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Variation in the Urokinase-Plasminogen Activator Gene Does Not Explain the Chromosome 10 Linkage Signal for Late Onset AD

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Linkage studies indicate that the same region of chromosome 10 contains a risk locus for late onset Alzheimer disease (LOAD) and a QTL for plasma Aβ42 levels suggesting that a single locus may influence risk for AD by elevating plasma Aβ42 [Ertekin-Taner et al., 2000; Myers et al., 2000]. A strong positional and biological candidate is the urokinase-plasminogen activator (*PLAU*) gene. Eight polymorphisms spanning the entire gene were examined using case control (CC) and family-based association methods. No association was observed by any method making it unlikely that variation in *PLAU* explains our linkage data.

KEY WORDS: Alzheimer disease; candidate gene; association study; urokinase-plasminogen activator; PLAU

INTRODUCTION

It has been suggested that faulty Aβ clearance might be the main risk factor for late onset Alzheimer disease (LOAD), rather than increased production of Aβ through changes in APP cleavage [Selkoe, 2001]. While the known mutations for the rare familial forms of AD produce more Aβ by increasing either β- or γ-cleavage of APP [Citron et al., 1992, 1997; Hsiao et al., 1996; Scheuner et al., 1996], APOE, the only known risk factor for LOAD, appears to act by affecting the deposition of fibrillar forms of Aβ [Holtzman et al., 2000].

Urokinase-plasminogen activator (PLAU, also known as u-PA, [OMIM 191840]) is a serine protease whose primary action is to convert the inactive zymogen plasminogen into its active form plasmin. Plasmin has been implicated in reduction of secreted forms of Aβ [Ledesma et al., 2000] and prevention of Aβ neurotoxicity [Tucker et al., 2000]. Thus, there is evidence that the plasmin system is involved in the clearance of secreted Aβ, and perhaps PLAU participates indirectly in this pathway by activating plasminogen.

Previously, we performed a two-stage full genome screen looking for novel genetic risk factors for LOAD. Our strongest finding was a multipoint lod score of 3.9 at 81 cM (D10S1211) on chromosome 10 [Myers et al.,

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2000, 2002]. A QTL study of plasma Aβ42 levels also reported linkage to this region, suggesting that the chromosome 10 LOAD loci may modify risk for disease by modulating Aβ42 levels [Ertekin-Taner et al., 2000]. The gene encoding PLAU is located within our peak. Thus, we were interested in *PLAU* both for its biological effects and physical location.

MATERIALS AND METHODS

Polymorphism Discovery

PLAU (AF377330, GI:14278713) is 6.4-kb long and is organized into 11 exons. We sequenced all exons plus 40 bases upstream and downstream for the entire coding region of *PLAU* in a pool of 96 cases (mean age of onset (AOO) = 74.5 ± 6.2 years, all AOO ≥ 65 years) and another pool of 96 controls (mean age at last assessment = 81.0 ± 8.1 years). The DNA pools were comprised of ethnicity and age matched cases and controls from the Memory and Aging Project (MAP) at the Washington University (WU) Alzheimer Disease Research Center (ADRC) [Kwon et al., 2000]. For exon 11, individual sequencing was performed on 11 additional individual samples, since clean reads could not be obtained from the pooled sequencing due to a deletion polymorphism located at the end of the exon.

Polymorphism Genotyping

Polymorphisms were typed either by pyrosequencing WU, RFLP digest (WU, Mayo), or by FRET (UK). Three different types of samples were genotyped: case-control (CC), discordant sibling pair (DSP), and a series of siblings taken from sibships in our linkage series where both siblings shared alleles identical by descent (IBD) for markers at our chromosome 10 peak (sharers).

Case-control samples were obtained from the WU Alzheimer Disease Research Center Memory and Aging Project (WU), the Mayo Clinic Rochester Memory Disorders Clinic (Mayo), and the Institute for Psychiatry in the United Kingdom (UK). Sample statistics are shown in Table I. All samples were Caucasians with an age of onset at least ≥60 years of age.

