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The Alzheimer's associated 5' region of the *SORL1* gene *cis*-regulates *SORL1* transcripts expression

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Abstract

SORL1 has been identified as a major contributor to Late-Onset Alzheimer's disease (LOAD). We test whether genetic variability in the 5' of *SORL1* gene modulates the risk to develop LOAD via regulation of *SORL1*-mRNA expression and splicing. Two brain structures, differentially vulnerable to LOAD pathology, were examined in 144 brain samples from 92 neurologically normal individuals. The temporal cortex, which is more susceptible to Alzheimer's pathology, demonstrated ~2-fold increase in *SORL1*-mRNAs levels in carriers of the minor alleles at SNPs, rs7945931 and rs2298525, compared to non-carriers. No genetic effect on total-*SORL1*-mRNA levels was detected in the frontal-cortex. However, rs11600875 minor allele was associated with significantly increased levels of exon-2 skipping, but only in frontal cortex. No correlation of *SORL1*-mRNAs expression was found between frontal and temporal cortexes. Collectively, indicating the brain-region specificity of the genetic regulation of *SORL1* expression. Our results suggest that genetic regulation of *SORL1* expression plays a role in disease risk and maybe responsible for the reported LOAD-associations. Further studies to detect the actual pathogenic variant/s are necessary.

Keywords

SORL1; Alzheimer's disease; mRNA; splicing; correlation to gene expression

1. Introduction

The neuronal sortilin-related receptor SORL1 (also known as *SORLA*, *LR11*) is a 250 kDa type 1 membrane glycoprotein mainly expressed in neurons in the brain (Jacobsen et al., 1996; Yamazaki et al., 1996). Several line of evidence implicates SORL1 in Alzheimer's

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disease (AD) pathogenesis. First, SORL1 has been recognized as a novel sorting receptor that regulates intracellular trafficking and processing of the amyloid precursor protein (APP) (Andersen et al., 2005; Offe et al., 2006). It was shown *in vitro* and *in vivo* that SORL1 can interact with APP, influence the trafficking of APP, and regulate β -amyloid ($A\beta$ production) (Andersen et al., 2005; Offe et al., 2006). Since then, accumulating evidence supports the notion that SORL1 protein directs APP into recycling endosomes which shuttle it to the cell membrane. When SORL1 is absent APP is misdirected to the late endosome where it is aberrantly processed and cleaved into $A\beta$ (Andersen et al., 2005; Rogaeva et al., 2007; Schmidt et al., 2007), the key component of senile plaques, the pathological hallmark of AD (Hardy and Selkoe, 2002; Mattson, 2004).

Second, it has been shown that SORL1 is markedly downregulated in and brain tissues of sporadic AD patients, particularly in neurons in the AD vulnerable areas of the cortex and hippocampus (Dodson et al., 2006; Scherzer et al., 2004). In support of this finding, a recent whole genome neuronal expression profile analysis identified significant regional differential *SORL1* expression in AD afflicted brains compared to controls. *SORL1* displayed decreased neuronal expression in specific regions (hippocampus, middle temporal gyrus, and posterior cingulate cortex) of AD brain (Liang et al., 2008). Of note, no alterations in SORL1 protein levels were found in either the autosomal-dominant form of AD or in a transgenic mouse model of AD (PS1/APP) (Dodson et al., 2006) suggesting that *SORL1* loss may be specific to late-onset AD (LOAD). Hence, its expression is not simply a reaction to amyloid accumulation, and its loss could not be simply explained by downstream events associated with amyloidosis and neurodegeneration (Dodson et al., 2006). Accordingly, the reduction in *SORL1* expression could be a proximal contributor to the disease process in LOAD.

Last, *SORL1* has been identified as a major genetic contributor to increased risk for LOAD (Bettens et al., 2008; Cellini et al., 2009; Lee et al., 2008; Lee et al., 2007; Li et al., 2008; Meng et al., 2007; Rogaeva et al., 2007; Tan et al., 2007). A growing number of association studies, based on candidate gene approaches, reported associations between genetic variants within the *SORL1* gene and LOAD in populations from different ethnic (*e.g.* Caucasian, Asian, African descent, Hispanic) and geographic backgrounds (Bettens et al., 2008; Cellini et al., 2009; Lee et al., 2008; Lee et al., 2007; Li et al., 2008; Rogaeva et al., 2007; Tan et al., 2007). Associations were observed with multiple SNPs and haplotypes in two distinct clusters of *SORL1*, designated as the 5' and the 3' associated regions, suggesting allelic heterogeneity at the *SORL1* locus (Bettens et al., 2008; Lee et al., 2008; Lee et al., 2007; Rogaeva et al., 2007). However, some sample sets have failed to replicate these findings (Li et al., 2008; Liu et al., 2007; Webster et al., 2008), possibly due to locus heterogeneity that has been observed for other complex diseases or to the presence of subtle allelic association rather than spurious positive-association.

