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The SORL1 Gene and Convergent Neural Risk for Alzheimer's Disease Across the Human Lifespan

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Conflict of Interest: Within the past five years, BGP has been a member of the advisory board of Lundbeck Canada (final meeting was May 2009) and Forest Laboratories (final meeting was March 2008). He has also served one time as a consultant for Wyeth (October 2008) and Takeda (July 2007), and was a faculty member of the Lundbeck International Neuroscience Foundation (LINF) (final meeting was April 2010). JLK has been a consultant to GlaxoSmithKline, Sanofi-Aventis, and Dianippon-Sumitomo. BHM has received travel support from Roche. AKM has served as a consultant for Genomind Inc. ANV had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Abstract

Prior to intervention trials in individuals genetically at-risk for late-onset Alzheimer's disease, critical first steps are identifying where (neuroanatomic effects), when (timepoint in the lifespan) and how (gene expression and neuropathology) Alzheimer's risk genes impact the brain. We hypothesized that variants in the sortilin-like receptor (SORL1) gene would affect multiple Alzheimer's phenotypes before the clinical onset of symptoms. Four independent samples were analyzed to determine effects of SORL1 genetic risk variants across the lifespan at multiple phenotypic levels: 1) microstructural integrity of white matter using diffusion tensor imaging in two healthy control samples (n=118, age 18-86, and n=68, age 8-40); 2) gene expression using the Braincloud postmortem healthy control sample (n=269, age 0-92); and 3) Alzheimer's neuropathology (amyloid plaques and tau tangles) using a postmortem sample of healthy, mild cognitive impairment (MCI), and Alzheimer's individuals (n=710, age 66-108). SORL1 risk variants predicted lower white matter fractional anisotropy in an age-independent manner, in fronto-temporal white matter tracts in both samples at 5% FWE-corrected thresholds. SORL1 risk variants also predicted decreased SORL1 mRNA expression, most prominently during childhood and adolescence, and significantly predicted increases in amyloid pathology in postmortem brain. Importantly, the effects of SORL1 variation on both white matter microstructure and gene expression were observed during neurodevelopmental phases of the human lifespan. Further, the neuropathological mechanism of risk appears to primarily involve amyloidogenic pathways. Interventions targeted toward the SORL1 amyloid risk pathway may be of greatest value during early phases of the lifespan.

Keywords

Alzheimer's disease; neuroimaging; genetics; SORL1; amyloid; gene expression

1. Introduction

Late-onset Alzheimer's disease (AD) (i.e. onset after 65 years of age) is the most common form of dementia and is expected to affect over 115 million individuals worldwide by 2050.[1] Recent evidence suggests that subtle deterioration of brain structure may be present decades before the late-life emergence of clinical signs and symptoms in people genetically at-risk for this disorder.[2, 3] The failure of phase 3 trials in early stages of AD has hastened calls for intervention prior to clinical disease onset in genetically at-risk groups in whom effects on brain structure or function might be present.[4] These brain alterations, detectable using advanced neuroimaging approaches, can then serve as markers of treatment efficacy during clinical trials. However, prior to the initiation of such trials, systematic investigation of where (neuroanatomic effects), when (timepoint in the lifespan) and how (gene expression and neuropathology) AD risk genes impact the brain is required. After the

Apolipoprotein E (APOE) gene, which has not always shown consistent neural effects prior to disease onset,[5, 6] a small number of confirmed risk genes[7] for late-onset AD require such systematic investigation.

Among these risk genes is the sortilin-related receptor, L(DLR class), A repeats containing (SORL1, sorLA, LR11) gene, which codes for an ApoE receptor.[8] SORL1 is thought to act within classical AD risk pathways by helping direct the preferential transport of amyloid precursor protein (APP) to endosomal recycling pathways, away from beta-secretase cleavage and subsequent beta-amyloid (1-42) ($A\beta_{42}$) formation.[9,10] Disruption of SORL1 has also been shown to influence tau-related cellular processes.[11] Furthermore, SORL1 operates at the interface of AD and vascular disease risk through its role as a modulator of lipoprotein lipase trafficking.[12]