Our DSP set was obtained from the NIMH series (see Appendix A) in which we found our original linkage results. To avoid possible censoring, only unaffected individuals that were shown to be cognitively normal at an age greater than the oldest age of onset for their family were selected. Hundred and twenty-six unaffected and 173 affected siblings from 100 families were typed (64% female, 13% autopsy confirmed cases, 0% autopsy confirmed controls). Hundred and sixty of those DSPs came from families typed in our linkage series.

The sharer series contained 39 affected individuals, each taken from a sibship in which the siblings shared alleles IBD ≥ 1.8 at each of the five markers located within the −1 LOD interval of our peak. We picked only one sharer from each family for our analysis, since siblings are not independent.

TABLE I. Case-Control Sample Statistics

	WU	MAYO	UK
Cases			
Total#	196	330	138
% female	57	65	77
% cases autopsied	30	16	N/A
Mean age of onset (yrs)	75.5	82.7	77
Controls			
Total#	193	493	151
% female	56	67	76
% controls autopsied	7	4	N/A
Mean age at assessment (yrs)	78.4	75.4	75.6

Shown are the sample statistics for the CC series obtained from Washington University (WU), the Mayo Clinic in Jacksonville, Florida (Mayo), and the series from the Institute for Psychiatry in the United Kingdom (UK).

Statistical Analysis

Single loci in the CC samples were analyzed using a standard Pearson's chi-square approach. Multi-locus genotypic analysis was performed using a stepwise logistic regression procedure. The probability that an individual is a case was modeled as a function of the relative risks of disease due to individual genotypes at each particular locus. First, each locus was tested individually using a standard chi-square test. The most significant locus was then fixed in the model. The effects of the other loci were tested allowing for the effects of this locus. This process was repeated until there was no significant effect of adding other loci to the model of risk. Finally, each locus in the final model was tested to see if it could be dropped from the model without significantly worsening the fit. This method is advantageous in that it can account for the interactions between multiple loci, which can help to distinguish loci of true effect from loci that are merely in linkage disequilibrium (LD) with the risk locus. Parameter estimates from the multi-locus regression analysis were used to calculate estimated genotypic and allelic relative risks (RR) for each locus and loci combinations. Interactions with the APOE locus were allowed for in the multiple genotypic logistic regression by forming a 3-level factor (levels: e4/e4, e4/x, x/x) and fitting this to the model of risk before incorporating the SNPs. Allelic haplotype analysis was performed using the hapipf and profhap commands written for STATA by Dr. Adrian Mander [Mander, 2001]. These commands use a log-linear model embedded within the EM algorithm. Haplotype frequencies for the phase-unknown samples were calculated using the EM algorithm, and then a log-linear approach was used to assess the risk contributions of each locus in the haplotypes. To assess the effects of particular haplotypes, odds ratios and confidence intervals were calculated.

The DSP series was used to analyze individual SNPs by treating the sibships as matched case-control sets, thus retaining robustness to population stratification. Both genotypic (no constraints on the relative risks for the genotypes; 2 df test of association) and allelic (genotypic relative risks constrained to follow a multiplicative model; 1 df test of association) analyses were performed. The program TRANSMIT [Clayton, 1999], which compares the numbers of alleles transmitted and

untransmitted to the affected individuals (unaffected sibs are used to infer transmission probabilistically), was used for comparison. *P*-values for all analyses were estimated by bootstrapping, to allow for the non-independence of transmissions to multiple affected siblings from the same sibship.

Power calculations for the PLAU mutations typed in the case-control series and the DSP sample were carried out assuming the SNP itself are a disease-susceptibility locus. Power calculations were also carried out for the case-control series assuming the SNP is in LD with a disease-susceptibility locus. Lewontin's disequilibrium coefficient (D′) was calculated to estimate the strength of linkage disequilibrium between the SNPs in our case-control samples.

RESULTS

Polymorphism Discovery

Six previously reported (http://pga.gs.washington.edu/data/plau/) polymorphisms were observed in intron 5, exon 6, intron 7, exon 8, and two in exon 11 of PLAU. No additional variants within or surrounding the coding region were found. See Figure 1 for genomic structure of *PLAU* and location of all the polymorphisms we typed.

