

Identification of Alzheimer Disease Risk Genotype That Predicts Efficiency of *SORL1* Expression in the Brain

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Objective: To identify *SORL1* risk genotypes that determine receptor protein expression in the human brain.

Design: DNA, RNA, and proteins were extracted from brain autopsies of Alzheimer disease cases and used for *SORL1* genotyping, RNA profiling, and SORLA protein quantification, respectively.

Setting: Specimens were provided by the MRC London Brain Bank for Neurodegenerative Diseases and the Netherlands Brain Bank.

Subjects: Brain autopsy material (frontal cortex) from 88 confirmed cases of sporadic Alzheimer disease.

Results: Our studies identified a *SORL1* haplotype in the 3' gene region consisting of single-nucleotide polymorphisms rs1699102 and rs2070045 that is associated with poor receptor expression in the brain of patients with Alzheimer disease. These gene variations alter the *SORL1* transcript sequence, resulting in a change from frequent to rare codon usage in the minor risk genotype. Studies in cultured cells confirm less efficient translation of the minor receptor transcripts into protein.

Conclusion: Our findings suggest a functional mechanism that correlates *SORL1* genotype with efficiency of receptor expression in the human brain.

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SORTING PROTEIN-RELATED RECEPTOR with A-type repeats (SORLA, also known as LR11) is a type 1 membrane protein highly expressed in neurons of the cortex, hippocampus, and cerebellum.^{1,2} It acts as a sorting receptor for the amyloid precursor protein regulating intracellular trafficking and proteolytic processing of this precursor into amyloidogenic and nonamyloidogenic products.³ Overexpression of SORLA reduces amyloid precursor protein processing and amyloid β peptide formation in cells.⁴⁻⁶ Loss of receptor activity in gene-targeted mice, on the other hand, increases amyloid β formation and senile plaque deposition.^{7,8}

Two lines of research implicate SORLA in Alzheimer disease (AD) in the human population. First, loss of receptor expression has been documented in patients with the sporadic form of the disease using gene expression and immunohistological analyses.^{9,10} Second, genetic variants in *SORL1* (the gene encoding SORLA) have been associated with late-onset AD in several population-based studies.¹¹⁻¹⁵ Cumula-

tive meta-analyses encompassing in excess of 30 000 individuals confirmed association of several markers in *SORL1* with AD.¹⁶

Thus, histopathological analyses of brain autopsies and association studies in human cohorts provide independent evidence for a role of SORLA in the onset and progression of AD. However, a direct link between distinct gene variants and neuronal receptor expression is lacking. Herein, we have correlated individual *SORL1* genotypes with protein expression in brain specimens from 88 confirmed cases of AD. Our studies identified a distinct *SORL1* single-nucleotide polymorphism (SNP) genotype that is associated with poor receptor expression in affected individuals. This SNP genotype alters the transcript sequence of *SORL1*, resulting in a change from frequent to rare codon usage. Studies in cultured cells confirmed less efficient translation of the receptor transcript into protein in the risk variant. Our findings suggest a functional mechanism that correlates *SORL1* genotype with efficiency of receptor expression in the human brain.

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Table 1. Characteristics of Sample Donors

Patient Characteristic	NBB	LBB
No. of subjects	44	44
Male, %	40.9	25.0
Age at death, y, mean (SD)	79 (10)	81 (9)
Braak stage, No. of individuals		
V-VI	36	36 ^a
III-IV	8	5 ^a
Unknown	0	3
<i>APOE4</i> allele frequency in population, %	39.8	36.0

Abbreviations: LBB, MRC London Brain Bank for Neurodegenerative Diseases; NBB, Netherlands Brain Bank.

^aBraak stage estimate based on Consortium to Establish a Registry for Alzheimer's Disease criteria diagnoses.

METHODS

BRAIN AUTOPSY MATERIAL

Brain autopsy specimens from the frontal cortex were obtained from the Netherlands Brain Bank (Netherlands Institute for Neuroscience, Amsterdam) and the MRC London Brain Bank for Neurodegenerative Diseases (Institute of Psychiatry, King's College London). The ethnicity of samples was white. All material was collected from donors for or from whom a written informed consent for a brain autopsy and the use of the material and clinical information for research purposes had been obtained by the Netherlands Brain Bank or the MRC London Brain Bank for Neurodegenerative Diseases. DNA, RNA, and membrane proteins were extracted from the snap-frozen tissues by standard procedures and used for genotyping, quantitative reverse transcriptase–polymerase chain reaction (PCR), or enzyme-linked immunosorbent assay (ELISA), respectively. Genotyping was performed using the TaqMan SNP Genotyping Assay, according to the manufacturer's recommendations, using the 7900HT Fast Real-Time PCR System (Applied Biosystems). RNA was extracted from brain specimens using the Trizol method and transcribed into complementary DNA (High Capacity RNA-to-cDNA Kit; Applied Biosystems). TaqMan gene expression assays were applied to quantify *SORLA* (Hs00983791_m1) messenger RNA (mRNA) levels. Human β -microglobulin (Hs00984230_m1) transcript levels were determined as the internal control.