Taken together, the evidence for *SORL1* as a causative gene is compelling. However, the exact identities of the genetic effectors in the *SORL1* locus and the mechanism through which they exert their effect have not yet been determined. In this study we focused on understanding the molecular basis underlying the observed association of SNPs located at the 5' region of *SORL1* with LOAD. In an attempt to link the current genetic and expression evidence, we examined the effect of the 5' associated region on the regulation of *SORL1* RNA expression that includes levels of total *SORL1* mRNA, and the novel splice variant at the 5' region of the gene that lacks exon 2 (Grear et al., 2009). Building on the successful approach with *SNCA* (Linnertz et al., 2009) and *SCARBI* (Chiba-Falek et al.) studies, the current study was carried out using two human brain structures frontal cortex and temporal cortex that are differentially vulnerable to AD pathology. Tissue was obtained from *post mortem* matched brain tissues of unaffected individuals, thus we can directly assess the

genetic contribution to the regulation of *SORL1* expression, avoiding other confounding factors arising from the neurodegeneration associated with AD.

2. Methods

2.1. Brain samples

Brain tissue samples, including frontal cortex (n=92), and a subset of matched temporal cortex (n=52), from neurologically healthy controls (Table 1) were obtained through the Kathleen Price Bryan Brain Bank (KPBBB) at Duke University, the Brain and Tissue Bank for developmental Disorders at the University of Maryland, the Layton Aging & Alzheimer's Disease Center at Oregon Health and Science University, and the National NeuroAIDS Tissue Consortium (NNTC). The ethnicity of all samples was white and all post mortem interval (PMI) were <24 hours. Demographics for these samples are included in Table 1.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from brain tissues by the standard Qiagen protocol. Genotypes within the human genome region of interest (chr 11: 120,834,164-120,898,894) were determined by mining previously published genome-wide genotyping database that was performed using Illumina Human Hap550K chips (Heinzen et al., 2008). For samples not in the database genotypes determination of each Single Nucleotide Polymorphism (SNP) was performed by allelic discrimination using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) using the ABI 7900HT and following the manufacture's protocol. All genotypes were tested for Hardy-Weinberg Equilibrium (Table 2).

2.3. RNA extraction and cDNA synthesis

Total RNA was extracted from brain samples (100 mg) using TRIzol reagent (Invitrogen, Carlsbad, CA) followed by purification with an RNeasy kit (Qiagen, Valencia, CA) following the manufacturer's protocol. RNA concentration was determined spectrophotometrically at 260nm, while the quality of the purification was determined by 260nm/280nm ratio that showed values between 1.9 and 2.1, indicating high RNA quality. Additionally, quality of sample and lack of significant degradation products was confirmed on an Agilent Bioanalyzer. For all samples that were included in the subsequent analysis the RNA Integrity Number (RIN) measurements were greater than 7 validating the RNA quality control. Next, cDNA was synthesized using MultiScribe RT enzyme (Applied Biosystems, Foster City, CA) under the following conditions: 10 min at 25 °C and 120 min at 37 °C.

2.4. Real time PCR

Real-time PCR was used to quantify the levels of human *SORL1* mRNAs, overall (total-*SORL1*) and the alternative splice variant lacking exon 2 (delta-2-*SORL1*). Briefly, duplicates of each sample were assayed by relative quantitative real-time PCR using the ABI 7900HT for analysis of the level of total-*SORL1* and delta-2-*SORL1* messages as compared in brain tissues to mRNAs encoding the human neuronal proteins Enolase 2 (*ENO2*) and Synaptophysin (*SYP*). ABI MGB probe and primer set assays were used to amplify the target cDNAs, total-*SORL1* (ID Hs00268342_m1, 72bp) and delta-2-*SORL1* (custom assay, 111bp); and the two RNA reference controls, *ENO2* (ID Hs00157360_m1, 77bp) and *SYP* (ID Hs00300531_m1, 63bp) (Applied Biosystems, Foster City, CA). The custom assay to detect specifically the alternative splicing variant, delta-2-*SORL1* was designed to target the novel exons 1 and 3 junction. Each cDNA (10 ng) was amplified in duplicate in at least two independent runs (overall 4 repeats), using TaqMan Universal PCR master mix reagent (Applied Biosystems, Foster City, CA) and the following conditions: 2 min at 50 °C, 10 min