Polymorphism Assessment

We performed several different analyses to determine whether variation in *PLAU* was a significant risk factor for LOAD. We first screened all the known coding changes within *PLAU*, since they are the most likely variants to result in functional changes. We then examined whether single nucleotide polymorphisms (SNPs) in combination contributed to LOAD risk. We performed two multi-locus analyses. In the first, we analyzed combinations of genotypes ignoring phase (the chromosomal arrangement of the alleles). We then considered phase by examining haplotypes. Finally, to examine whether any of the polymorphisms could account for our linkage peak on chromosome 10, we typed a series of individuals taken from sibships in which each sibling shared alleles IBD ≥ 1.8 for all five markers located underneath our linkage peak (sharers).

Coding Variants

Three previously reported non-synonymous variants were genotyped in our CC series. Two rare variants (frequency of the minor allele ~1%) in exons 2 (V15L) and 8 (K231Q) were genotyped in two case-control series of age and gender matched Caucasian samples. The coding change in the signal peptide (exon 2, V15L) was typed in 176 cases and 175 controls from WU and the exon 8 SNP (K231Q) was typed in 271 cases and 458 controls from the Mayo clinic. Neither SNP showed evidence of association either by allelic tests [exon 2 V15L *P*-value = 0.78, $\chi^2 = 0.08$ (1 df); exon 8 K231Q *P*-value 0.63, $\chi^2 = 0.23$ (1 df)] or by genotypic tests [exon 2 V15L *P*-value = 0.85, $\chi^2 = 0.32$ (2 df), exon 8 K231Q *P*-value = 0.63 $\chi^2 = 0.23$ (2 df)]. Exon 8 (K231Q) *P*-values and χ^2 are the same as the allelic analysis, since there were no individuals that were homozygous for the rare allele.

The coding change in the kringle domain of PLAU (exon 6, P141L) has been shown to affect single chain PLAU activity in that constructs with this mutation have a reduced affinity for fibrin clots [Yoshimoto et al., 1996]. Additionally, this mutation was more common than the coding changes in exons 2 and 8 (frequency of the minor allele ~25%). Thus, the exon 6 mutation was a better candidate than the other coding SNPs and so it was typed in an extensive series of cases and controls from three different sites (n = 652 cases, 824 controls).

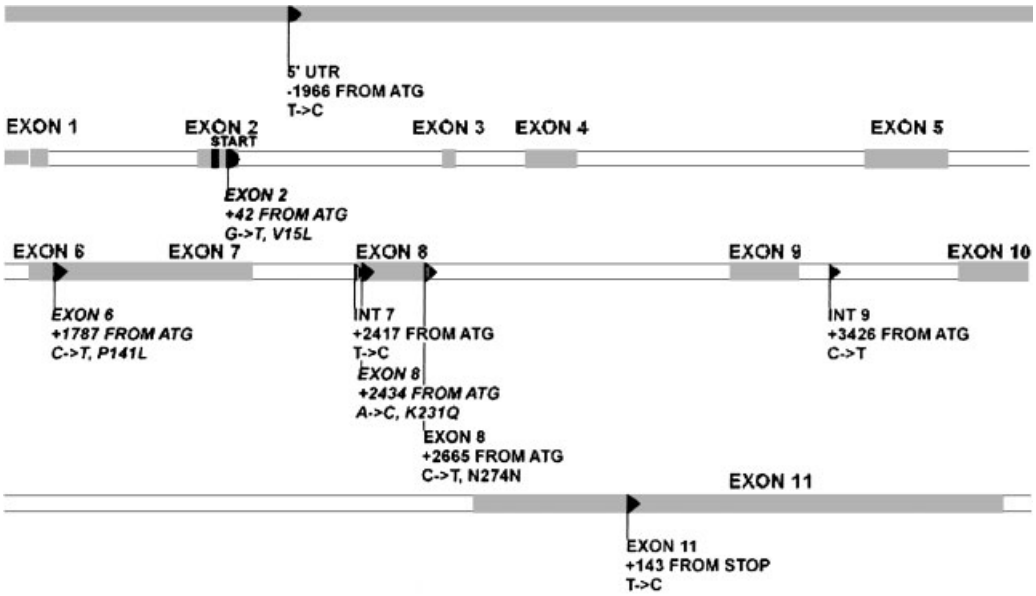

Fig. 1. *PLAU* genomic structure and polymorphisms. Shown in the figure is the genomic structure of *PLAU* and the eight variants that were typed. Non-synonymous variants are in italics. Gray bar at the top of the figure depicts the 5′ UTR of *PLAU*. Gray boxes indicate exons; open boxes introns.