ENZYME-LINKED IMMUNOSORBENT ASSAY

For quantification of *SORLA*, we developed a custom-made ELISA in 96-well plates (Nunc Maxisorb F96; Thermo Fisher Scientific) coated with 1 μ g/mL of rabbit anti-*SORLA* IgG (IgG 5387) in coating buffer (100mM sodium bicarbonate, pH 9.8) at 4°C overnight. After rinsing in wash buffer (150mM sodium chloride, 7.5mM disodium phosphate, 2.8mM monosodium phosphate, and 0.05% Tween, pH 7.4), coated wells were blocked with wash buffer supplemented with 2.5% casein (Sigma-Aldrich) at 4°C overnight. Protein lysates or recombinant *SORLA* protein standards (purified from stably transfected Chinese hamster ovary [CHO] cells) were loaded on the wells and incubated overnight at 4°C. The next day, the plates were washed and incubated with mouse monoclonal anti-*SORLA* antibody (IgG 20c11) at a final concentration of 1 μ g/mL in wash buffer overnight and followed by rabbit antimouse polyhorseradish peroxidase IgG for 2 hours at room temperature (1:2500 in wash buffer; Dako). Colorimetric staining was

performed by adding 100 μ L of peroxide buffer (Thermo Fisher Scientific) with *o*-phenylenediamine (Pierce) for 30 minutes at room temperature and measured on an ELISA reader at 450 nm.

CELL CULTURE EXPERIMENTS

The major allele variants of rs2070045 and rs1699102 present in the human *SORLA* complementary DNA⁶ were converted into the minor genotypes by PCR-based site-directed mutagenesis using primers 5'-cag gga ctg gtc Gga tga ag-3' and 5'-ctt cat cCg acc agt ccc tg-3' for rs2070045 and 5'-ctg ccc aaa Cgg cac ttg ca-3' and 5'-tgc aag tgc cGt ttg ggc-3' for rs1699102. Both allele variants were introduced into pcDNA3.1zeo vector and transiently transfected into CHO cells using Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, cells were harvested for RNA and protein preparation. *SORLA* transcript and protein levels were quantified by TaqMan and ELISA as described earlier. For determination of transfection efficiencies, zeozin resistance cassette transcripts and 18s ribosomal RNA were amplified by primers 5'-CGC CGC TAG AGG TGA AAT TC-3' and 5'-TGG GCA AAT GCT TTC GCT C-3' (18s ribosomal RNA) and 5'-AGT TGA CCA GTG CCG TTC-3' and 5'-GAT GAA CAG GGT CAC GTC G-3' (zeozin resistance cassette transcripts) applying the Power SYBR Green Real-Time PCR System (Applied Biosystems). Differences in expression levels were calculated using the Pfaffl method.¹⁷

STATISTICAL ANALYSES

Statistical analyses were performed with GraphPad Prism software (GraphPad Software). Nonparametric tests were used to evaluate all data. The Mann-Whitney test was applied to differences between 2 experimental groups. Differences between more than 2 groups were tested using a Kruskal-Wallis 1-way analysis of variance test with a Dunn post hoc test. To account for multiple comparison, we applied a permutation-based method to control the experiment-wise error rate, as described by Churchill and Doerge,¹⁸ considering that the tests are highly correlated owing to strong linkage disequilibrium between SNPs. Haplotype distribution in *SORL1* was estimated using MaCH.¹⁹ Linkage disequilibrium is shown as *D'* and significance for deviation from Hardy-Weinberg equilibrium was obtained from a χ^2 test (Haploview; MIT/Harvard Broad Institute).

