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No association of *SORL1* SNPs with Alzheimer's disease

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ABSTRACT

SORL1 is an element of the amyloid precursor protein processing pathway and is therefore a good candidate for affecting Alzheimer's disease (AD) risk. Indeed, there have been reports of associations between variation in *SORL1* and AD risk. We examined six statistically significant single-nucleotide polymorphisms from the initial observation in a large Caucasian American case–controls cohort (1000 late-onset AD [LOAD] cases and 1000 older controls). Analysis of allele, genotype and haplotype frequencies revealed no association with LOAD risk in our cohort.

It is estimated that up to 79% of the risk for late-onset Alzheimer's disease (LOAD) is attributable to genetics [4]. Thus far, only variation in *APOE* has been definitively associated with LOAD [2], but only 20–29% of risk is attributable to this variation [20,22]. Association between variation in an excellent biological candidate gene, sortilin-related receptor 1 (*SORL1*), and the risk of LOAD has been reported [18]. We genotyped and analyzed six of the single nucleotide polymorphisms (SNPs), listed in Table 1, that were reported to be associated with LOAD in multiple case–control cohorts or family-based samples [18] to verify this report.

Case subjects were Caucasian Americans with LOAD ($n = 1009$; mean age-at-onset [AAO] 72.8 ± 6.2 [S.D.] years; 67.7% female; 7.3% autopsy-confirmed) recruited by the University of Pittsburgh Alzheimer's Disease Research Center. All cases were evaluated clinically and met criteria for probable or possible AD [11] or by autopsy and met neuropathological criteria for definite AD [13,14]. Controls were Caucasian Americans of age 60 or above with no psychiatric or neurological disorders ($n = 1009$; mean age-at-baseline 74.1 ± 6.2 [S.D.] years; 59.9% female; 1.3% autopsy-confirmed). All experiments on human subjects were conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written consent of the subjects. The genetic study was approved by the University of Pittsburgh Institutional Review Board.

Genotypes for the six *SORL1* SNPs were ascertained from genomic DNA using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA). Case and control samples were present on each 384-well plate used in genotyping. To estimate genotyping error rates, 10% of the samples were selected at random and repeated. Genotypes for *APOE* were determined either as previously described [5] or using TaqMan SNP genotyping assays.

Allele and genotype frequencies were calculated by the direct allele-counting method. Goodness of fit to Hardy–Weinberg equilibrium was tested using the χ^2-test. Differences between genotype and allele frequencies in cases and controls were tested with the χ^2-test. Differences between cases and controls stratified by *APOE*4* carrier status were also tested with the χ^2-test. Haplotype frequencies were estimated in cases and controls, and the global difference in frequencies was tested. Linkage disequilibrium (LD) between the SNPs was estimated by calculating D' and r^2 between each pair. Haplotype frequencies were then again compared after eliminating highly correlated SNPs (those with $r^2 > 0.8$). These statistics were calculated using R 2.2.0 with the genetics and haplo.stats packages attached [16,21,24]. Power to detect associations was determined with PS 2.1.30 [3].

The allele and genotype frequencies for the *SORL1* SNPs are shown in Table 1. Neither cases nor controls had genotype frequencies which differed significantly from those expected under Hardy–Weinberg equilibrium. The differences between cases and controls do not rise to statistical significance (range: $p = 0.177$–0.980).

Among, non-*APOE*4* carriers, the differences were not statistically significant (range: $p = 0.357$–0.922). Among *APOE*4* carriers, *rs661057* was associated with AD risk (genotype frequency differences, $p = 0.022$; alleles, $p = 0.008$). None of the other SNPs were

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Table 1
Examined *SORL1* markers, their genotype and allele frequencies, and statistical significance