TABLE II. Analysis of the Mutation in Exon 6 (P141L)

			Allele			Genotype				Association	
Site	Size		C	T	TOT	CC	CT	TT	TOT	Allelic	Genotypic
WU	377	# cases	286	86	372	116	54	16	186		
		Frequency	0.77	0.23		0.62	0.29	0.09		$P = 0.94$; $\chi^2 = 0.006$; 1 df	$P = 0.58$; $\chi^2 = 1.10$; 2 df
		# controls	295	87	382	115	65	11	191		
		Frequency	0.77	0.23		0.60	0.34	0.06			
MAYO	810	# cases	491	165	656	180	131	17	328		
		Frequency	0.75	0.25		0.55	0.40	0.05		$P = 0.94$; $\chi^2 = 0.009$; 1 df	$P = 0.26$; $\chi^2 = 2.71$; 2 df
		# controls	734	230	964	282	170	30	482		
		Frequency	0.76	0.24		0.59	0.35	0.06			
UK	289	# cases	212	64	276	80	52	6	138		
		Frequency	0.77	0.23		0.58	0.38	0.04		$P = 0.52$; $\chi^2 = 0.4$; 1 df	$P = 0.50$; $\chi^2 = 1.4$; 2 df
		# controls	225	77	302	79	67	5	151		
		Frequency	0.75	0.25		0.52	0.44	0.03			
COMB	1,476	# cases	989	315	1,304	376	237	39	652		
		Frequency	0.76	0.24		0.73	0.46	0.07		$P = 0.88$; $\chi^2 = 0.02$; 1 df	$P = 0.95$; $\chi^2 = 0.1$; 2 df
		# controls	1,254	394	1,648	476	302	46	824		
		Frequency	0.76	0.24		0.58	0.37	0.06			

Shown in the table are the allele and genotype counts and frequencies for the P141L mutation located in exon 6. All *P*-values were calculated allowing for possible effects of APOE genotype. This mutation was not significantly associated with LOAD risk in any of the series we examined. WU, Washington University; MAYO, Mayo Clinic, Jacksonville, Florida; UK, Institute of Psychiatry, UK; COMB, combined sample; Size, total sample size.

None of the CC series showed evidence of association either individually or when pooled into a single sample (Table II).

Although the CC method is generally more powerful than family-based association methods, we could not be certain that the chromosome 10 loci would show such a large effect in these independent samples. We, therefore, examined the more common P141L SNP in a series of discordant sib pairs derived from the linkage sample. The P141L SNP showed no evidence of association in the DSP series either when it was analyzed as a matched case-control sample (allelic P-value $= 0.21$, $\chi^2 = 1.65$, df 1; genotypic P-value $= 0.34$, $\chi^2 = 2.16$, df 2) or when the transmission of alleles was assessed ($P = 0.27$, $\chi^2 = 1.42$ 1 df using TRANSMIT [Clayton, 1999].

Multi-Locus Analysis

To evaluate the putative role of non-coding changes within the *PLAU* locus, we analyzed our CC series using multi-locus genotypic and haplotypic methods. Seven SNPs spanning the entire gene from −1,966 upstream of the start site to +143 beyond the stop codon were genotyped in 196 cases and 193 controls from WU.