RESULTS

We compiled a sample set of brain autopsy material (frontal cortex) from 88 confirmed cases of AD. Donors were of white ancestry. Specimens were provided by the MRC London Brain Bank for Neurodegenerative Diseases (44 individuals) and the Netherlands Brain Bank (44 individuals). Because no discernable differences in sex, age, or clinical scores were evident, both sample sets were combined into 1 study (**Table 1**). DNA, RNA, and membrane proteins were extracted from snap-frozen tissues of each individual by standard procedures and used for genotyping, RNA profiling, and *SORLA* protein quantification, respectively.

For genotyping, we tested 12 SNPs in the *SORL1* region that have previously been associated with AD in white populations¹¹⁻¹⁵ (**Figure 1A**). These SNPs cover the 5' and 3' regions of the gene that have both been genetically linked to disease.¹¹ Single-nucleotide polymor-

phism genotyping replicated the reported distribution of major and minor allele frequencies in our sample set (**Table 2**). Also, linkage disequilibrium analysis confirmed the existence of 2 distinct haplotype blocks in the 5' and 3' regions of *SORL1* (Figure 1B).

In parallel, we determined SORLA protein concentrations in brain specimens of our sample set. To do so, we developed a sensitive sandwich ELISA using 2 different antihuman SORLA antisera for antigen capture and detection, respectively (see the "Methods" section for details). Replicate determination of SORLA levels in brain extracts demonstrated high interassay precision and reproducibility (eFigure 1A, <http://www.archneurology.com>). Also, SORLA concentrations determined by ELISA correlated well with semiquantitative analysis of receptor levels by Western blotting in selected samples (eFigure 1B and C).

To explore whether SORLA expression may be associated with distinct genotypes, we next correlated the 12 SNPs in *SORL1* with protein concentration in the brain. In doing so, we identified 2 SNPs (rs1699102 and rs2070045) that were significantly associated with protein levels in affected individuals ($P = .01$ and $P = .03$, respectively). In line with poor receptor expression being considered disease promoting, the minor (risk) genotype was consistently correlated with lower receptor levels. SORLA levels in heterozygous carriers were intermediary, further supporting a link between genotypes and receptor expression (**Figure 2A** and B). The strength of correlation between risk genotype and expression levels for both SNPs was further improved when samples were adjusted for age, sex, and *APOE4* status using linear regression analysis ($P = .006$ and $P = .03$, respectively) (Figure 2C and D). No statistically significant association of receptor level with genotype was observed for the remaining 10 SNPs (**Figure 3**). Analyses adjusted for multiple testing yielded similar findings with statistically significant association of SNP rs1699102 with SORLA brain levels ($P = .02$) and a trend for association with rs2070045 ($P = .10$) (see the "Methods" section for details).

Single-nucleotide polymorphisms rs2070045 (SNP 19) and rs1699102 (SNP 22) clustered in a haplotype block (block 2) in the 3' region of *SORL1* in our sample set (Figure 1B). Haplotype analysis replicated association of the major or minor haplotype with higher or lower SORLA levels, respectively, as shown in **Table 3**. In detail, haplotype distribution of SNPs in the 5' haplotype block (block 1) did not show association with SORLA levels, in line with our previous results in the individual SNPs in this region (SNPs 8, 9, and 10) (Table 3). In contrast, significant association of the 3' haplotype block (block 2) with the major variant TTCCAT (haplotype frequency, 58.7%) correlating with high SORLA levels ($P = .049$) and the minor variant GCTTCC (haplotype frequency, 26.5%) correlating with low levels ($P = .04$) was reproduced. All other haplotypes were estimated with frequencies less than 5%. However, the strength of association of the minor allele variant ($P = .04$) did not improve as compared with the analysis of the individual SNPs rs2070045 ($P = .03$) and rs1699102 ($P = .01$) on a nominal significance level.

