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Neurobiology of Disease

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Neurobiology of Disease 209 (2008) 293–296

SORL1 variants and risk of late-onset Alzheimer's disease

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Received 17 May 2007; revised 31 August 2007; accepted 9 September 2007
Available online 16 September 2007

A recent study reported significant association of late-onset Alzheimer's disease (LOAD) with multiple single nucleotide polymorphisms (SNPs) and haplotypes in *SORL1*, a neuronal sortilin-related receptor protein known to be involved in the trafficking and processing of amyloid precursor protein. Here we attempted to validate this finding in three large, well characterized case–control series. Approximately 2000 samples from the three series were individually genotyped for 12 SNPs, including the 10 reported significant SNPs and 2 that constitute the reported significant haplotypes. A total of 25 allelic and haplotypic association tests were performed. One SNP rs2070045 was marginally replicated in the three sample sets combined (nominal $P=0.035$); however, this result does not remain significant when accounting for multiple comparisons. Further validation in other sample sets will be required to assess the true effects of *SORL1* variants in LOAD.

Keywords: Alzheimer's disease; Single nucleotide polymorphism; Haplotype; Association study; *SORL1*

Introduction

Late-onset Alzheimer's disease (LOAD) is a complex, neurodegenerative disease that primarily manifests itself in the deterioration and loss of cognitive abilities. Susceptibility to the disease is strongly affected by genetic factors (Gatz et al., 2006), with apolipoprotein E4 (*APOE4*) likely to play a major role in the predisposition to the condition (Strittmatter et al., 1993). There is no consensus on the identities of other genetic risk factors, although many others have been proposed that have either failed replication or not yet been tested in independent sample sets.

Recently, Rogaeva et al. (2007) reported a role of the neuronal sortilin-related receptor *SORL1* in the genetics of LOAD, after testing single nucleotide polymorphisms (SNPs) in several members of the vacuolar protein sorting gene family for association with the disease. They tested multiple LOAD sample sets from different ethnic backgrounds and identified 10 SNPs (SNP numbers 4, 8–10, 12, 17, 19, and 23–25 in the Rogaeva publication) and 4 haplotypes (8-9-10, 9-10-11, 22-23-24, and 23-24-25) in *SORL1* that are significantly associated with LOAD in at least one individual sample set or a meta-analysis of several sample sets combined. *SORL1* is known to be involved in intracellular transport and processing of the amyloid precursor protein (Andersen et al., 2005; Offe et al., 2006), thus genetic variation in *SORL1* may affect predisposition to LOAD. However, genetic association studies have often produced spurious findings, and thus require validation in multiple other sample sets (Ioannidis, 2005; Moonesinghe et al., 2007). In this study, we specifically tested all the reported significant *SORL1* markers and haplotypes in ~2000 Caucasian samples from three well-characterized LOAD case–control series.

Materials and methods

Samples

Three LOAD case–control sample sets, collected with informed consent/assent from the participating individuals and approvals from the participating institutions, were used in the current study. The WU sample set (WU) was obtained from the Washington University Alzheimer's Disease Research Center, and the UK1 and UK2 sample sets were obtained from the Cardiff University. These sample sets consist of 377 cases and 376 controls (WU), 343 cases and 346 controls (UK1), and 278 cases and 311 controls (UK2), respectively. Cases had a minimum age at disease onset of 60 years and a diagnosis of probable or definite Alzheimer's disease (NINCDS-ADRDA), with mean age at onset of 76.2±6.9 years (WU), 75.8±6.9 years (UK1), and 76.3±7.1 years (UK2). Controls were ascertained at the age of ≥65 years and screened for evidence of dementia (MMSE =28, clinical dementia rating=0, or full neurological exam); the mean age at examination is 77.4±7.5 years (WU), 76.4±6.1 years (UK1), and 76.5±5.6 years (UK2). All individuals are of Caucasian origins, and females account for 63.9% of cases and 62.8% of controls in the WU sample set, 77.6% of cases and 75.1%

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2007.09.001

of controls in the UK1 sample set, and 73.0% of cases and 57.2% of controls in the UK2 sample set. The *APOE4* allele frequencies in cases/controls are 32.8%/12.5% (WU), 38.0%/13.1% (UK1), and 37.2%/12.1% (UK2).

Genotyping and statistical analyses

Genotyping of SNPs was carried out by allele-specific real-time PCR for individual samples using primers that were designed and validated in-house (Germer et al., 2000). Cases and controls were run on the same plate in a blinded fashion, and genotypes were assigned using an automated algorithm. Assay quality was manually scored by an individual who had no access to the sample phenotypes, before the genotyping results were subjected to statistical analysis. To estimate the genotyping accuracy of the 12 SORL1 assays, approximately 12% samples were genotyped twice for each marker. The concordance rate of duplicate genotypes ranges from 99.1% to 100% (average ± SD: 99.8 ± 0.3%).