Multi-Locus Genotypic Analysis

First, we analyzed combinations of genotypes in our CC series-ignoring phase to determine the relative contributions of genotypic combinations from each locus to disease risk. Examining each locus on its own (first stage of building the genotypic model of risk), only one locus demonstrated a slight trend. The T → C variant 3′ of the STOP codon in exon 11 gave a P-value $= 0.15$ (Table III). This variant could potentially be functional, because the 3′ UTR of *PLAU* has been implicated in regulating turnover of mRNA transcripts of *PLAU* [Nanbu et al., 1994]. To further analyze this result, we continued to build a model of risk by fixing this locus in our model and determining the effects at the other loci. When this analysis was completed, we obtained a P-value $= 0.063$, $\chi^2 = 8.94$ (4 df). If we included both intron 7 and exon 11 in our model, it appeared that the CC genotype at the intron 7 locus decreased risk, while the CC genotype at exon 11 increased risk. To examine whether this was a true minor effect that was not reaching significance because of low power in our series, or whether this was a spurious result, we typed an additional 96 controls and 96 cases for these two polymorphisms. This result did not replicate (replication sample: $P = 0.77$, $\chi^2 = 1.8$, 4 df, combined sample n = 290 controls, 291 cases: $P = 0.22$, $\chi^2 = 5.71$, 4 df,

TABLE III. Multi-Locus Genotypic Analysis: Step 1

		Allelic		Genotypic	
Locus	Sample size	χ^2	P-value	χ^2	P-value
5′UTR	376	0.001 (1 df)	0.97	1.86 (2 df)	0.4
Exon 2	351	0.08 (1 df)	0.78	0.32 (2 df)	0.85
Exon 6	377	0.006 (1 df)	0.94	1.10 (2 df)	0.58
Intron 7	389	0.12 (1 df)	0.73	0.83 (2 df)	0.66
Exon 8	389	1.34 (1 df)	0.25	2.36 (2 df)	0.31
Intron 9	389	0.001 (1 df)	0.97	0.12 (2 df)	0.94
Exon 11	379	0.21 (1 df)	0.65	3.73 (2 df)	**0.15**

Shown are the allelic and genotypic χ^2 and P-values from the first step of our multi-locus analysis of each *PLAU* variant. No significant differences between cases and controls were found for any locus. The variant +143 from the stop codon in exon 11 showed a slight trend (highlighted in bold). All results calculated allowing for effects of *APOE* genotype (see Methods).

allowing for *APOE*). No significant interactions were observed between *APOE* genotype and any of the loci we examined.

Haplotype Allelic Analysis

Haplotype analysis involves identifying a group of neighboring SNPs that are located on the same chromosome and co-segregating together from generation to generation. This group of SNPs (the haplotype) is then assessed to determine if it is at a different frequency in case and controls. In certain cases, haplotype analysis is more powerful than the study of single loci [Schork et al., 2000; Bader, 2001]. Indeed, there are cases in the literature where no association was found looking at single SNPs, but there was a significant haplotypic effect [Drysdale et al., 2000].

We tested for evidence of association between LOAD and *PLAU* haplotypes in the WU series of 196 cases and 193 controls. Since parental DNAs were not available, we estimated haplotypes using the EM algorithm. The effects of each SNP on the allelic haplotype were then determined using a log-linear model [Mander, 2001]. No haplotype showed evidence of association before correcting for multiple testing (Table IV).

TABLE IV. Haplotype Analysis

Locus	χ^2	*P*-value
All	15.82 (31 df)	0.98
Intron 7/intron 9/exon 11	6.89 (7 df)	0.44
Intron 7/intron 9	4.9 (3 df)	0.18
Intron 9/exon 11	2.45 (3 df)	0.48
Exon 6/exon 8/exon 11	10.43 (7 df)	0.17
Exon 6/exon 11	2.14 (3 df)	0.81

The calculated χ^2 and *P*-values are given for different haplotype combinations. Only five loci (exon 6, intron 7, exon 8 (synonomous change), intron 9, and exon 11) were typed in this analysis. The 5′ UTR polymorphism located −1,966 from the start site and the rare exon 2 mutation were not used. No significant difference between cases and controls was seen for any haplotype combination found in our series of 196 cases and 193 controls from WU.

Sharers

To determine whether variation in PLAU could explain our linkage data, we examined five of the eight SNPs (all but the 5′ UTR SNP and the rare coding polymorphisms in exons 2 and 8) spanning the 5′ end of PLAU in our sharer series. There was no significant difference in the genotypic or allelic frequencies comparing the sharers and a series of age, gender, and ethnically matched controls (Table V).