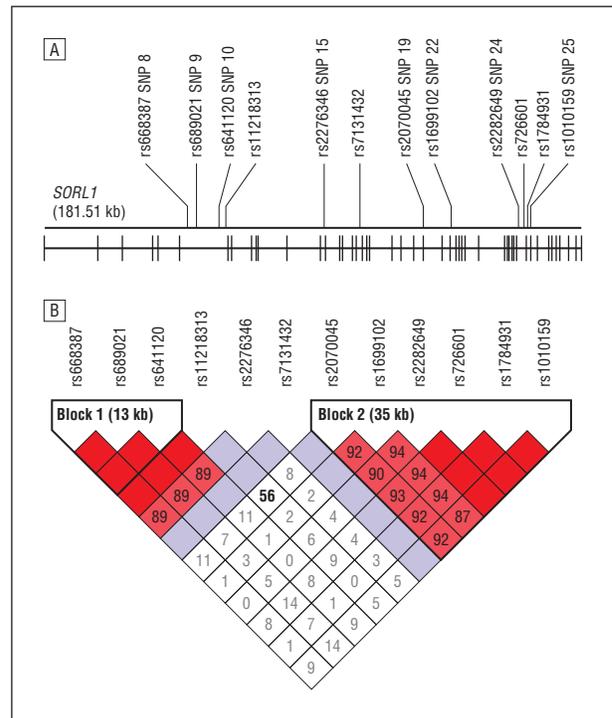


Figure 1. Genotypes of individuals in the sample set. A, Map of the *SORL1* locus highlighting the single-nucleotide polymorphisms (SNPs) used for genotyping.¹¹⁻¹⁵ The numbers above the SNPs refer to the nomenclature of SNPs used in Rogaeva et al.¹¹ B, Linkage disequilibrium and haplotype structure for 12 SNPs in the sample set. Haplotype color scheme indicates logarithm of the odds ratio (LOD) > 2 and $D' = 1$ (red), LOD < 2 and $D' = 1$ (blue), and LOD < 2 and $D' < 1$ (white). kb indicates kilobase.

Remarkably, rs1699102 (SNP 22) and rs2070045 (SNP 19) were the only 2 SNPs among the 12 sequence variations tested that were exonic. Although both SNPs represent silent mutations, alteration of the primary transcript sequence may potentially affect *SORL1* expression. No significant correlation of genotype with *SORL1* mRNA levels ($P = .22$) was seen in a selected set of samples of major or minor SNP genotypes, arguing against an influence of these polymorphisms on transcription efficiency or mRNA stability (**Figure 4**).

To explore alternative effects of *SORL1* sequence variations on receptor expression, we generated *SORL1* complementary DNA constructs encoding the major or minor alleles of rs1699102 and rs2070045 (eFigure 2A). *SORL1* was efficiently expressed from both constructs following transient transfection of Chinese hamster ovary (CHO) cells (eFigure 2B). To accurately quantify SORLA expression, we transiently transfected replicate CHO cell layers with both constructs and determined SORLA mRNA and protein levels using quantitative reverse transcriptase-PCR and ELISA, respectively. As the internal control, we quantified the levels of zeozin resistance cassette transcripts encoded by the vector backbone (eFigure 2A). Levels of zeozin resistance cassette transcripts relative to 18S ribosomal RNA were identical in major and minor haplotype transfectants, demonstrating equal transfection efficiency (**Figure 5A**). Also, no difference in SORLA transcript levels relative to zeozin resistance cassette transcripts was observed, indicating identical transcription efficiency of both *SORL1* minigene variants (Figure 5B).

Table 2. Distribution of *SORL1* Genotypes in Sample Set^a

SNP ID	Genotypes			HW P Value
rs668387	C/C	C/T	T/T	.90
	0.35	0.50	0.15	
rs689021	G/G	A/G	A/A	> .99
	0.35	0.48	0.17	
rs641120	G/G	A/G	A/A	.90
	0.35	0.50	0.15	
rs11218313	A/A	A/G	G/G	> .99
	0.83	0.16	0.01	
rs2276346	G/G	G/T	T/T	> .99
	0.41	0.45	0.14	
rs7131432	T/T	A/T	A/A	> .99
	0.99	0.01	0.00	
rs2070045	T/T	G/T	G/G	.80
	0.50	0.43	0.07	
rs1699102	T/T	C/T	C/C	.96
	0.38	0.49	0.14	
rs2282649	C/C	C/T	T/T	.97
	0.45	0.43	0.11	
rs726601	C/C	C/T	T/T	> .99
	0.41	0.47	0.13	
rs1784931	A/A	A/C	C/C	.89
	0.36	0.47	0.17	
rs1010159	T/T	C/T	C/C	> .99
	0.39	0.47	0.15	

Abbreviations: HW, Hardy-Weinberg equilibrium; SNP, single-nucleotide polymorphism.
^aThe values under the genotypes are the frequency of that genotype in the sample set.