SORL1 genetic variants have been associated with risk for AD in several ethnic groups.[13–15] These studies have implicated single nucleotide polymorphisms (SNPs), primarily within two haplotype blocks at the 5′ and 3′ ends of the gene. Recently identified mutations at both ends of the SORL1 gene have been described in early-onset AD,[16] suggesting a potentially causative role for this gene. SORL1 risk variants have also been associated with SORL1 expression in postmortem brain,[17, 18] and down-regulation of SORL1 in AD and mild cognitive impairment (MCI) brain has also been shown.[19, 20] Furthermore, these variants have been associated with white matter atrophy and hyperintensities in late-life,[21] as well as hippocampal volume in early adult life.[22] However, white matter microstructure (i.e. fractional anisotropy (FA)) was recently identified as the best MRI-based predictor of conversion from normal cognitive state to amnestic cognitive impairment,[23] underscoring the potential of this neuroimaging phenotype to improve detection of early risk for late-onset AD.

In four independently collected samples, we assessed the effects of SORL1 risk variants on gene expression, AD neuropathology, and white matter microstructure *in vivo*, using a lifespan approach. We hypothesized that SORL1 risk variants would influence both white matter microstructure and SORL1 gene expression, decades prior to the timeframe of typical AD-onset. Given the putative effect of decreased SORL1 expression on the APP pathway, we also hypothesized that SORL1 risk variants would predict increased amyloid-β plaque levels in postmortem brain.

2. Material and Methods

2.1. Neuroimaging (CAMH and Zucker Hillside Samples)

CAMH Sample—142 healthy volunteers were recruited at the Centre for Addiction and Mental Health (Toronto, Canada). All individuals (age 18-85) completed extensive clinical assessment protocols including the structured clinical interview for DSM-IV Axis I disorders (SCID-I), the Mini-Mental State examination,[24] physical exam, and laboratory investigations to rule out the presence of any psychiatric disorder or a dementia, and a neuropsychological test battery (see Supplementary Methods) as previously described.[25, 26] Genotypic groups were matched for socio-demographic factors (see Table 1). The protocol was approved by the local Research Ethics Board, and all participants provided informed,

written consent. All participants were genotyped for six single nucleotide polymorphisms (SNPs) in the SORL1 gene (sequentially numbered SNPs 8-10 and 23-25 as defined by Rogaeva and colleagues, see Table 2)[15] and two in APOE (rs429358 and rs7412) using previously published methods.[27] Genotype calls were made manually, with two laboratory personnel independently verifying results. 10% of sample genotypes underwent quality control duplication. Diffusion tensor imaging (DTI) was conducted on a 1.5T GE Echospeed scanner (General Electric, Milwaukee, WI) using a single-shot spin echo planar sequence with diffusion gradients applied in 23 noncollinear directions and B=1000 s/mm^2 (isotropic 2.6 mm voxels). The entire sequence repeated 3 times to improve signal to noise ratio (Supplementary Methods).

Zucker Hillside Sample—To better characterize the effects of SORL1 during white matter development which plateaus in the 4th decade of life,[28] 68 healthy Caucasian subjects (age 8-40) were examined from an ongoing study at the Zucker Hillside Hospital, Glen Oaks, NY, by advertisement and word of mouth. Exclusion criteria included serious medical illness and any history of psychosis or major mood disorders, as determined by structured and semistructured assessments.[29–31] Genotypic groups were matched for socio-demographic factors (see Table 1). Further details on sample characteristics, inclusion and exclusion criteria have been previously published.[29] Genotyping for all subjects was performed using the Illumina (San Diego, CA, USA) HumanOmniExpress-12v1.0 BeadChips assay, which contained information for SNP 8 and SNP 9 (see Table 2). Missing genotypes were imputed using data from HapMap 3. APOE ε4 status was derived from rs4420638 (a proxy for APOE rs429358, where the rs4420638 G allele is linked to ε4). DTI was performed on a GE Signa HDx scanner using 8 channel head coils. The acquisition sequence included 31 noncollinear directions with B=1000 s/mm^2 and 2.5mm × 1.88mm × 1.88mm voxels (see Supplementary Methods).