Power Calculations

To determine whether our analysis of *PLAU* was sufficiently powered, we performed two tests. First, assuming that one of the coding changes in *PLAU* actually was a disease locus, we determined what was

TABLE V. Sharer Analysis

	Allele			Genotype				Association	
SNP	C	T	TOT	CC	CT	TT	TOT	Allelic *P*-value	Genotypic *P*-value
Exon 6									
# sharers	58	20	78	23	12	4	39	*P*-value = 0.60;	*P*-value = 0.63;
Frequency	0.74	0.26		0.59	0.31	0.10		$\chi^2 = 0.28$; 1 df	$\chi^2 = 0.92$; 2 df
# controls	289	85	374	113	63	11	187		
Frequency	0.77	0.23		0.60	0.34	0.06			
Intron 7									
# sharers	39	39	78	11	17	11	39	*P*-value = 0.41;	*P*-value = 0.37;
Frequency	0.50	0.50		0.28	0.44	0.28		$\chi^2 = 0.68$; 1 df	$\chi^2 = 2.01$; 2 df
# controls	176	210	386	38	100	55	193		
Frequency	0.46	0.54		0.20	0.52	0.28			
Exon 8									
# sharers	67	11	78	29	9	1	39	*P*-value = 0.30;	*P*-value = 0.53;
Frequency	0.86	0.14		0.74	0.23	0.03		$\chi^2 = 1.09$; 1 df	$\chi^2 = 1.26$; 2 df
# controls	203	75	278	71	61	7	139		
Frequency	0.73	0.27		0.51	0.44	0.05			
Intron 9									
# sharers	41	37	78	11	19	9	39	*P*-value = 0.22;	*P*-value = 0.47;
Frequency	0.53	0.47		1.08	2.38	1.49		$\chi^2 = 1.49$; 1 df	$\chi^2 = 1.53$; 2 df
# controls	177	209	386	42	93	58	193		
Frequency	0.46	0.54		0.22	0.48	0.30			
Exon 11									
# sharers	36	42	78	10	16	13	39	*P*-value = 0.62;	*P*-value = 0.37;
Frequency	0.46	0.54		0.26	0.41	0.33		$\chi^2 = 0.24$; 1 df	$\chi^2 = 2.01$; 2 df
# controls	162	216	378	34	94	61	189		
Frequency	0.43	0.57		0.18	0.50	0.32			

Shown are the allelic and genotypic frequencies and counts for the 39 individuals from our linkage series who were from sibships that contributed to our linkage peak on chromosome 10 and the individuals from the WU control series. There was no significant difference, indicating that these *PLAU* variants could not explain our linkage peak.

the likelihood that we would have found an association given our sample sizes and the frequencies of each variant. Second, assuming that none of the variants we typed were the chromosome 10 locus, we attempted to determine whether we would have missed an association given that we would be looking for a locus with an effect of $\lambda_s = 1.5$ (calculated from our linkage data, see Myers et al., 2000), and that we would be looking for that locus relying upon LD between the variants we typed and the true disease locus.

Coding Changes Are the Disease Allele

In our series of 652 cases and 824 controls used to examine the exon 6 P141L mutation (minor allele frequency = 25%), we had a 99% likelihood (α, false positive error rate = 0.05%) to detect dominant relative risk (RR Dd) of 1.5 or recessive relative risk (RR DD) of 2, using a multiplicative model of risk. For comparison, the Dd RR for *APOE* is approximately 4 and the DD RR is approximately 10; therefore, using this CC series, we could detect even smaller effects than the only known risk locus for LOAD. In the DSP series used to examine this polymorphism, we had an 84% likelihood of detecting an effect of a dominant disease allele if the relative risk was 3 ($\alpha = 0.05$), and a 99% chance if the relative risk was 5 ($\alpha = 0.05$).