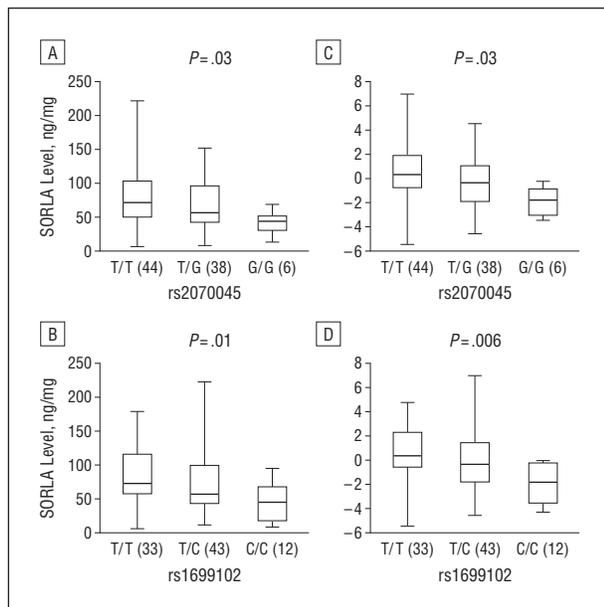


Figure 2. Association of SORLA levels with single-nucleotide polymorphisms rs1699102 and rs2070045. Statistically significant association of the minor allele genotype with lower concentrations of SORLA in the brain of patients is seen for the 2 indicated single-nucleotide polymorphisms. Analyses in the uncorrected sample set (A and B) and following correction for age, sex, and *APOE4* status (number of $\epsilon 4$ alleles) in linear regression (C and D) are shown. In panels C and D, expression measurements have been normalized (square root transformation). The numbers of samples in each genotype group are given in parentheses.

However, a significant 30% decrease in receptor protein levels was detected in transfectants expressing the minor compared with the major haplotype in multiple rep-

licate experiments (Figure 5C). Because the sequence variations encoded by rs2070045 (SNP 19) switch serine codon usage from frequent to rare, our findings argue that this *SORL1* haplotype affects brain SORLA expression by altering translation efficiency.

COMMENT

In recent years, major efforts have been dedicated to unraveling the mechanisms that may predispose carriers of *SORL1* risk alleles to poor receptor expression in the brain. Yet, the results have been confusing. Herein, we present the first study, to our knowledge, that correlates *SORL1* genotypes with protein levels in the brain of patients with AD. Our data uncovered a 2-SNP risk genotype in *SORL1* that predicts receptor expression in patients likely by affecting the efficiency of translation of the receptor transcript.

SORL1 is one of the most extensively characterized AD risk genes, with a total of 50 SNPs studied previously. Although not all SNPs have been replicated in all cohorts, most reports agree on the existence of 2 haplotype blocks in the 5' and 3' gene regions that are associated with risk of sporadic AD.^{11,16} In particular, a 3' haplotype block consisting of 5 SNPs (SNPs 19-23) (Figure 1A) has been associated with AD in several European populations.¹¹ Our analysis replicates the existence of this haplotype block structure in our sample set (Figure 1B).

Because gene expression profiling in lymphoblasts had uncovered 2.5-fold lower levels of *SORL1* transcripts in patients with AD compared with controls, an effect of