2.2. Postmortem SORL1 mRNA (BrainCloud Sample)

The BrainCloud postmortem dataset consists of 269 human subjects, ranging from fetal to late-life, each with genomic data and transcriptomic data for the prefrontal cortex. All subjects had no history of significant psychological problems or psychological care, psychiatric admissions, or drug detoxification and no known history of psychiatric symptoms or substance abuse, as determined by both telephone screening and medical examiner documentation, as previously described.[32] All individuals from the BrainCloud dataset were genotyped using either Illumina (San Diego, CA, USA) Infinium II 650K or Illumina Infinium HD Gemini 1M Duo BeadChips and mRNA quantified with the Illumina Human 49K Oligo array (HEEBO-7 set) according to previously published methods.[33]

2.3. Postmortem Amyloid Load and Tangles (Religious Orders Study {ROS} and Memory and Aging Project {MAP} Sample)

Participants from ROS are older nuns, priests and brothers from across the US,[34] and those from MAP are residents of approximately 40 senior housing facilities in the Chicago metropolitan area, including subsidized housing facilities, retirement communities, and retirement homes as previously described.[35] Both studies, approved by the Institutional Review Board of Rush University Medical Center, enroll older persons without dementia

who agree to annual evaluation and autopsy. All subjects were assessed with a comprehensive decision tree algorithm as well as a uniform, structured, clinical evaluation that included a self-report medical history obtained by trained nurses and research technicians, a neurologic examination by trained nurses and cognitive function testing by trained neuropsychological test technicians. Please see Bennet et al. (2006)[36] for further detail. The follow-up rate exceeds 95% and the autopsy rate exceeds 90%. At the time of analysis, genomic data were available from n=710 autopsied subjects in total (249 HC, 182 MCI, 279 AD). For regional quantification of Aβ plaques and paired helical filament tau (PHFtau) tangles in postmortem brains, tissue blocks were analyzed from entorhinal cortex proper, hippocampus (CA1/subiculum), superior frontal cortex, dorsolateral prefrontal cortex, inferior temporal cortex, angular gyrus cortex, anterior cingulate cortex, and calcarine cortex. Immunohistochemical analysis was performed to quantify Aβ and PHFtau for two average measures of pathology across all regions. Details of autopsy procedure and quantification of neuropathological measures have been previously published.[37] Genomic data was generated using the Affymetrix (Santa Clara, CA, USA) Genechip 6.0 platform, with APOE and SORL1 SNP 8-10 genotypes imputed from MACH (version 1.0.16a) and HapMap release 22 CEU (build 36), as previously published (see Table 2).[38] Genotype groups were matched for socio-demographic characteristics as described in Table 3.

2.5. Statistical Analysis

2.5.1. Neuroimaging (CAMH and Zucker Hillside Samples)—Each sample was analyzed independently using the same approach. Whole-brain voxelwise differences in FA between genotypic groups were assessed using tract-based spatial statistics (TBSS, see Supplementary Methods) with general linear models, co-varying for age, APOE ε4 status, and sex. Due to near-perfect linkage of the 5′ SORL1 haplotype (SNPs 8-10), all individuals were grouped according to rs689021 genotype using dominant (major allele [G] homozygotes vs. minor [A] carriers) and recessive (minor allele [A] homozygotes vs. major allele [G] carriers) models to determine the direction of effect. 5000 permutations were performed for each contrast and voxels were deemed significant if $p<0.05$ after threshold-free cluster enhancement (TFCE) correction for multiple comparisons across space. In both samples, *post hoc* analysis was performed for peak voxels within select tracts using OLS regression (R statistical software v.2.15.1) to visualize how genotype related to FA across age, using voxel FA as the dependent measure, co-varying for sex and APOE ε4 status.

2.5.2. Postmortem SORL1 mRNA (BrainCloud Sample)—The only SORL1 SNP (within the SNP 8-10 haplotype) available in the Braincloud sample was rs689021 (SNP 9). Raw data were extracted and analyzed externally using R. Ordinary least squares (OLS) regression models were used, including restricted cubic splines to evaluate non-linear effects and interactions of genotype and age within ethnic subgroups (Caucasian and African American (AA)) together and separately, co-varying for sex, postmortem interval, and sample pH. Samples with an RNA integrity number (RIN)[39] of less than 7.0 were excluded from analysis to help reduce confounding due to poor RNA quality.

