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No association between very low density lipoprotein receptor (VLDL-R) and Alzheimer disease in American Caucasians

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Abstract

The very low density lipoprotein receptor gene (VLDL-R) is a receptor for apolipoprotein-ε (APOE)-containing lipoproteins, and thus has been suggested as a possible risk factor for Alzheimer disease (AD). Recently, Okuizumi et al. [Nature Genet., 11 (1995) 207–209] reported an association between the 96 bp allele at the VLDL-R locus and AD in a Japanese population. The association resulted in a two-fold increase of risk that decreased with increasing age. We have examined this association in 316 Caucasian sporadic AD patients, comparing their findings to 160 Caucasian AD spouse controls. We also investigated 53 late-onset Caucasian AD families for association and linkage. Our data failed to confirm linkage and/or association to the VLDL-R locus. Stratification by age at onset or APOE genotype also failed to show significant results.

Keywords: Alzheimer disease; Very low density lipoprotein receptor gene; Association; Apolipoprotein-ε

The apolipoprotein-ε (APOE)-4 allele has been demonstrated to be a risk factor in both sporadic and late-onset familial Alzheimer disease (AD) by numerous laboratories in several different ethnic groups (reviewed in [8]). The APOE-4 allele acts to increase risk in a dose-related manner and decrease age at onset in AD in the homozygous state [1]. APOE is the single most important biological factor involved in AD risk defined to date. Despite its significant effect, studies have shown that APOE accounts for only 45–55% of the genetic variation seen in late-onset AD [10]. The very low density lipoprotein receptor gene (VLDL-R) is a receptor for APOE-containing lipoproteins. Hence, it has been hypothesized that VLDL-R is a potential additional risk factor in AD.

Recently, an association between an allele at the VLDL-R locus and increased risk of AD was reported in a Japanese population of sporadic AD patients. The authors found a two-fold increase in risk of AD for the 96 bp allele (OR = 2.1, 95% CI = 1.1–4.2) [7]. The strength of the association and its effect on risk decreased with increasing age. We investigated this association in a large, Caucasian, clinic-based population of sporadic AD patients ($N = 316$) ascertained through the Joseph and Kathleen Bryan Alzheimer Disease Research Center (ADRC). We also examined 53 late-onset (mean age of onset ≥60 years) familial AD patients and their families for evidence of linkage and/or association to VLDL-R.

DNA was obtained on 316 sporadic patients (42% male) who were ascertained through the Joseph and Kathleen Bryan ADRC. All AD-diagnosed individuals exhibited standard clinical definitions of either dementia or AD based on the NINDS-ADRDA criteria [6]. There was no obvious family history of AD upon personal interview of family members and/or other knowledgeable informants. DNA was also obtained from 160 spouses of AD patients (43% male) to serve as a control population. Upon initial contact and interview, the spouse controls in this study showed no evidence or history of dementia. The mean age of examination for the sporadic patients

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was 72.2 ± 8.0 years (age at onset 67.2 ± 8.4 years), and the mean age of examination for the spouse controls was 69.6 ± 8.0 years. Although this difference is statistically significant ($P = 0.0004$), it is most likely an artifact of large sample size and not clinically relevant. However, in order to take this difference into account, we controlled for age in our analyses. All sporadic AD cases and spouses used in this study were Caucasian.

Fifty-three families with late-onset AD were also included in the study. This dataset has been described previously [4]. The family data consisted of 115 AD-affected and 199 unaffected individuals. All diagnosed affected individuals met the NINDS-ADRDA diagnostic criteria for AD [6]. Mean age of examination for the affecteds in the family data was 75.7 ± 8.2 years (onset 69.2 ± 8.8 years).

Amplification of the CGG polymorphism was carried out using primers designated by the probe A34R/UA (A34R, GCAGCCAGAGCGCCCAGAGCG and UA, AGGGCTGGTAACTTGTTGTGCGGAG) [7]. Genotyping was performed as previously described [13]. A comparison of our sizing to the coding used by Okuizumi et al. [7] is: 96 bp, 5 repeat; 99 bp, 6 repeat; 105 bp, 8 repeat; 108 bp, 9 repeat; 111 bp, 10 repeat; 114 bp, 11 repeat. These data are based on a series of identical DNA samples independently genotyped by both our laboratory and by Okuizumi et al. [7]. APOE genotyping was performed as described in Saunders et al. [11].

Allele frequencies were compared between the control and AD groups using the χ^2 statistic. For the family data association one affected per family was chosen at random. Odds ratios with corresponding 95% confidence intervals were calculated to estimate risk of AD for each allele, where risk of AD was compared for that allele versus all other alleles [3]. The Bonferroni correction for multiple comparisons on the same data was used in the calculation of confidence intervals for the odds ratios [2].

Affected Pedigree Member (APM) linkage analysis and two point LOD scores were calculated as previously described [9]. The Fastlink 2.2 version of the LINKAGE computer program [5] was used in the LOD score analysis. Both an autosomal dominant and autosomal recessive model of inheritance were tested. Because of the late and variable age of onset in AD, both age-adjusted and low penetrance analyses were performed for each of the models examined. Probabilities were assigned for at-risk individuals in the age-adjusted analysis assuming a standard normal distribution with mean and standard deviation as determined from the sample population [9]. For the low penetrance analysis, phenotypic information on disease status was included for affected individuals only, while genotypic (marker) data was included on all participating family members. APM analysis was performed using the APM computer package, using the $1/\sqrt{(p)}$ weighting function for allele frequencies [9,14].