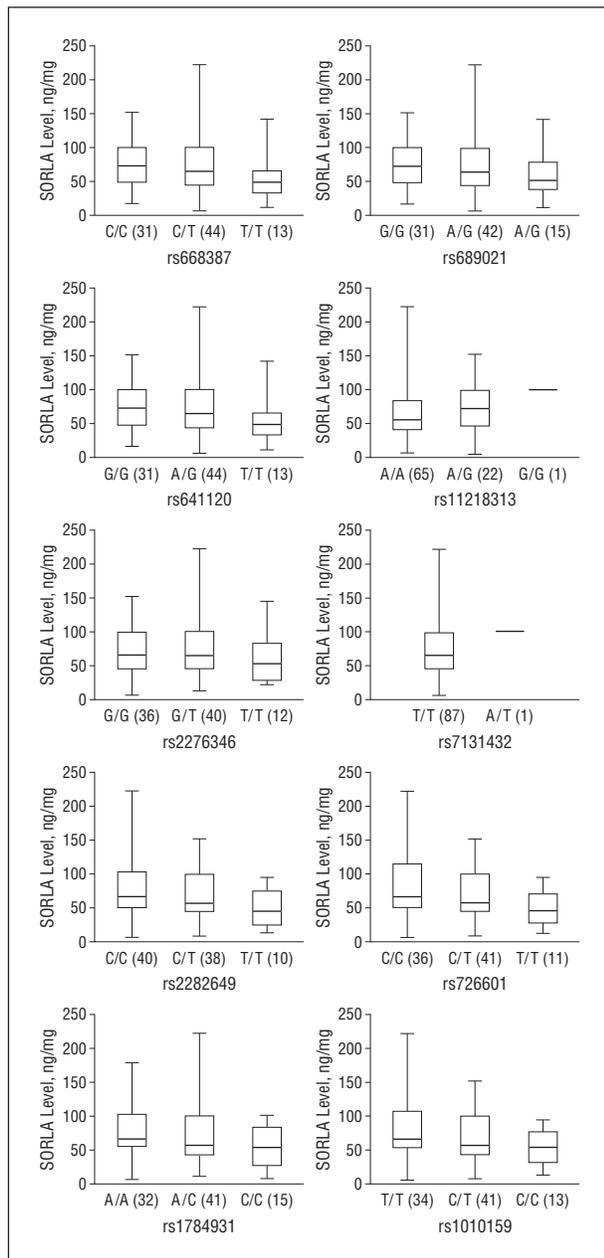


Figure 3. No association of SORLA levels with additional *SORL1* single-nucleotide polymorphisms. No statistically significant association of genotype with concentrations of SORLA in the brain of patients with Alzheimer disease is seen for the single-nucleotide polymorphisms tested here. The numbers of samples in each genotype group are given in parentheses.

SORL1 sequence variations on gene transcription had been proposed.⁹ In line with this hypothesis, Grear and colleagues²⁰ identified association of *SORL1* mRNA levels with rs661057 (SNP 4) in the 5' intronic gene region in AD cases. Similarly, McCarthy et al²¹ reported association of *SORL1* transcripts with SNPs rs7945931 and rs2298525 in the 5' haplotype block in a cohort of healthy controls. However, in the latter study, association was only seen in the temporal but not the frontal cortex, with the minor allele variants being associated with a 2-fold increase in *SORL1* mRNA.²¹ Finally, Rogava and colleagues¹¹ identified a haplotype in the 3' *SORL1* region

Table 3. Correlation of *SORL1* Haplotype With Receptor Levels

	No. (%)	P Value
Haplotype Block 1		
CGG	114 (58.2)	.42
TAA	80 (40.8)	.13
CAG	2 (1.0)	.06
Haplotype Block 2		
TTCCAT	115 (58.7)	.049
GCTTCC	52 (26.5)	.04
All others	(<5)	>.05

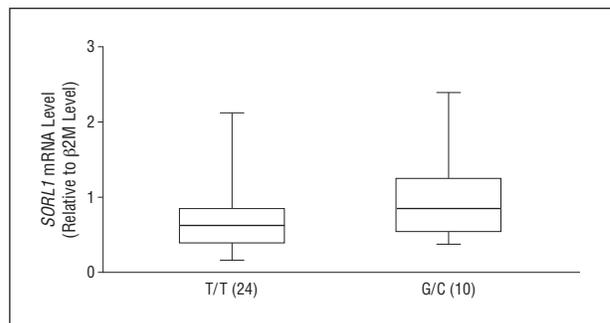


Figure 4. No correlation of single-nucleotide polymorphism genotype with *SORL1* transcript levels in rs2070045 and rs1699102. Quantitative reverse transcriptase-polymerase chain reaction was used to determine *SORL1* transcript levels in subjects with the minor (10) or major (24) haplotype. *SORL1* levels are indicated relative to the levels of β 2-microglobulin (β 2M) transcripts determined as the internal control. The RNA integrity values as determined by Bioanalyzer platform (Agilent Technologies) were comparable in both genotype groups (mean [SD], 5.54 [0.26] in the major vs 5.45 [0.52] in the minor genotype). The numbers of samples in each genotype group are given in parentheses. mRNA Indicates messenger RNA.

(SNPs 22-24) associated with a 50% reduction in mRNA in lymphoblasts. Interestingly, regression analysis also suggested that this genotype accounts for only 14% of the variance, implicating additional genetic or nongenetic factors in modulation of SORLA expression.