2.5.3. Postmortem Amyloid Load and Tangles (Religious Orders Study {ROS} and Memory and Aging Project {MAP} Sample)—Of the total 710 subjects, 5 (0.7%)

had non-conforming SORL1 5′ haplotypes and were therefore excluded from analysis, resulting in a final n=705 for which SNPs 8-10 were in perfect LD. For neuropathology measures, the distributions of Aβ and PHFtau were heavily right skewed. We therefore performed median splits of each measure to create binary factors with values corresponding to zero-low and moderate-high pathology levels. The resulting data were analyzed using logistic regression to model these levels of Aβ and PHFtau as a function of SORL1 rs689021 (SNP 9) genotype group, using an additive model with three genotypic groups, then using dominant (major allele [C] homozygotes vs. minor [T] carriers) and recessive (minor allele [T] homozygotes vs. major allele [C] carriers) models, co-varying for age, APOE ε4 status, sex, and education. Analysis was performed separately within each diagnostic group (HC, MCI, AD), and correction for multiple comparisons (2 pathological measures × 3 diagnostic groups=6 independent tests) was performed using FDR with q=0.05.

Following consistent evidence in existing literature of a strong and robust LD structure within the 5′ region of SORL1 (specifically the haplotype defined by SNPs 8-10),[17, 40–43] as well as our own findings of near perfect LD within each analyzed sample (rare haplotype group frequencies were prohibitively low (<1%) and only present in the CAMH and ROS/MAP datasets), we chose to analyze one representative SNP across all four samples (SNP9, rs689021).

3. Results

3.1. Neuroimaging Samples (CAMH and Zucker Hillside Samples)

In both the CAMH and Zucker Hillside samples, the 5′ haplotype block (SNPs 8-10) showed significant associations with white matter FA (Figure 1A), with rs689021 A allele ho mo zygotes showing reduced FA primarily in fronto-temporal white matter tracts, including the bilateral superior longitudinal fasciculus, uncinate fasciculus, inferior fronto-occipital fasciculus, and cingulum bundle, as well as right inferior longitudinal fasciculus, and the genu and splenium of the corpus callosum in both samples at 5% family-wise error (FWE) corrected thresholds. Additionally, the Zucker Hillside sample showed effects of genotype within the internal capsule. No effects of SNPs 23-25 were found in the CAMH sample. *Post hoc* analysis revealed a pattern of reduced FA in rs689021 A-allele homo zygotes that was consistent across the age-range of both samples (i.e. no interaction with age) (Figure 1B).

3.2. Postmortem SORL1 mRNA (BrainCloud Sample)

After removing observations with RIN<7.0, age<0, and missing sample PH information, ethnic subgroup sample sizes were 3(Asian), 5(Hispanic), 90(Caucasian), and 99(AA). Based on these group sizes, analysis was conducted in the Caucasian and AA subgroups only. In the combined Caucasian and AA sample (n=189), a significant non-linear genotype by age interaction was found ($F_{12,176}$=4.06, p=0.008), co-varying for ethnicity, pH, PMI and sex, whereby major differences in SORL1 mRNA levels were prominent during childhood and adolescence into early adulthood. During this period, the A-allele homozygotes demonstrated reduced prefrontal SORL1 mRNA. Analyzing ethnic subgroups separately

revealed that the effect was driven by Caucasians ($F_{11,78}$=7.03, p=0.0003) (Figure 2). No effect of SORL1 variation was found in the AA group ($F_{11,87}$=0.1, p=0.97).

3.3. Postmortem Amyloid Load and Tangles (Religious Orders Study {ROS} and Memory and Aging Project {MAP} Sample)

Associations of SORL1 rs689021 (SNP 9) genotype with Aβ were found in both MCI (dominant model, GG genotype<A carriers, O.R.=0.34 (95% C.I.=0.16-0.73), p=0.0056 (p_{adj}=0.03)) and AD (recessive model, AA genotype>G carriers, O.R. = 3.05 (95% C.I.=1.29-7.22), p=0.011 (p_{adj}=0.03)) subjects, but not in the HC group (genotypic model O.R.=1.2, p=0.65). For PHFtau, an association trend with rs689021 genotype was found in HC subjects (genotypic model, AA>GG, O.R.=2.26 (95% C.I.=0.98-5.21), p=0.055 (p_{adj}=0.11)), but not in the MCI (genotypic model O.R.=0.96, p=0.93) or AD (genotypic model O.R.=1.41, p=0.42) groups. While the PHFtau result did not survive FDR correction, it is worth noting that the same T allele associated with greater Aβ pathology was also associated with increased PHFtau.