Unfortunately, our tests of the two rare mutations (exons 2 and 8, minor allele frequency = 1%) were not as powerful. For the case-control series used to examine the exon 2 polymorphism (n = 176 cases, 175 controls), we had an 88% likelihood of detecting an effect at an $\alpha = 0.05$, but only if the disease allele had a large, dominant effect on risk (RR Dd = 5). We had more power in our larger series of 271 cases and 458 controls used to type the exon 8 mutation. We simulated that there was a 76% likelihood of detecting either a dominant or recessive allelic effect with RR = 3 ($\alpha = 0.05$) and a 99% likelihood of detecting either a dominant or recessive allelic effect with RR = 5 ($\alpha = 0.05$). All of our genotypic tests were underpowered for both of these rare mutations because of the paucity of homozygous rare genotypes. See Table VI for all of the power calculations obtained simulating different relative risks and disease models (recessive, dominant, multiplicative) for different significant levels for the CC series typed for all the known *PLAU* coding mutations.

Disease Allele is in LD With our Variants

We also wanted to determine what was the likelihood of finding a disease locus given that it was in LD with one of the non-coding polymorphisms we examined. In addition to the three coding polymorphisms we typed, there were an additional five non-coding polymorphisms typed in the WU CC series (n = 196 cases, 193 controls). We calculated the minimum amount of LD that would need to exist between our SNP markers and the disease loci to give us a power of 80% to detect a locus that had a $\lambda_s = 1.5$ with either a dominant or recessive mode of inheritance for an $\alpha = 0.05$. For the two non-coding polymorphisms where the minor allele was moderately frequent (5′UTR SNP, minor allele = 18%, exon 8, synonymous change, minor allele = 25%; we tested a marker allele frequency = 25%), the minimum D′ between the disease locus and the SNP marker needed to obtain 5% significance and 80% power if the disease allele was dominant was 0.31, and for a recessive disease allele, the minimum D′ was 0.40. The other non-coding variants we examine had approximately equi-frequent alleles (intron 7, minor allele = 46%; intron 9, minor allele = 46%; exon 11, minor allele = 43%, we tested a marker allele frequency = 50%). For these more common polymorphisms, the minimum D′ for a dominant disease allele (80% power, $\alpha = 0.05$) was 0.48, and for a recessive disease allele the minimum D′ = 0.32.

D′ varies between 0 and 1.00, and a score of 1.00 indicates perfect LD, i.e., alleles for two marker loci are consistently co-occurring on the same chromosome. To achieve an 80% likelihood that we would not miss a disease locus that had an effect approximately equivalent to our estimated effect size for the chromosome 10 locus ($\lambda_s = 1.5$), for all polymorphisms we examined in *PLAU*, we did not need perfect disequilibrium to exist between our SNPs and the putative disease locus. To examine the extent of LD across *PLAU*, we calculated the D′ between each SNP locus we examined. As shown in Table VII, for the most part, there was considerable more LD between each of the loci we typed than what we calculated would be necessary to find the chromosome 10 loci. Unfortunately, this is no guarantee that we would have not missed this locus by relying on LD, since our power calculations rely on the LD between the disease locus and our SNPs, which is unknown, and not upon the LD between the SNPs we typed. Additionally, for a given D′ and SNP allele frequency, power to detect association is highest when the frequency of the disease allele is equal to that of the associated SNP allele [Muller-Myhsok and Abel, 1997]. This assumption was made to obtain the minimum values of D′ quoted here. If, in fact, the frequencies of SNP and disease alleles are very different, the levels of D′ required for our sample to have power to detect association may be much higher.

DISCUSSION

We have examined polymorphisms spanning the entire length of *PLAU* in several different case-control series, a discordant sibling pair series, and a group of individuals taken from sibships that contributed to our linkage signal on chromosome 10 (see summary Table VIII). We did not find evidence for association with Alzheimer disease in any series examined with any of the variants we typed. From this study, we conclude that variation in the *PLAU* gene is not a significant risk factor for LOAD as seen from our negative results in the CC series. Additionally, variation in PLAU does not account for the linkage to chromosome 10 as seen from the analysis of our sharer sample.

