.68)	0.2
	UCSD	26	113	46	185	0.446	0.0018	66	168	118	352	0.426	0.66	0.74[b]	0.99 (0.56:1.75)	1.71 (1.02:2.85)	0.014
	ALL	165	464	302	931	0.426	0.59	237	529	330	1096	0.458	0.36	0.031	1.36 (1.05:1.75)	1.32 (1.04:1.57)	0.041
LT75	UK	15	71	52	138	0.366	0.27	42	66	42	150	0.500	0.14	0.0016	3.47 (1.69:7.09)	3.01 (1.53:5.93)	0.0017
	WashU	27	74	73	174	0.368	0.26	35	69	41	145	0.479	0.62	0.0032[b]	2.31 (1.23:4.34)	1.39 (0.76:2.53)	0.023
	UCSD	11	72	25	108	0.435	0.00037	22	56	32	110	0.455	0.85	0.33[b]	1.56 (0.64:3.82)	2.57 (1.15:5.74)	0.042
	ALL	53	217	150	420	0.385	0.079	99	191	115	405	0.480	0.27	0.0001	2.43 (1.61:3.67)	2.15 (1.46:3.17)	0.00006
GE75	UK	47	99	76	222	0.435	0.17	46	126	74	246	0.443	0.61	0.80	1.01 (0.60:1.69)	0.77 (0.47:1.25)	0.36
	WashU	50	107	55	212	0.488	1	48	100	55	203	0.483	0.89	0.56[b]	0.96 (0.56:1.65)	1.03 (0.64:1.66)	0.96
	UCSD	12	31	20	63	0.437	1	44	112	86	242	0.413	0.51	0.68[b]	0.85 (0.38:1.90)	1.01 (0.48:2.15)	0.85
	ALL	109	237	151	497	0.458	0.42	138	338	215	691	0.444	0.82	0.90	0.97 (0.69:1.35)	0.91 (0.67:1.24)	0.81

[a]Individual counts and sum of genotypes 11, 12, and 22 and minor allele frequency (MAF) are presented. The UK case-control series was used in the exploratory study. The WashU and UCSD samples were used in the replication study.
[b]One-sided p-value.
ALL, All samples were combined data from the three sample series; LT75, age of onset < 75 years in patients, age at exam < 75 years in controls; GE75, age of onset ≥ 75 years in patients, age at exam ≥ 75 years in controls.

TABLE 4. Association of hCV1558625 with LOAD

	Case						Control									
Sample	11	12	22	Sum	MAF	HWE p	11	12	22	Sum	MAF	HWE p	p (log-additive)	OR Hom (95% Cl)	OR Het (95% Cl)	P (genotypic. 2 df)
UK	95	169	95	359	0.500	0.291	67	207	119	393	0.434	0.182	0.010	1.78 (1.18:2.68)	1.02 (0.73:1.43)	0.0077
WashU	90	186	102	378	0.484	0.758	66	164	117	347	0.427	0.512	0.015[a]	1.56 (1.03:2.37)	1.30 (0.93:1.83)	0.09
UCSD	32	70	50	152	0.441	0.414	78	178	83	339	0.493	0.386	0.937[a]	0.68 (0.40:1.17)	0.65 (0.42:1.02)	0.15
ALL	217	425	247	889	0.483	0.202	211	549	319	1079	0.450	0.389	0.024	1.37 (1.06:1.77)	1.02 (0.82:1.26)	0.026

[a]One-sided p-value.