4. Discussion

We found that SORL1 risk variants influenced microstructure of white matter tracts with known susceptibility in AD, in two imaging-genetics datasets, with consistent effect from childhood onward. We then bridged the gap from genetic risk variants to brain structure by demonstrating that the same SORL1 risk variant predicted lower levels of mRNA expression, most prominently in childhood and adolescence, demonstrating a temporal consistency of onset of neural risk with our findings in both neuroimaging samples. Finally, we demonstrated that variation at the SORL1 gene predicts amyloid-β plaque levels, thus conferring neuropathological risk via the amyloidogenic pathway.

In both the CAMH and Zucker Hillside samples, SORL1 risk variants were associated with lower white matter FA in structures vulnerable in MCI and the earliest phases of AD. Conventional MRI studies show brain changes in AD typically occur first in medial temporal structures, spreading globally as the disease progresses.[44–46] DTI studies in AD have shown that this gray matter neurodegeneration is paralleled by impairment in white matter tract microstructure (i.e. FA), primarily in association fibers connecting to the medial and lateral temporal lobes.[47] These changes are also present in MCI individuals who have not yet developed dementia.[48, 49] The results of a recent study, which identified parahippocampal white matter FA (part of the cingulum bundle in the medial temporal lobe), as the single best neuroimaging predictor of incipient cognitive impairment[50] raise the possibility that white matter changes may precede gray matter changes in the sequence of preclinical AD-related neural events. Our data support that the very earliest forms of genetically-mediated neural risk for AD may occur through white matter pathways, from childhood onward. A previous examination of SORL1 and white matter found increased risk for postmortem white matter atrophy and white matter hyperintensities in elderly individuals *in vivo* in the elderly white MIRAGE (Multi-Institutional Research in Alzheimer's Genetic Epidemiology) cohort.[21] Although white matter hyperintensities can be present earlier in adult life, they are generally uncommon in healthy young individuals,[51] and as such, may

not be as useful as microstructural integrity of white matter when assessing subtle forms of early neural risk for AD.

Our lifespan analysis using BrainCloud demonstrates that the effects of SORL1 risk variants on SORL1 mRNA expression are most prominent from childhood through to early adulthood (i.e. during neurodevelopmental phases of the lifespan). Minor allele homozygotes showed reduced mRNA expression during this period, consistent with our findings of reduced microstructural integrity of white matter already present from childhood onward in the Zucker Hillside sample and from late adolescence onward in the CAMH sample. Previous studies have found allelic differences in SORL1 protein[17] and mRNA levels[52] in elderly postmortem brain; however, by using a lifespan approach, we provide the first evidence that the temporal impact of SORL1 risk variants on SORL1 mRNA expression occurs during neurodevelopmental phases of the lifespan, rather than in late-life.

Our association of SORL1 genotype with Aβ plaque levels provides direct neuropathological evidence that SORL1 confers risk for AD through the amyloidogenic pathway. Our results confirm those of *in vitro* studies which have found that increased levels of SORL1 result in decreased APP processing[53] and greater production of intracellular $A\beta_{42}$.[53, 54] Loss of SORL1 expression in histologically normal late-onset AD brain-derived neurons[20, 55] suggests that this is a primary event in late-onset AD pathology and may precede disease onset. SORL1's role in amyloid accumulation supports its role as a risk factor for AD rather than as a marker of disease progression. Our findings do not support SORL1 as a marker of disease progression (i.e. accumulation of tau pathology) in AD populations, which has recently been shown to be due to an entirely different set of genetic factors.[56] Although it is possible that subtle changes in $A\beta_{42}$ concentration resulting from allelic differences in SORL1 expression drive changes in microstructural integrity of white matter early in life, our study cannot directly answer this question. Indirect evidence for this possibility is provided by inverse correlations of CSF levels of SORL1 protein with $A\beta_{42}$ in MCI subjects,[57] and association of CSF levels of $A\beta_{42}$ with medial frontal FA.[58]