at 95 °C, 40 cycles: 15 sec at 95 °C, and 1 min at 60°C. As a negative control for the specificity of the amplification, we used RNA control samples that were not converted to cDNA (no-RT) and no-cDNA/RNA samples (no-template) in each plate. No observable amplification was detected. Data were analyzed with a threshold set in the linear range of amplification. The cycle number at which any particular sample crossed that threshold (Ct) was then used to determine fold difference, whereas the geometric mean of the two control genes served as a reference for normalization. Fold difference was calculated as $2^{-\Delta\Delta Ct}$; $\Delta Ct = [Ct(\text{target}) - Ct(\text{reference})]$. $\Delta\Delta Ct = [\Delta Ct(\text{sample})] - [\Delta Ct(\text{calibrator})]$. The calibrator was a particular brain RNA sample used repeatedly in each plate for normalization within and across runs.

For assay validation we generated standard curves for *SORL1*, delta-2-*SORL1* and each reference assay, *ENO2* and *SYP* using different amounts of human brain total RNA (0.1-100 ng). In addition, the slope of the relative efficiency plot for *SORL1* and for delta-2-*SORL1* with each internal control (*ENO2* or *SYP*) was determined to validate the assays. The slope in the relative efficiency plot for *SORL1*, delta-2-*SORL1* and the reference genes were <0.1, showing a standard value required for the validation of the relative quantitative method.

2.5. Statistical analysis

All analyses were carried out using SAS statistical software, Version 9.1 (SAS Institutes, Cary, NC). *SORL1* mRNA and delta-2-*SORL1* mRNA fold expression values of each sample were analyzed repetitively and the results of all replicates were averaged. All average values were expressed as mean±SE. Tissues comparisons were done by paired 2 tailed T-tests. Correlations of *SORL1* genotypes with expression traits (fold levels of total *SORL1* mRNA and delta-2-*SORL1* mRNA) were assessed using the Generalized Linear Models procedure (PROC GLM). A log transformation (log2) was performed on all mRNA levels to assure normal distribution (Bengtsson et al., 2005). SNP genotypes were coded as a dominant model pooling homozygous for minor alleles and heterozygous genotypes, as homozygosity for the minor allele was rare for most SNPs. All models controlled for gender, age, PMI, and tissue source.

Linkage disequilibrium (r^2) within the studied 5' region of *SORL1* was determined using Haploview software (version 4.2) (Barrett et al., 2005).

3. Results

3.1. The effect of secondary (non-genetic) variables on *SORL1*-mRNA level

Recently, two novel *SORL1* splice variants were identified one of which, in frame exon 2 skipping, was more abundant (represented 1-5% of total mRNA)(Grear et al., 2009). Using RT-PCR for amplification from exon1 through exon 3 followed by direct sequencing of the shorter product detected on a gel we confirmed the generation of *SORL1*-mRNA isoform lacking exon 2 in human brain (Supplementary Material 1 and Supplementary Fig1). In order to understand the regulation of *SORL1* expression we focused on the major mRNA isoforms, *i.e.* the full length message and the relatively abundant, delta exon 2 splicing variant. *SORL1*-mRNAs fold levels [total *SORL1*/(*SYP*, *ENO*); delta-2-*SORL1*/(*SYP*, *ENO*)] were measured in 144 brain tissue samples obtained from 92 subjects (all white, 60% males; Table 1). One sample was removed as an outlier from the association analyses. First, we assessed the correlations with confounding factors that might affect RNA levels. No significant associations of total *SORL1* and delta-2-*SORL1* mRNAs levels were observed in temporal (n= 51) and frontal cortex (n= 91) with sex, age, or *post mortem* interval (PMI). Tissue source was significantly associated with the expression levels of frontal cortex overall and splice variant ($p < 0.0001$ and $p = 0.0004$, respectively). All of the subsequent

analyses were adjusted for tissue source, sex, age and PMI. Next, the effect of specificity of the brain tissue region on *SORL1* expression was assessed. To carry out this analysis we used matched samples, *i.e.* samples of the two different brain structures obtained from the same cadaver (n=51). No correlations in *SORL1* mRNAs expression were found between frontal and temporal cortexes for total ($r^2=0.0004$; Fig. 1A) or delta-2 splice form, ($r^2=0.005$; Fig. 1B). Frontal cortex showed significantly higher average levels of delta-2-*SORL1* mRNA compared with temporal cortex ($p=0.003$). The average delta-2-*SORL1* mRNA levels observed in the frontal cortex samples was nearly two fold higher compared to the average delta-2-*SORL1* mRNA levels detected in the temporal cortex (Fig. 1D). On the contrary, comparison of the average total *SORL1*-mRNA fold levels between matched samples of temporal and frontal cortexes revealed no significant differences ($p=0.53$, Fig. 1C).