It is worth noting that this finding does not address the issue of whether PLAU is involved in the pathogenesis of Alzheimer disease. It remains possible that PLAU is indeed involved in the clearance of Aβ. It is also possible that rare, genetic variability at the locus

TABLE VI. Coding Variant Power Analysis

	Disease model	Relative risk		Power calculations allelic test (1 df)	
		RR(Dd)	RR(DD)	$\alpha = 5\%$	$\alpha = 1\%$
A.[a] Mutation (sample size, minor allele frequency)					
Exon 6, P141L (652 cases/824 controls, 25%)	REC	1	1.5	0.29	0.13
		1	2	0.76	0.55
		1	3	1.00	0.99
	DOM	1.5	1.5	0.94	0.82
		2	2	1.00	1.00
	MULT	**1.5**	**2**	**0.99**	0.97
		1.73	3	1.00	1.00
Exon 2, V15L (176 cases/175 controls, 1%)	REC	1	100	0.18	0.06
	DOM	2	2	0.16	0.05
		3	3	0.43	0.18
		5	**5**	**0.88**	0.66
	MULT	1.73	3	0.11	0.02
		2.24	5	0.23	0.08
		3.16	10	0.49	0.24
Exon 8, K231Q (271 cases/458 controls, 1%)	REC	1	100	0.34	0.21
	DOM	2	2	0.34	0.18
		3	**3**	**0.76**	0.58
		5	**5**	**0.99**	0.97
	MULT	1.73	3	0.22	0.11
		2.24	5	0.46	0.28
		3.16	10	0.82	0.66
B.[b]					
	REC	1	1	0.052	
		1	2	0.195	
		1	3	0.45	
		1	5	0.97	
	DOM	2	2	0.47	
		3	3	**0.84**	
		5	5	**0.99**	
	MULT	1.73	3	0.63	
		2.24	5	0.945	
		3.16	10	1	

[a]Shown are the power calculations using various models of disease risk (REC, recessive; DOM, dominant; MULT, multiplicative) for two different false positive error rates ($\alpha = 0.05$ or 0.01) for each CC sample used to analyze the three *PLAU* mutations in exons 6, 2, and 8. Highlighted in bold are the likelihoods discussed in the text.
[b]Shown are the power calculations using various models of disease risk (REC, recessive; DOM,dominant; MULT, multiplicative) for our DSP series used to analyze the *PLAU* mutations in exon 6. Highlighted in bold are the likelihoods discussed in the text.

may influence disease in some cases. However, our data strongly suggests that common genetic variability at the locus does not underpin the report of linkage of AD to chromosome 10 markers and, if variability at the locus underpins the reported genetic linkage of plasma Aβ to the same chromosomal location, these results suggest that that marker has doubtful relevance to the etiology of typical late onset AD.

TABLE VII. LD Between all Eight SNP Loci Typed in *PLAU*

	5′UTR	Exon 2	Exon 6	Intron 7	Exon 8	Intron 9	Exon 11
5′UTR	×	**1**	**0.87**	**0.96**	**1.00**	**0.96**	**0.96**
Exon 2		×	**1**	**0.75**	**1**	**0.75**	0.16
Exon 6			×	**0.97**	**0.94**	**0.99**	**1.00**
Intron 7				×	**0.96**	**0.94**	**0.92**
Exon 8					×	**0.92**	**0.96**
Intron 9						×	**0.93**
Exon 11							×

Shown are the D′ calculations for each of the eight *PLAU* SNPs we typed. All D′ highlighted in bold have a *P*-value ≤ 0.05.

TABLE VIII. Summary of Analyses Performed for Each *PLAU* Variant

Variant	Position from ATG	Sample	Analysis				
5′UTR	−1,966	WU case-control	Single locus	Multilocus genotypic regression			
Exon 2	+42 coding	WU case-control	Single locus	Multilocus genotypic regression			
Exon 6	+1,787 coding	WU case-control	Single locus	Multilocus genotypic regression	Haplotypic analysis		
		MAYO case-control	Single locus				
		UK case-control	Single locus				
		DSP				Matched case control	Transmit
Intron 7	+2,417	WU case-control	Single locus	Multilocus genotypic regression	Haplotypic analysis		
Exon 8	+2,434 coding	MAYO case-control	Single locus				
Exon 8	+2,665 noncoding	WU case-control	Single locus	Multilocus genotypic regression	Haplotypic analysis		
Intron 9	+3,426	WU case-control	Single locus	Multilocus genotypic regression	Haplotypic analysis		
Exon 11	+143 from stop	WU case-control	Single locus	Multilocus genotypic regression	Haplotypic analysis		

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APPENDIX A

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