Genotypic distributions of each SNP were assessed for Hardy–Weinberg equilibrium in cases and controls separately in each of the three sample sets. Allelic association for each individually typed marker was tested using a standard two-sided chi-square test. Meta-analysis was performed with SAS version 9 (SAS Institute Inc. Cary, NC, USA) using fixed effects Mantel–Haenszel methods to combine odds ratios across studies and the Breslow–Day test to assess homogeneity of the odds ratios. The haplo.stats package available for the R language was used for haplotype analysis (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm). The statistical power of the WU, UK1, and UK2 studies was estimated for a two-sample chi-square test of equal proportions as

Table 1
Allelic association of *SORL1* variants with Alzheimer's disease in the WU, UK1, and UK2 sample sets

SNP number[a]	SNP ID	Allele 1	Allele 2	Sample set	Case[b]					Control[b]					
					11	12	22	Sum	MAF[c]	11	12	22	Sum	MAF[c]	Allelic P
4	rs661057	C	T	All	201	487	306	994	0.447	191	533	308	1032	0.443	0.804
				UK1	76	166	101	343	0.464	71	178	97	346	0.462	0.952
				UK2	50	136	92	278	0.424	62	160	89	311	0.457	0.268
				WU	75	185	113	373	0.449	58	195	122	375	0.415	0.179
8	rs668387	T	C	All	195	484	313	992	0.441	197	524	306	1027	0.447	0.682
				UK1	72	172	97	341	0.463	67	180	99	346	0.454	0.721
				UK2	52	130	95	277	0.422	69	150	91	310	0.465	0.147
				WU	71	182	121	374	0.433	61	194	116	371	0.426	0.776
9	rs689021	A	G	All	197	489	311	997	0.443	204	522	303	1029	0.452	0.562
				UK1	74	174	95	343	0.469	69	178	99	346	0.457	0.635
				UK2	52	131	95	278	0.423	71	150	89	310	0.471	0.096
				WU	71	184	121	376	0.434	64	194	115	373	0.432	0.935
10	rs641120	A	G	All	196	483	316	995	0.440	198	527	307	1032	0.447	0.631
				UK1	72	170	99	341	0.460	67	178	100	345	0.452	0.759
				UK2	52	131	95	278	0.423	70	150	91	311	0.466	0.133
				WU	72	182	122	376	0.434	61	199	116	376	0.427	0.794
12	rs12285364	T	C	All	3	93	900	996	0.050	3	85	944	1032	0.044	0.398
				UK1	0	31	311	342	0.045	1	30	315	346	0.046	0.929
				UK2	1	26	251	278	0.050	0	26	285	311	0.042	0.483
				WU	2	36	338	376	0.053	2	29	344	375	0.044	0.407
17	rs556349	T	G	All	97	454	441	992	0.327	99	442	487	1028	0.311	0.296
				UK1	39	150	153	342	0.333	35	144	166	345	0.310	0.358
				UK2	18	129	131	278	0.297	28	130	152	310	0.300	0.901
				WU	40	175	157	372	0.343	36	168	169	373	0.322	0.389
19	rs2070045	G	T	All	58	383	550	991	0.252	51	357	619	1027	0.223	0.035
				UK1	25	129	188	342	0.262	16	116	214	346	0.214	0.037
				UK2	11	110	156	277	0.238	17	110	184	311	0.232	0.785
				WU	22	144	206	372	0.253	18	131	221	370	0.226	0.223
23	rs3824968	A	T	All	89	438	463	990	0.311	97	422	510	1029	0.299	0.416
				UK1	36	155	151	342	0.332	32	133	181	346	0.285	0.058
				UK2	20	120	135	275	0.291	31	129	150	310	0.308	0.523
				WU	33	163	177	373	0.307	34	160	179	373	0.306	0.945
24	rs2282649	T	C	All	89	416	487	992	0.299	81	407	536	1024	0.278	0.131
				UK1	35	146	160	341	0.317	26	128	191	345	0.261	0.022
				UK2	20	113	144	277	0.276	27	124	160	311	0.286	0.703
				WU	34	157	183	374	0.301	28	155	185	368	0.287	0.551
25	rs1010159	C	T	All	111	469	412	992	0.348	119	453	458	1030	0.335	0.389
				UK1	40	156	145	341	0.346	34	144	168	346	0.306	0.117
				UK2	27	134	116	277	0.339	37	136	136	309	0.340	0.960
				WU	44	179	151	374	0.357	48	173	154	375	0.359	0.937

[a] Based on Rogaeva et al. Nat Genet 39, 168, 2007.
[b] Counts of genotype 11, 12, and 22.
[c] Minor allele frequency.