3.2. Defining the genomic region of interest

In the present work we focused on genetic functional consequences that are primarily controlled by transcription and/or splicing mechanisms. The new identified splicing event, exon 2 exclusion, was included within the *SORL1* 5' LOAD- associated region. Therefore, we hypothesized that our putative expression regulatory variants are most likely positioned within the 5' region of the gene. Based on the reproducible LOAD associations reported within the putative regulatory genomic region (Bettens et al., 2008; Cellini et al., 2009; Lee et al., 2008; Lee et al., 2007; Li et al., 2008; Meng et al., 2007; Rogaeva et al., 2007; Tan et al., 2007), we narrowed down the analysis to contain only the region between the original SNP4 (rs661057; chr 11: 120,834,164) to SNP13 (rs2298813; chr 11: 120,898,894)⁵, encompassing the broader *SORL1* 5' genomic region reported to be associated with LOAD (Bettens et al., 2008; Cellini et al., 2009; Lee et al., 2008; Lee et al., 2007; Li et al., 2008; Meng et al., 2007; Rogaeva et al., 2007; Tan et al., 2007). Ten tagging SNPs in the Illumina Human Hap550K chip were mapped within the region of interest (Table 2 and Fig. 2).

3.3. The effect of genotypes on total *SORL1*-mRNA expression levels in human brain tissues

We tested the effect of variants in the 5' region of *SORL1* on total *SORL1*-mRNA levels with ten tagging SNPs (Table 2). Two SNPs, rs7945931 and rs2298525, showed a significant effect on total *SORL1*-mRNA expression in the temporal cortex ($p=0.008$ and 0.01 ; Fig. 3). These two SNPs are in high LD ($r^2=0.78$), hence likely tagging a common genetic effector. In the temporal cortex carriers of the SNP rs7945931 minor allele, 'A' (AA and AG; n=10), showed higher average expression levels of total *SORL1* mRNA than the non-carriers (GG, n= 41), amounting to a nearly two fold increase (Fig. 4). A similar effect in the temporal cortex was observed with SNP rs2298525, where carriers of the minor allele 'A' had increased overall levels of *SORL1*-mRNA (data not shown). In the frontal cortex, on the contrary, no association was identified between total *SORL1*-mRNA and the 5' region genotypes, neither in the analyses of the entire frontal cortex samples set (n=91), nor when constrained to matched samples only (n=51) (Fig. 3 and Table 2).

3.4. The effect of genotypes on alternative splicing of *SORL1*-mRNA in human brain tissues

Next, we evaluated whether SNPs at *SORL1* 5' region associated with the expression levels of the novel identified splice variant, delta-2-*SORL1*.

Genotypes of SNP rs11600875 showed a significant effect on *SORL1* exon 2 splicing levels in frontal cortex (n=91, $p=0.005$, Table 2). The correlation remains significant when the analysis was limited to the matched frontal samples (n=51, $p=0.003$; Fig. 3 and Table 2).

Carriers of the minor allele (TT and TC; n=9) had approximately two fold higher delta-2-*SORL1*-mRNA average levels than non carrier (CC; n= 42) (Fig. 5). The temporal cortex region, however, did not show a significant correlation with delta-2-*SORL1*-mRNA folds levels with any of the studied SNPs within the *SORL1* 5' region (Fig. 3 and Table 2). This SNP was not in linkage disequilibrium with either of the SNPs that were associated with overall expression (Fig. 2).

4. Discussion

In previous studies, down regulation of *SORL1* expression was reported in sporadic AD patients compared to control, particularly in neurons in the cortex and hippocampus, areas vulnerable to AD pathology (Dodson et al., 2006; Scherzer et al., 2004),(Liang et al., 2008). Analysis of afflicted brains may introduce interpretative challenges that arise from the massive cell loss, particularly neuronal loss, along with other pathologic processes accompanying neurodegeneration that may influence expression. Nevertheless, interesting associations between genetic variants and gene expression levels do not necessarily require a comparison between tissues from both cases and controls, since significant differences in gene expression levels were also shown to be associated with different genotypes in disease relevant tissues of unaffected individuals (Emilsson et al., 2008; Myers et al., 2007). In the current study we analyzed variations in *SORL1* mRNA expression in the brains of unaffected people, aiming to identify such genetic expression effects in AD relevant tissue. Specifically, we looked for variations in the levels of total *SORL1* mRNA and the novel exon 2 skipped transcripts and tested for association with one of the two suggested LOAD-associated regions within *SORL1* locus(Bettens et al., 2008; Cellini et al., 2009; Lee et al., 2008; Lee et al., 2007; Li et al., 2008; Meng et al., 2007; Rogaeva et al., 2007; Tan et al., 2007). We hypothesized that the 5' LOAD-associated interval of the gene might be relevant in the regulation of its RNA expression, *i.e.* overall levels and/or efficiency of exon 2 splicing, and therefore chose to focus on variants within the 5' *SORL1* LOAD-associated region.