There are several potential limitations to this study. First, in healthy control samples, it is possible that subclinical symptomatology might be present, and this caveat should be taken into consideration when interpreting our results. However, the similar results in both of our neuroimaging samples, which were from different countries and of different age range, provide added confidence in our results. Second, as with any group-wise analysis of means, the relatively small group sizes of risk allele homozygotes in some of our samples can be considered a limitation. However, statistically significant associations were found in each sample, and the direction of effect was consistent across samples. Third, due to the cross-sectional nature of our analyses, we cannot unequivocally conclude that the imaging results are specific to risk for AD, as white matter impairments are prevalent in other disorders, such as depression, that are known to affect older adults.[59] It is important to note, however, that SORL1 is considered an Alzheimer's risk gene, based both on genome-wide analysis and meta-analysis.[14] Furthermore our findings align with previous investigations of regions/tracts that are first affected in early AD and MCI, such as the cingulum bundle,[50, 60] uncinate fasciculus,[61, 62] and corpus callosum.[63]

Importantly, our findings must be viewed in context of the existing literature. In the initial Rogaeva et al. (2007)[15] study (as well as the Reitz et al. (2011) meta-analysis[14]), the SNP 8-10 haplotype associated with increased risk for AD diagnosis was CGC. In the Cuenco et al. (2008)[21] imaging study, it is the A allele at SNP 9 (corresponding to the TAT haplotype) that is associated with increased risk for AD-associated imaging phenotypes (notably white matter atrophy and hyperintensities), and the T allele at SNP 8 (belonging to the same TAT haplotype) was associated with smaller hippocampal volumes in the only other imaging investigation of SORL1 gene variants by Bralten et al. (2011).[22] Our neuroimaging results, along with our results of mRNA expression and Aβ, are in agreement with these existing structural imaging findings within the 5′ region of SORL1. Therefore, when all genetic investigations of SORL1 are taken together, it appears that allelic heterogeneity may be operating at these loci.

The demonstrated effects of SORL1 variation on brain structure, SORL1 mRNA, and amyloid pathology coupled with our lifespan approach, provide answers about when, where, and how this gene confers neural risk for AD. Our study identifies SORL1-related risk mechanisms and neuroimaging biomarkers that can be utilized in potential intervention studies targeted toward risk carriers, yet our findings also raise questions regarding when in the lifespan such interventions should be tested. At the same time, it is clear that variation at the SORL1 gene, except for rare cases of identified mutations, is unlikely to act as a causative factor alone for late-onset AD. Therefore, systematic assessment of other risk genes using similar multi-level lifespan approaches are first required to move closer toward targeted genetically-based interventions in healthy individuals at-risk for late-onset AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The BrainCloud postmortem data used for the analysis described in this manuscript were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/gap through dbGaP accession number phs000417.v1.p1. Submission of the data, phs000417.v1.p1, to dbGaP was provided by Drs. Barbara Lipska and Joel Kleinman. Collection of the data was through a collaborative study sponsored by the NIMH Intramural Research Program. Initial report on this dataset is from Colantuoni et al. (2011). We would also like to thank all of the study participants and acknowledge the essential contributions of Chaya Gopin and Kimberly Cameron to the recruitment and clinical assessments of those participants. We are indebted to the participants in the Religious Orders Study and the Rush Memory and Aging Project. We thank the staff of the Rush Alzheimer's Disease Center. Work from Rush was supported in part by grants P30AG10161, R01AG15819, R01AG17917, R01AG30146, the Illinois Department of Public Health, and the Translational Genomics Research Institute. Work from Hillside was supported by NIMH grant P50MH080173. Work from CAMH was supported in part by the CAMH Foundation thanks to the Kimel Family, Koerner New Scientist Award, and Paul E. Garfinkel New Investigator Catalyst Award, as well as the Canadian Institutes of Health Research, Ontario Mental Health Foundation, the Alzheimer's Society of Canada, and NIMH grant R01MH099167. No sponsor or funder played any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. Supplementary information is available at *Molecular Psychiatry*'s website.