RefSNP	Variation	Gene element	AD status	*n*	Genotype frequencies			*p*	Allele frequencies		*p*
					TT	TC	CC		T	C	
rs661057	c.285 + 5629T > C	Intron 1	Cases	1000	0.336	0.490	0.174	0.980	0.581	0.419	0.852
			Controls	1001	0.339	0.491	0.171		0.584	0.416	
					CC	CT	TT		C	T	
rs668387	c.939 + 163C > T	Intron 6	Cases	1005	0.353	0.473	0.174	0.177	0.590	0.410	0.526
			Controls	1004	0.323	0.514	0.163		0.580	0.420	
					GG	GA	AA		G	A	
rs689021	c.939 + 3362G > A	Intron 6	Cases	1000	0.345	0.174	0.178	0.370	0.584	0.417	0.786
			Controls	1003	0.326	0.163	0.167		0.579	0.421	
					GG	GA	AA		G	A	
rs641120	c.940 − 2747G > A	Intron 6	Cases	1004	0.348	0.480	0.172	0.462	0.588	0.412	0.518
			Controls	999	0.324	0.507	0.169		0.578	0.422	
					TT	TG	GG		T	G	
rs2070045	c.3561T > G	Exon 26	Cases	994	0.607	0.344	0.049	0.711	0.779	0.221	0.823
			Controls	1001	0.608	0.335	0.057		0.776	0.224	
					TT	TA	AA		T	A	
rs3824968	c.4752T > A	Exon 34	Cases	1001	0.498	0.480	0.084	0.674	0.707	0.293	0.420
			Controls	1007	0.485	0.507	0.094		0.695	0.305	

observed to be associated (range: $p = 0.109$–0.765). Haplotype frequencies calculated for all six SNPs did not differ with statistical significance ($p = 0.627$) (data not shown). LD between the SNPs is shown in Table 2. Because the three SNPs in intron 6 (*rs668387*, *rs689021* and *rs641120*) were highly correlated ($r^2 = 0.988$–0.996), haplotype frequencies were recalculated between just four of the SNPs (*rs661057*, *rs668387*, *rs2070045* and *rs3824968*). The frequency differences were not significant ($p = 0.858$).

Given the minor allele frequencies at $\alpha = 0.05$, we had 80% power to detect a risk odds-ratio of 1.23 with statistical significance for *rs2070045* which had the lowest minor allele frequencies and 1.20 for *rs641120* which had the highest.

Sortilin-related receptor 1 (also known as sorLA and LR11) is an excellent biological candidate gene due to its role in amyloid precursor protein (APP) processing and evidence that variation in SORL1 levels are associated with both APP [1,15] and LOAD [19]. Association between variation in *SORL1* and LOAD has been reported in Northern Europeans, Caucasian Americans, Caribbean Hispanics and Israeli Arabs [18]; Caribbean Hispanics and Caucasian Americans [6]; Han Chinese [23]; and Caucasian Americans with Down syndrome [7]. However, the same large study that reported association in multiple groups [18] found no association in Caucasian American or African American families. Two groups examined *SORL1* SNPs in publicly available data from the same genomewide association studies of AD [17] and found marginal associations with some variants in specific regions of *SORL1*: Meng et al. [12] observed associated SNPs in the interval from exon 7 to exon 18; Webster et al. [25], from intron 25 to intron 39 (*SORL1* comprises 48 exons). However, neither replicated any of Rogaeva et al.'s [18] significant SNPs in particular. Another high-density genomewide association study of AD failed to find any association between disease risk and any of 41 SNPs included from the *SORL1* region [8].

Liu et al. [10] also failed to find evidence of association between *SORL1* and LOAD in a large Dutch pedigree, although they did observe evidence of linkage nearby at 11q25 that they believe to be associated not with *SORL1* but with *OPCML* and *HNT*. Li et al. [9] examined several SNPs and haplotypes in a population of size and demographics similar to ours, and observed only marginal association with AD risk for a single SNP (*rs2070045*) which they dismissed because it is not statistically significant after correcting for multiple testing. We did not find evidence of association in our Caucasian American cohort. It is possible that the effect of *SORL1* variation on AD risk is specific to particular ethnic groups or that the effect is not large enough to be detected reliably by a cohort of our size. Further examinations into this gene and the region surrounding it are necessary to determine the role of *SORL1* if any in modulating LOAD risk.

Table 2
Linkage disequilibrium between examined *SORL1* SNPs

		D'				
	rs661057	0.6363	0.6373	0.6437	0.0989	0.0437
	0.4014	*rs668387*	0.9956	0.9900	0.1414	0.0680
	0.4044	0.9780	*rs689021*	0.9876	0.1470	0.0642
r^2	0.4141	0.9720	0.9704	*rs641120*	0.1487	0.0640
	0.0039	0.0081	0.0086	0.0088	*rs2070045*	0.8643
	0.0011	0.0014	0.0013	0.0013	0.5019	*rs3824968*

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