Prior *SORL1*-LOAD association studies have described the major alleles at the 5' SNPs as risk alleles(Bettens et al., 2008; Lee et al., 2008; Rogaeva et al., 2007; Webster et al., 2008). Although we analyzed different SNPs we looked at the same genomic interval; and in agreement to the correlation found between AD and reduced *SORL1* expression, we revealed that the major alleles, presumably disease-risk alleles, correlate with lower overall *SORL1* expression level. Rogaeva et al.(Rogaeva et al., 2007) also attempted to show that the LOAD associated haplotype might be attributed to some extent to lower *SORL1* expression supporting the concept that reduction in *SORL1* levels is likely to be a primary and pathogenic event. They were able to provide tentative support for the hypothesis that LOAD-associated haplotype in *SORL1* may be associated with reduced *SORL1* transcription, however the analysis was performed in lymphoblasts and was limited to the 3' LOAD-associated block (insufficient samples to test the 5' SNPs effect). Here for the first time we report the genetic effect of the 5' LOAD-associated interval on the *SORL1* transcription in disease relevant tissues.

In addition, we found a genetic effect on the splicing regulation of *SORL1* exon 2 that was independent of the effect on overall expression. The associated SNP rs11600875 is positioned within intron 3, ~20 Kb downstream from exon 2 and a bioinformatics search predicted neither creation nor disruption of putative splicing elements (Pupasuite2, data not shown). It is unlikely that this is the actual regulatory variant, and possible serves as a marker for a proximate variant that harbors a direct effect on exon 2 splicing event. The delta-2 isoform encodes a *SORL1* protein that lacks amino acids V96-D134 that contains the vacuolar protein sorting 10 (VPS10) domain. This region may be involved in amyloid

precursor protein (APP) processing, but the functional consequences of the loss of this domain are not known. Biochemical experiments are needed in order to interpret the functional relevance of changes in delta-2 *SORL1* levels to AD risk.

Our analysis included functionally and anatomically distinct brain regions from unaffected age-matched brains. Frontal cortex and temporal cortex are known to have differential susceptibility to AD pathology, ranging from severely afflicted temporal cortex, to the frontal cortex that is involved in a later stages of disease (Braak and Braak, 1991). Interestingly, the genetic control identified in this study was not global (across the two brain regions) but brain-region specific, indicating regionally differential regulation of *SORL1*-mRNAs expression in both transcriptional and splicing levels. Cuenco *et al.* found that distinct *SORL1* variants were associated with MRI and neuropathological changes related to AD in different brain regions, supporting the hypothesis of functional importance of multiple *SORL1* variants (Cuenco et al., 2008). Here we provide functional evidence to further support the importance of distinct *SORL1* genetic variants in different regions of the brain. Furthermore, from a subset of subjects (n=12) hippocampus samples were available for measuring *SORL1*-mRNAs levels. In the hippocampus (n=12) a 50% increase in the average expression level of delta-2 *SORL1*-mRNA was detected with the minor allele carriers genotypes (n= 3) when compared to non carrier genotype (n=9) (Supplementary Fig. 2B). This result suggested a possible effect of the minor allele on increased levels of delta-2 *SORL1*-mRNA, which will need to be explored with a larger group. Interestingly this trend of the effect supports the significant genetic association observed in the frontal cortex. Using this small group of hippocampus tissues, on the other side, identified no associations between total *SORL1*-mRNA and the 5' region genotypes (Supplementary Fig. 2A).