In our studies, we have tested association of *SORL1* risk alleles with SORLA protein levels in the brain, more directly correlating genotype with neuronal receptor expression. We focused our analyses on the frontal cortex, the brain region in which reduction in receptor protein has been shown by Western blot and immunohistological analysis.^{9,10} In our studies, we identified a novel haplotype composed of 2 SNPs wherein the minor (risk) variant significantly correlates with low receptor expression (Figure 2). Intentionally, we performed our studies in samples from patients with AD because we reasoned that genotype effects might be promoted by a sensitized background of the diseased brain, a situation seen for many multifactorial diseases. Obviously, neurodegenerative processes may affect neuronal protein expression secondarily. However, such impact on SORLA expression has been ruled out in previous studies in individuals with familial forms of AD and in aged AD mouse models.¹⁰

As documented in Figure 2, the 2 proximal SNPs (rs2070045 and rs1699102) in the 3' haplotype block are significantly associated with SORLA protein levels. A similar trend of association of the minor allele variant with

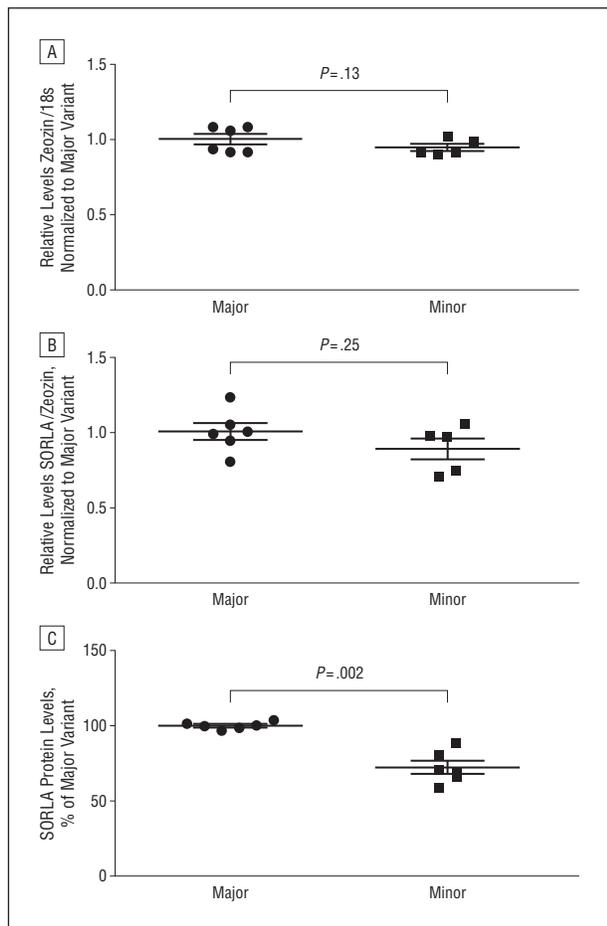


Figure 5. *SORL1* genotype determines posttranscriptional efficiency of receptor expression. Replicate layers of Chinese hamster ovary cells were transiently transfected with constructs encoding minor or major *SORL1* haplotype variants and zeozin resistance cassette transcripts. Levels of zeozin transcripts relative to 18s ribosomal RNA (A), levels of *SORL1* transcripts relative to zeozin resistance cassette transcripts (B), and concentration of SORLA protein as determined by enzyme-linked immunosorbent assay (C) are shown for both genotypes. A total of 6 experiments were performed and measured in triplicate.

lower protein levels is observable for the remaining 4 SNPs in this haplotype block (rs2282649, rs1784931, rs726601, and rs1010159), although this trend did not reach significance (Figure 3). The most likely explanations for this difference in strength of association of the 6 SNPs in linkage disequilibrium block 2 are differences in allele frequencies (Table 2) and the nonperfect linkage disequilibrium between rs2070045, rs1699102, and the remaining SNP markers in this haplotype block (Figure 1B).

The relevance of our 2-SNP haplotype for AD is supported by several lines of evidence. First, this genotype has been found to be relevant for European populations in previous association studies^{11,16} and in the present work. Second, both SNPs independently show association with low expression levels in carriers with 1 or 2 minor (risk-bearing) alleles. Third, both SNPs change the primary mRNA sequence, suggesting an effect of sequence variation on RNA structure, stability, or translation efficiency. In particular, rs2070045 changes codon usage for serine from frequent (TCT, 18.5%) to rare (TCG, 5.6%)²² in the

minor allele, suggesting reduced translation efficiency as the molecular basis of insufficient SORLA expression. Although we cannot exclude the existence of additional sequence variations in haplotype block 2 that affect receptor expression, a critical contribution of SNPs 19 and 22 receives strong support from experiments in CHO cells, demonstrating a 30% decrease in protein translation in the minor compared with the major gene variant. The effect of sequence variation on receptor expression in cultured cells correlates well with the 25% reduction seen in the frontal cortex of patients with AD by Western blotting.⁹

Clearly, our data do not exclude an impact of additional genetic and environmental factors on SORLA expression in this risk genotype, a situation seen for *SORL1* in other studies.¹¹ *APOE* genotype, sex, and age do not contribute to this variance (data not shown), but impaired viability of neurons in the diseased brain may aggravate the impact of codon usage on efficiency of protein expression. Also, we cannot rule out a moderate effect of the risk haplotype on *SORL1* transcription, which may only be detected in a much larger data set (not available for the current study).