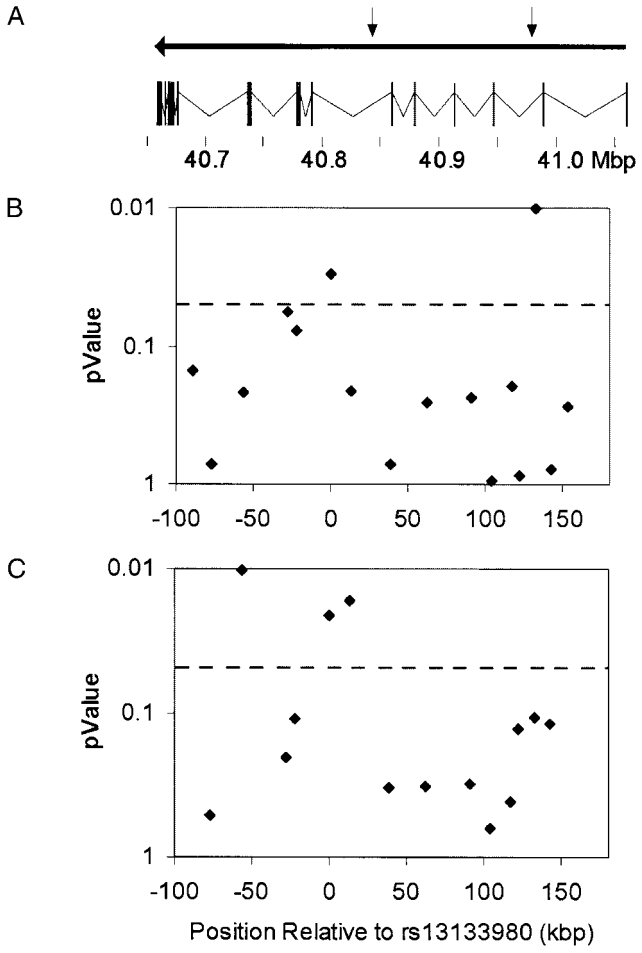

FIGURE 1. **Genotyping of APBB2 markers. A: The APBB2 gene structure. The orientation of transcription is denoted with the horizontal arrow, relative to the chromosomal positions given in Mbp. Exons are denoted with vertical bars. The two significant markers are indicated with vertical arrows. B: Log-additive genotypic P-values for all 16 APBB2 markers in the UK sample. C: Global P-value for a three-marker sliding haplotype window. The relative position relates to the marker in the center. The dotted line denotes P = 0.05. The marker order is identical to the markers listed in Figure 2.**

combined sample (P = 0.0025). Age of onset tended to be older for the GG genotype (median = 77.5 years) than for the CG genotype (median = 75.0 years) or the CC genotype (median = 74.5 years).

These results, and the fact that the *APBB2* gene covers a large genomic region, prompted us to design additional assays for other *APBB2* SNPs. Another 14 SNPs, prioritized for putative functional SNPs (e.g., transcription factor binding sites, SNPs in HMCS), with an average spacing of 16.2 kbp (range: 4.4–29.0 kbp) were genotyped in the UK case–control series. One additional significant marker, hCV1558625 (P = 0.01, unstratified analysis), which is 132.6 kbp downstream of rs13133980, was identified by this analysis (Fig. 1B). This marker did not show interaction with age of disease onset, ApoE status, or gender ($P_{Interaction} > 0.05$). The association of hCV1558625 with LOAD was replicated in the WashU (P = 0.015) but not in the UCSD case–control series (Table 4). A meta-analysis of the combined data from the three sample series showed that the marker was significantly associated with LOAD (P = 0.024), but showed no interaction with the subgroups.

We next examined LD for the SNPs typed in the UK series. Pairwise measures of LD (D′ and r^2) were calculated (Fig. 2). Both significant markers, rs13133980 and hCV1558625, have only limited LD (D′ = 0.23) and appear to reside in two adjacent regions of high LD, with some markers showing stronger LD across these two regions. Several of the genotyped SNPs are in high LD with both rs13133980 and hCV1558625, but none of them was significantly associated with LOAD in the UK series. From our three-marker sliding window analysis, we identified three haplotypes with significant global P-values, two of which included rs13133980 (Fig. 1C). The individually significant haplotypes containing rs13133980 are T/G/C (P = 0.012) and T/C/C (P = 0.031; rs12509038/rs13133980/rs4861358) and G/C/A (P = 0.0063) and C/C/A (P = 0.024; rs13133980/rs4861358/hCV1558549), respectively.