Our study examined several SNPs in two brain tissues for association with expression, and as such, is subject to false positive associations. Therefore, P values should be interpreted with caution. After Bonferroni adjustment for 20 hypotheses (10 variants \times 2 tissues), the associations with all variants became non significant. However, given that these were not entirely independent hypotheses (SNPs in linkage disequilibrium), this may be an overly conservative interpretation. Moreover, the coincidental location of *SORL1* SNPs associated with both expression (our study) and AD (other studies) lends support to a putative functional role of this locus that warrants further investigation in a larger cohort. Furthermore, we suggest using deep re-sequencing analysis of the expression associated region to identify potential causal variant/s that will be pursued in follow up studies, *e.g. in vitro* in cell based system and *in vivo* using animal models, and which will further our understanding of the underlying biology of associations with LOAD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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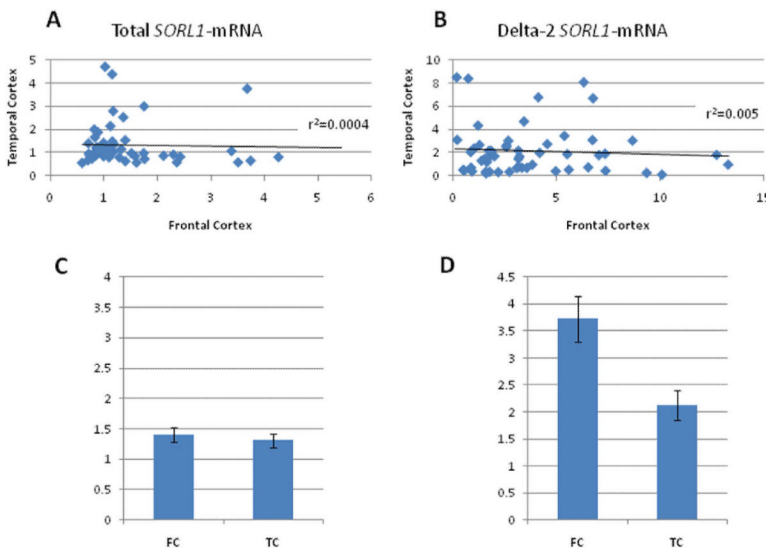


Figure 1. Comparison of SORL1 RNA levels between matched brain regions obtained from the same subjects (n=51)

Fold levels of total (A, C) and delta-2 (B, D) -*SORL1*-mRNAs were assayed by real-time RT-PCR using TaqMan technology and calculated relative to the geometric mean of *SYP* and *ENO*-mRNAs reference control using the $2^{-\Delta\Delta C_t}$ method. The analysis included matched frontal (FC) cortex and temporal cortex (TC) samples obtained from 51 subjects. (A) Total *SORL1*-mRNA levels in FC were not correlated with total *SORL1*-mRNA levels in TC ($r^2=0.0004$). (B) Delta-2 *SORL1*-mRNA levels in FC were not correlated with delta-2 *SORL1*-mRNA levels in TC ($r^2=0.005$). (C and D) The bar graph presents the average of *SORL1*-mRNA s fold expression (mean \pm SE) of the 51 subjects for each brain region. (C) No significant differences in the levels of total *SORL1*-mRNA levels were found between FC and TC ($p=0.53$). (D) delta-2-*SORL1* mRNA levels were significantly higher in the FC compared with TC ($p=0.003$).