In conclusion, our study identified correlation of *SORL1* risk genotypes with SORLA expression in the brain, further supporting the genetic basis of insufficient receptor activity in the brain as an important risk factor in AD.

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REFERENCES

- Jacobsen L, Madsen P, Moestrup SK, et al. Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein. *J Biol Chem*. 1996;271(49):31379-31383.

2. Yamazaki H, Bujo H, Kusunoki J, et al. Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member. *J Biol Chem.* 1996;271(40):24761-24768.
3. Willnow TE, Carlo AS, Rohe M, Schmidt V. SORLA/SORL1, a neuronal sorting receptor implicated in Alzheimer's disease. *Rev Neurosci.* 2010;21(4):315-329.
4. Andersen OM, Reiche J, Schmidt V, et al. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc Natl Acad Sci U S A.* 2005;102(38):13461-13466.
5. Offe K, Dodson SE, Shoemaker JT, et al. The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments. *J Neurosci.* 2006;26(5):1596-1603.
6. Schmidt V, Sporbert A, Rohe M, et al. SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1. *J Biol Chem.* 2007;282(45):32956-32964.
7. Rohe M, Carlo AS, Breyhan H, et al. Sortilin-related receptor with A-type repeats (SORLA) affects the amyloid precursor protein-dependent stimulation of ERK signaling and adult neurogenesis. *J Biol Chem.* 2008;283(21):14826-14834.
8. Dodson SE, Andersen OM, Karmali V, et al. Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease. *J Neurosci.* 2008;28(48):12877-12886.
9. Scherzer CR, Offe K, Gearing M, et al. Loss of apolipoprotein E receptor LR11 in Alzheimer disease. *Arch Neurol.* 2004;61(8):1200-1205.
10. Dodson SE, Gearing M, Lipka CF, Montine TJ, Levey AI, Lah JJ. LR11/SorLA expression is reduced in sporadic Alzheimer disease but not in familial Alzheimer disease. *J Neuropathol Exp Neurol.* 2006;65(9):866-872.
11. Rogaeva E, Meng Y, Lee JH, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat Genet.* 2007;39(2):168-177.
12. Meng Y, Lee JH, Cheng R, St George-Hyslop P, Mayeux R, Farrer LA. Association between SORL1 and Alzheimer's disease in a genome-wide study. *Neuroreport.* 2007;18(17):1761-1764.
13. Bettens K, Brouwers N, Engelborghs S, De Deyn PP, Van Broeckhoven C, Sleegers K. SORL1 is genetically associated with increased risk for late-onset Alzheimer disease in the Belgian population. *Hum Mutat.* 2008;29(5):769-770.
14. Webster JA, Myers AJ, Pearson JV, et al. Sorl1 as an Alzheimer's disease pre-disposition gene? *Neurodegener Dis.* 2008;5(2):60-64.
15. Lee JH, Cheng R, Honig LS, Vonsattel JP, Clark L, Mayeux R. Association between genetic variants in SORL1 and autopsy-confirmed Alzheimer disease. *Neurology.* 2008;70(11):887-889.
16. Reitz C, Cheng R, Rogaeva E, et al; Genetic and Environmental Risk in Alzheimer Disease 1 Consortium. Meta-analysis of the association between variants in SORL1 and Alzheimer disease. *Arch Neurol.* 2011;68(1):99-106.
17. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 2001;29(9):e45.
18. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics.* 1994;138(3):963-971.
19. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol.* 2010;34(8):816-834.
20. Gear KE, Ling IF, Simpson JF, et al. Expression of SORL1 and a novel SORL1 splice variant in normal and Alzheimers disease brain. *Mol Neurodegener.* 2009;4:46.
21. McCarthy JJ, Saith S, Linnertz C, et al. The Alzheimer's associated 5' region of the SORL1 gene cis regulates SORL1 transcripts expression. *Neurobiol Aging.* 2010;(Dec):22.
22. Zeeberg B. Shannon information theoretic computation of synonymous codon usage biases in coding regions of human and mouse genomes. *Genome Res.* 2002;12(6):944-955.