Table 2
Association of *SORL1* haplotypes with Alzheimer's disease in the WU, UK1, and UK2 sample sets combined

Haplotype SNP# [a]	Haplotype	Case frequency	Control frequency	*P* (haplotype)	*P* (global)
8-9-10	CGG	0.554	0.546	0.623	0.837
	TAA	0.438	0.446	0.587	
9-10-11	AAT	0.415	0.421	0.722	0.779
	GGG	0.394	0.399	0.717	
	GGT	0.161	0.147	0.239	
	AAG	0.022	0.025	0.495	
22-23-24	TTC	0.643	0.663	0.196	0.216
	CAT	0.284	0.262	0.121	
	CTC	0.041	0.036	0.393	
	CAC	0.014	0.021	0.095	
	TAT	0.011	0.014	0.472	
23-24-25	TCT	0.650	0.661	0.484	0.396
	ATC	0.295	0.275	0.166	
	TCC	0.034	0.038	0.515	
	ACC	0.015	0.021	0.140	

SNP#11 is rs4935775; SNP#22 is rs1699102; see Table 1 for others.
[a] Based on Rogaeva et al. Nat Genet 39, 168, 2007.

described by Lachin (Lachin et al., 1981) assuming an odds ratio of 1.2, allele frequencies as observed in Rogaeva et al., and an alpha level (two-sided) of 0.05.

Results

We genotyped a total of 12 SNPs, including the 10 reported individually significant markers and two others that constitute the reported significant haplotypes, in the WU, UK1, and UK2 sample sets. None of the 12 markers showed significant deviation from Hardy–Weinberg equilibrium in any of the three sample sets. Allelic association tests identified one SNP, rs2070045, that reached marginal significance in the three sample sets combined ($P=0.035$) (Table 1). This marker and rs2282649, which have a pairwise r^2 of 0.63, were significant in the UK1 ($P=0.037$ and 0.022, respectively) but not other sample sets (Table 1). In a meta-analysis, including our and Rogaeve et al's Caucasian sample sets, rs2070045 showed an odds ratio of 1.20 (95% CI: 1.10–1.31; heterogeneity: $P=0.30$). No other *SORL1* marker reached significance in any of the three sample sets, either individually or combined. Power to detect an odds ratio of 1.2 or greater was >70% for 8 of the 9 SNPs in Table 1 showing non-significant results among the combined WU, UK1, and UK2 sample sets.

We next tested whether any of the 4 reported haplotypes (8-9-10, 9-10-11, 22-23-24, and 23-24-25) was significant in our sample sets. None of these haplotypes yielded a significant global or haplotype-specific *P*-value ($P<0.05$) in an analysis of all three sample sets combined (Table 2).

Discussion

Although multiple SNPs and haplotypes were reported to be significantly associated with LOAD in the initial study by Rogaeva and colleagues, no consistent association of single SNPs or haplotypes has been observed across all sample sets or sample sets of identical ethnicity (Rogaeva et al., 2007). Testing these markers in other sample sets may help resolve whether the observed associations are genuine, assess their true effect size, and pinpoint likely causal markers. In our replication study, only one putative association was confirmed in the analysis of our three sample sets combined. While association of rs2070045 with LOAD cannot withstand a multiple-testing correction for the number of markers we tested, it is noteworthy that this marker had a similar odds ratio and *P*-value in the combined Caucasian case–control sample sets in the Rogaeva et al. publication. Furthermore, this marker remained significant when all Caucasian sample sets, including ours, were analyzed together. Its overall effect size is a moderate 1.20, which is similar to reports from other groups in large sample sets, including ours, for markers in *DAPK1* (Li et al., 2006), *GALP* (Grupe et al., 2007), *GAPD* (Li et al., 2004), and *LOC439999* (Grupe et al., 2006). While markers of such effect sizes individually are likely to have limited predictive utility, their aggregation, together with *APOE*, may present a useful predictor of LOAD risk. Furthermore, they provide insight into the molecular mechanism of LOAD.

Although our data singled out rs2070045 among the initial reported markers for significant association with LOAD, it is unlikely that it is the causal variant since it corresponds to a silent mutation (Ser1187Ser). Four other tested markers are in relatively strong LD with rs2070045 ($r^2>0.5$ with rs1699102, rs3824968, rs2282649, and rs1010159), but none is significantly associated with LOAD in our sample sets. Two haplotype combinations (22-23-24 and 23-24-25) derived from the latter 4 markers were reported to be significant in the Rogaeva et al. study, but not so in our sample sets. The other two haplotype combinations (8-9-10 and 9-10-11) were not significant either. Thus other untested variants in strong LD with rs2070045 should be examined in future studies.

In summary, we marginally replicated one SNP (rs2070045) from the Rogaeva et al. report in 3 Caucasian case–control sample sets combined. Further validation of this and the other markers in additional large sample sets will be required to assess whether *SORL1* is a genuine risk factor for LOAD.

Acknowledgments

We thank the families/individuals for their invaluable participation in this study and our colleagues for expert technical contributions. We acknowledge Pamela Moore and Dragana Turic for providing clinical/DNA samples from the MRC UK Genetic Resource for LOAD and Mary Coats and Elizabeth Grant for coordinating the Washington University material. Funding for this work was partly provided by the Medical Research Council, UK, and the Alzheimer's Research Trust to J.W., M.O., M.O'D., and S.L., and the National Institute of Health grants P50 AG05681 and PO1 AG03991 to J.M. and RO1 AG16208 to A. Goate. Statement of conflict of interest: Y.L., C.R., J.C., and A. Grupe are employed by Celera. A. Goate received research funding from and was a consultant to Celera. M.O. and J.W. received research funding from Celera.

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