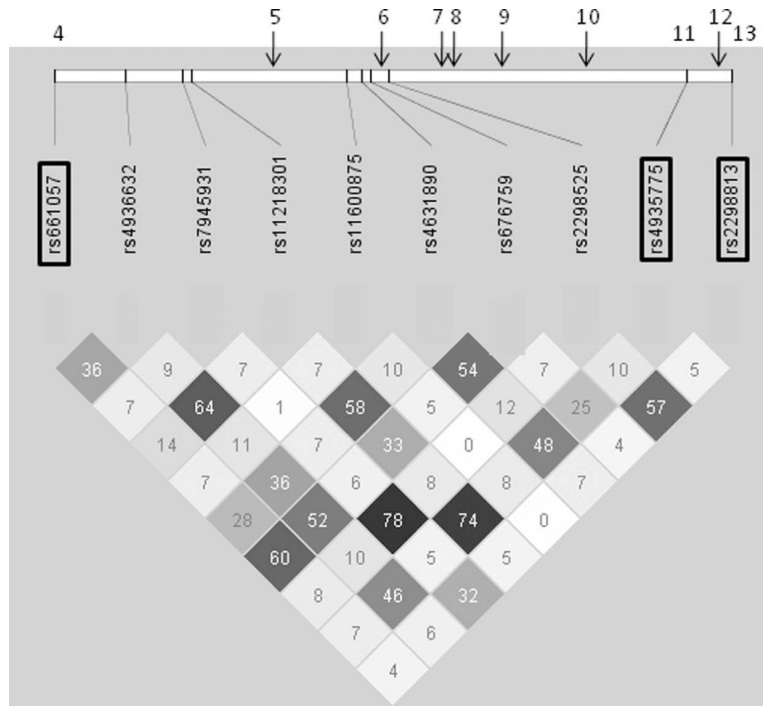


Figure 2. Linkage disequilibrium (LD) structure of the 5' region of *SORL1* gene
 Genotypes of ten SNPs from the total sample set of this study (N=92) were used to determine LD (r^2) within the *SORL1* 5' region using Haploview software. r^2 values are shown within cells. Black cells, strong LD; shaded of grey cells, intermediate; white cells evidence of recombination. SNPs that were included in the original LOAD association study (Rogaeva et al., 2007) are marked in rectangles. Upper panel- the relative positions of the original SNPs that were not analyzed in this study are designated in arrows and the SNPs are marked in numbers according to the original order⁵.

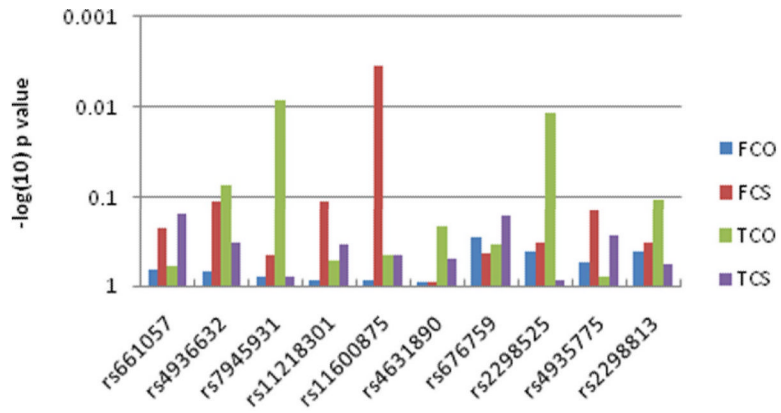


Figure 3. Correlations of *SORL1* genotypes with expression traits

Fold levels of total *SORL1* mRNA and delta-2-*SORL1* mRNA in FC and TC were assessed using the Generalized Linear Models procedure (GLM). A log transformation (log2) was performed on all mRNA levels to assure normal distribution (Bengtsson et al., 2005). SNP genotypes (n=10) were coded as a dominant model pooling homozygous for minor alleles and heterozygous genotypes, as homozygosity for the minor allele was rare for most SNPs. The bars represent the p values.

FCO- frontal cortex, total *SORL1* mRNA; FCS- frontal cortex, delta-2-*SORL1* mRNA; TCO-temporal cortex, total *SORL1* mRNA ; TCS- temporal cortex, delta-2-*SORL1* mRNA.

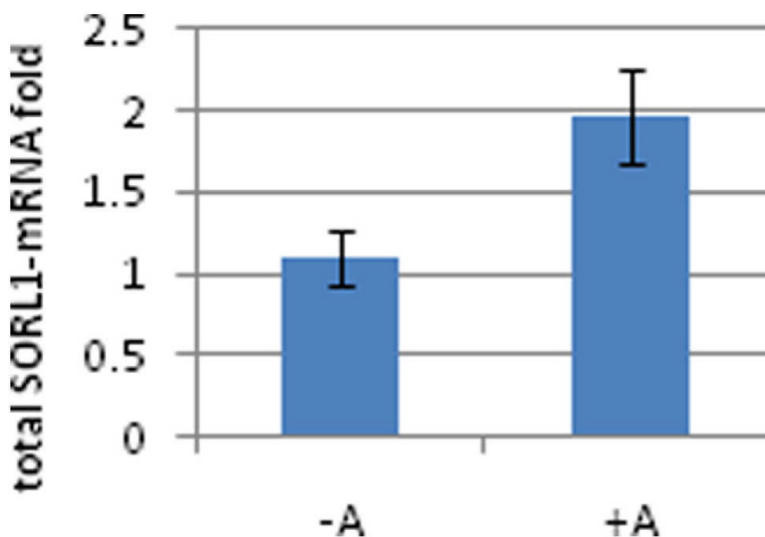


Figure 4. Effect of SNP rs7945931 genotypes on human total *SORL1*-mRNA expression levels in human temporal cortex

Individuals were genotyped for SNP rs7945931 and fold levels of human total *SORL1*-mRNA were assayed by real-time RT-PCR using TaqMan technology and calculated relative the geometric mean of *SYP*- and *ENO*-mRNAs reference control using the $2^{-\Delta\Delta C_t}$ method. Analysis of the temporal cortex showed that the minor allele carriers '+A' genotypes (AA, AG; n=10) correlate with significantly higher total *SORL1*-mRNA levels than the non carrier, '-A' genotype (GG; n=41) ($p=0.008$). The same significant trend was observed in the analysis of SNP rs2298525 ($p=0.01$).