DISCUSSION

The risk for complex diseases, including LOAD, is influenced by environmental and genetic factors. Contrary to Mendelian diseases with one major genetic risk factor of very high penetrance, it is thought that genetic risk factors for complex diseases are of lower penetrance and heterogeneous. While replication of genetic risk factors in independent sample collections is critical to validate a reported genetic association, replication may occur in some, but not all, sample sets, due to the low relative risk and both environmental and genetic etiological heterogeneity. We tested whether SNPs in the APP binding protein APBB2 show evidence of genetic association with LOAD and identified two significant markers. The evidence for association was replicated in one of the two other sample sets we tested. Based on our results, we hypothesize that genetic variation in APBB2 may affect LOAD susceptibility, particularly among those with age of disease onset before 75 years of age. This hypothesis is supported by our genotyping data of SNP rs13133980, one of the two significant markers identified in this study. First, we detected a significant association of rs13133980 with LOAD in two of the three sample sets we analyzed (P = 0.03 and P = 0.037, respectively). While this SNP was not replicated in the third LOAD case–control series when testing for a log-additive effect, it showed a significant association using a two degrees of freedom genotypic test that does not place a constraint on the pattern of association (P = 0.014). The genotypic distribution of the SNP in the UCSD sample violated HWE in cases only (P = 0.0018). An analysis of each sample set using the program Structure [Pritchard et al., 2000] and careful examination of genotyping results suggest that the observed HWE violation is unlikely due to population structure or genotyping errors. Deviation from HWE in cases but not in controls can indicate association between the marker and LOAD [Nielsen et al., 1998]. Thus, the observed HWE violation in cases of the UCSD sample set may suggest the presence of selection forces in cases and may be indicative of an association with another marker that is in LD with the one typed here [Nielsen et al., 1998]. It may also suggest that the LD profile in cases differs between the UCSD and UK or WashU samples. Second, we observed a significant interaction of rs13133980 with age of disease onset ($P_{Interaction} = 0.0016$). This marker has a much stronger effect in subjects with disease onset before 75 years of age (P = 0.0001), using combined data from all three sample sets, while we found no significant effect in the older subset. Even though rs13133980 shows no interaction with age of disease onset in the UCSD sample set, we observed that the HWE violation