The results of this association study found no difference in the VLDL-R allele frequencies between controls and either sporadic or familial AD patients (Table 1).

Table 1
VLDL-R frequencies (Caucasians only)

	Sporadics				χ^2	df	P	Families ($N = 53$)
	All ($N = 316$)	<65 years ($N = 53$)	65–74 years ($N = 137$)	≥75 years ($N = 124$)				
96	0.36	0.30	0.38	0.37				0.48
102	0.00	0.00	0.00	0.00				0.00
105	0.28	0.25	0.26	0.30	8.75	8	0.36	0.28
108	0.35	0.44	0.35	0.31				0.22
114	0.01	0.01	0.00	0.02				0.02
	Controls							
	All ($N = 160$)	<65 years ($N = 41$)	65–74 years ($N = 80$)	≥75 years ($N = 38$)				
96	0.38	0.35	0.39	0.42				
102	0.00	0.00	0.00	0.00				
105	0.32	0.32	0.32	0.29	3.69	6	0.72	
108	0.28	0.33	0.26	0.28				
114	0.02	0.00	0.03	0.01				
χ^2	6.23	3.55	8.20	1.05				3.31
df	4	3	4	4				3
P	0.18	0.31	0.08	0.90				0.35

N, number of persons.

Table 2
Odds ratios for VLDL-R alleles

No. of alleles	96	105	108	114
0	1.0	1.0	1.0	1.0
1	0.84 (0.57, 2.46)	0.71 (0.35, 1.44)	1.65 (0.81, 3.38)	0.60 (0.07, 4.98)
2	0.81 (0.29, 2.28)	0.87 (0.27, 2.78)	1.51 (0.48, 4.70)	–

Sample size too small to do calculations for 102 allele or for 2 alleles category for 114 allele. All values corrected for multiple comparisons.

Subdividing by age category (<65, 65–74, and ≥75 years) also showed no significant difference between the sporadics and control data for VLDL-R allele frequencies. The family association data was not divided by age due to the small sample size. The results of the association studies were also not significant stratifying by the number of APOE-4 alleles. In addition, there was no difference in allele frequencies in VLDL-R between controls and the sporadic AD patients when controlling for the number of APOE-4 alleles in conjunction with age (data not shown).

Odds ratios were calculated for each of the five VLDL-R alleles (Table 2). No increase in risk was reported in the 96 bp allele as had been seen in the Okuizumi et al. study [7]. An increase in risk of AD was suggested for the 108 allele, but when the results were corrected for multiple comparisons the confidence interval was no longer significant (OR = 1.65, 95% CI = 0.81–6.38). Similarly, when logistic regression was performed on these data adjusting for age and sex, there was no significant increase or decrease in risk of AD for any of the VLDL-R alleles. In addition, for each VLDL-R allele, there was no evidence for interaction between the allele and presence of at least one APOE-4 allele.

Linkage analysis was also performed on 53 late-onset AD families. Two-point LOD scores excluded at least 13 cM on either side of the VLDL-R gene for all models tested (Table 3). Results of the APM linkage analysis [14] also gave no evidence of linkage or association between AD and VLDL-R.

In summary, no significant association was found in these data for the VLDL-R locus versus AD in a series of Caucasian AD sporadic individuals versus Caucasian AD spouse controls. Also, examination of linkage and association in late-onset Caucasian AD families failed to show significant results.

Neither our sporadic nor familial AD data confirm the results of Okuizumi et al. [7] where significant association was seen. Several possibilities could account for these differences. First and foremost, our study was performed on American Caucasians while the Okuizumi et al. [7] study population was Japanese. Thus, true ethnic differences underlying AD risk in the two populations could exist. The allele frequencies for our Caucasian controls did differ significantly from the Japanese control frequencies reported by Okuizumi et al. [7] ($P < 0.0001$). It may also be that the finding in the Japanese data was a chance occurrence due to population stratification. In addition, the rare possibility exists that a gene in linkage disequilibrium with VLDL-R in the Japanese population represents the true biological association. Exclusion of this possibility would need to be examined in the study of Okuizumi et al. However, it is extremely unlikely that another gene is the cause of their significant findings. Finally, the result could represent the failure to identify underlying differences between the patient and control populations sampled and compared. Although additional study is necessary in both of these ethnic groups, it seems unlikely that this polymorphism of VLDL-R explains a substantial portion of the genetic etiology of AD in American Caucasians.

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Table 3
LOD scores for VLDL-R in families

Model	Theta						
	0.00	0.05	0.10	0.15	0.20	0.30	0.40
Dominant	−25.10	−10.07	−6.12	−3.90	−2.42	−0.82	−0.18
Dominant/no age curve	−14.01	−5.00	−2.61	−1.34	−0.62	−0.04	−0.21
Recessive	−54.83	−15.63	−9.27	−5.82	−3.65	−1.28	−0.27
Recessive/no age curve	−40.47	−12.33	−6.99	−4.17	−2.47	−0.75	−0.14

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