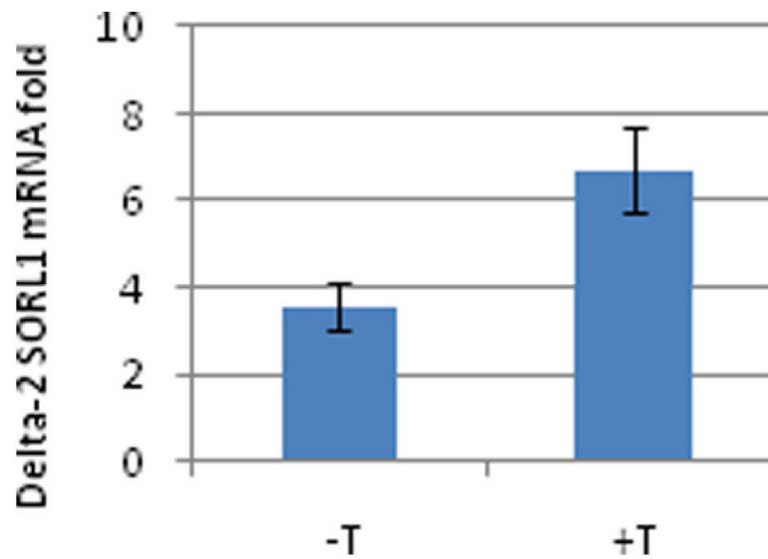


Figure 5. Effect of SNP rs11600875 genotypes on human delta-2 *SORL1*-mRNA expression levels in human frontal cortex

Individuals were genotyped for SNP rs11600875 and fold levels of human delta-2 *SORL1*-mRNA were assayed by real-time RT-PCR using TaqMan technology and calculated relative the geometric mean of *SYP*- and *ENO*- mRNAs reference control using the $2^{-\Delta\Delta C_t}$ method. Analysis of the frontal cortex showed that the minor allele carriers '+T' genotypes (TT, TC; n=9) correlate with significantly higher delta-2 *SORL1*-mRNA levels than the non carrier, '-T' genotype (CC; n=42) ($p=0.003$).

Table 1

Demographic description of the brain samples

	FC	TC
N _{total}	92	52
N _{male}	55	29
N _{female}	37	23
Age mean±SD	74.1±16.5	77.6±14.1
PMI mean±SD	14.3 ± 9.6	11.2±7.7
White %	100	100

FC- frontal cortex, TC-temporal cortex,

PMI- post mortem interval.

Table 2

The analyzed SNPs at *SORL1* 5' region

rs Number	Map position(bp)	Distance from previous	SNP type	Orig. order(Rogaeva et al., 2007)	MAF	HW (p)	FC total (p ^a ,p ^b)	FC delta2 (p ^a ,p ^b)	TC total (p)	TC delta2 (p)
rs661057	120834164		Intron1	4	0.413	0.98	0.97,0.65	0.38,0.23	0.59	0.16
rs4936632	120840938	6774	Intron1		0.473	0.42	0.81,0.67	0.12,0.11	0.07	0.32
rs7945931	120846472	5534	Intron2		0.098	0.39	0.52,0.78	0.33,0.45	0.008	0.79
rs11218301	120847297	825	Intron2		0.418	0.28	0.27,0.87	0.13,0.11	0.52	0.34
rs11600875	120862178	14881	Intron3		0.092	0.32	0.16,0.87	0.005,0.003	0.44	0.46
rs4631890	120863571	1393	Intron3		0.495	1.00	0.83,0.88	0.83,0.90	0.22	0.50
rs676759	120864475	904	Intron4		0.364	0.52	0.42,0.28	0.76,0.44	0.35	0.16
rs2298525	120866225	1750	Intron5		0.109	1.00	0.14,0.40	0.26,0.33	0.01	0.86
rs4935775	120894712	28487	Intron8	11	0.451	0.38	0.65,0.53	0.1,0.14	0.77	0.27
rs2298813	120898894	4182	T528A	13	0.065	0.64	0.43,0.42	0.32,0.33	0.11	0.57

FC, frontal cortex; TC, temporal cortex; total, total *SORL1*-mRNA; delta2, delta-2-*SORL1*-mRNA.

^a p value for the entire sample size, n=91;

^b p value when only matched sample were included in the analysis, n=51.