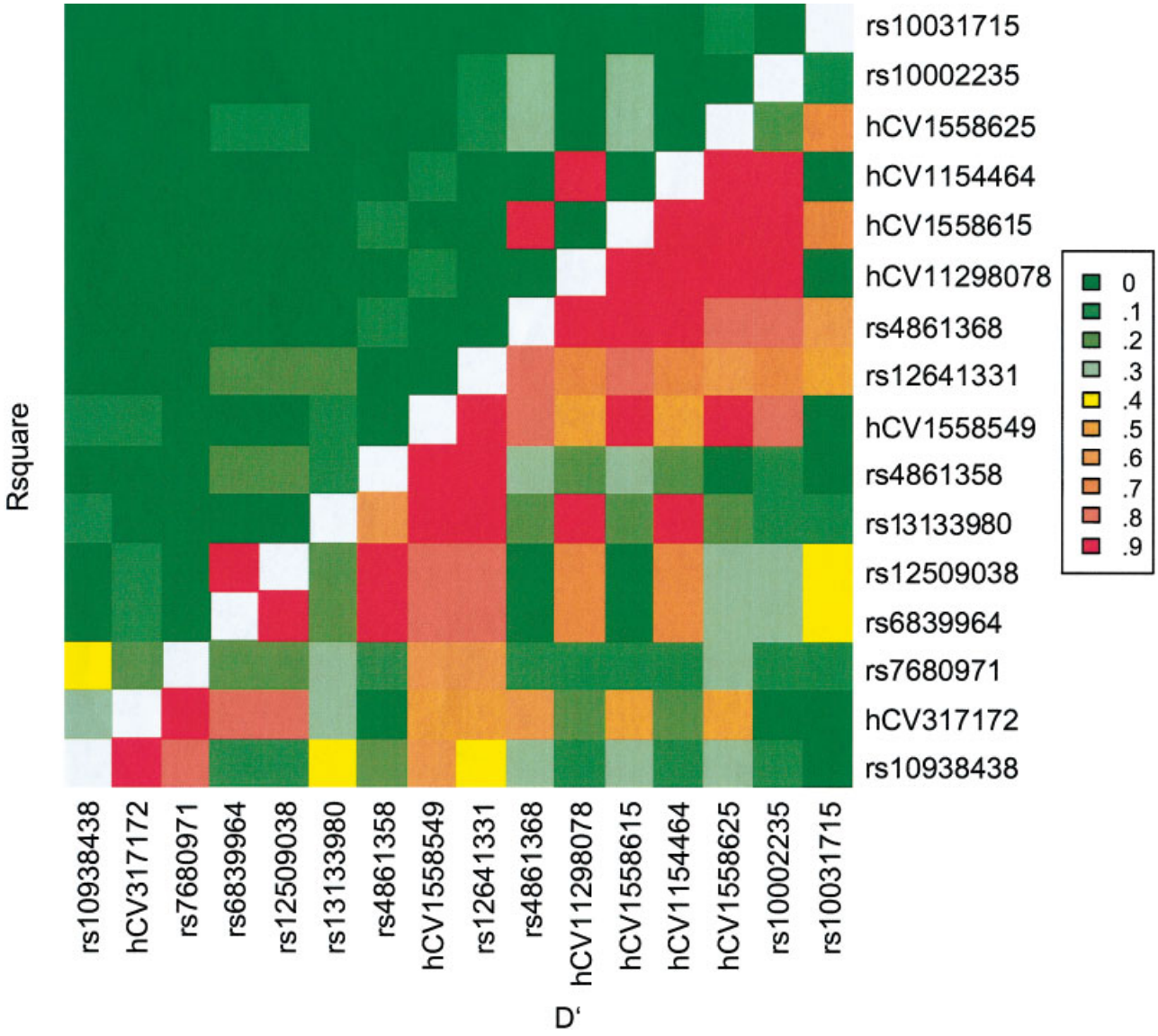

FIGURE 2. **Pairwise LD metrics between the genotyped APBB2 SNPs in the UK controls.**

came entirely from the younger patient samples ($P = 0.00037$ for HWE). Third, after adjusting for multiple testing of all 16 markers and three age subgroup analyses (ALL, LT75, and GE75) using the very conservative Bonferroni correction, this marker remains significant in the age subgroup LT75 under the log-additive model ($P_{adjusted} = 0.0048$, 48 tests) and the two degrees of freedom genotypic test ($P_{adjusted} = 0.0029$, 48 tests). The second significant marker, hCV1558625, does not remain significant after adjusting for testing of all 16 markers ($P_{adjusted} = 0.384$, 16 tests).

rs13133980 and hCV1558625 are both intronic SNPs, but rs13133980 is in a region that is conserved between human and mouse genomes; however, its functional significance has not been determined. Northern blot analysis shows that two major transcripts of *APBB2* exist in most tissues including brain [Guenette et al., 1996]. These two transcripts of 3.7 and 7.5 kbp, respectively, are likely to be derived from alternative splicing. Our RT-PCR analysis shows that *APBB2* is expressed in most brain regions, including hippocampus and others important in learning and memory (data not shown). Further functional evaluation of both SNPs may include testing whether they affect the relative levels of these transcripts and whether the transcript ratio alters the activity of APBB2.

In any study, it is possible that association between disease and markers in one gene reflect LD between the markers and true susceptibility variants in adjacent genes. In the present study, we think this unlikely, though the possibility cannot be fully discounted. *APBB2* was selected as a functional rather than a positional candidate, and therefore the prior probability for association within this gene is much higher than for adjacent genes. Second, both replicated markers from this study reside in LD blocks that are entirely within the genomic region of the *APBB2* gene, and therefore it is probably less likely that other mutations in neighboring genes, which are in LD with these variants, account for our finding. A genetic association of the *APBB2* gene with LOAD would be consistent with its known biological function and the high frequency of rs13133980 would be compatible with the common disease–common variant hypothesis. Additional genotyping of other LOAD case–control series will be

necessary to confirm genetic mutations in *APBB2* as LOAD risk factors and may perhaps help to identify other potentially causal variants for further biological characterization.

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