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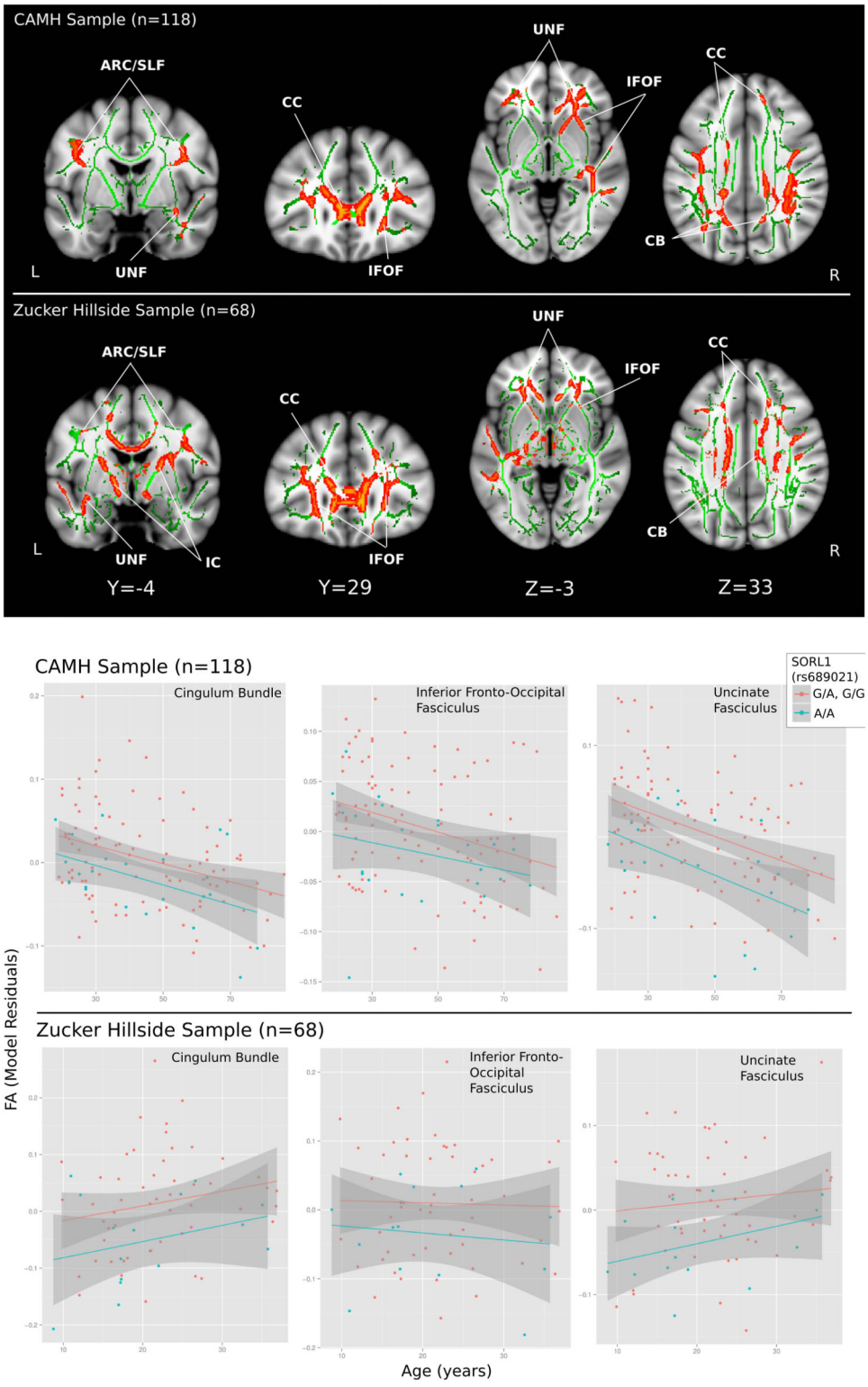

Figure 1.

1A. Results of TBSS white matter analysis for CAMH (A) and Zucker Hillside (B) imaging-genetics datasets. The average white matter FA skeletons for each sample have been

overlaid on the MNI152 1mm T1-weighted brain standard and significant voxels are indicated by yellow-red colouring, corrected for multiple comparisons using TFCE at $p<0.05$. Only voxels within the mean FA skeleton (Green) were analyzed, surrounding voxels have been colo ured for emphasis. UNF = uncinate fasciculus; IFOF = inferior fronto-occipital fasciculus; CB = cingulum bundle; CC = corpus callosum; IC = internal capsule; ARC/SLF = arcuate fasciculus/superior longitudinal fasciculus; (R) = right; (L) = left.

1B. Regression model residuals of white matter fractional anisotropy at select peak voxels (as determined using TBSS) plotted against age, according to SORL1 rs689021 genotypic group ([A] allele homozygotes vs. [G] allele-carriers) in both the CAMH and Zucker Hillside samples. Models co-varied for sex and APOE ε4 status.

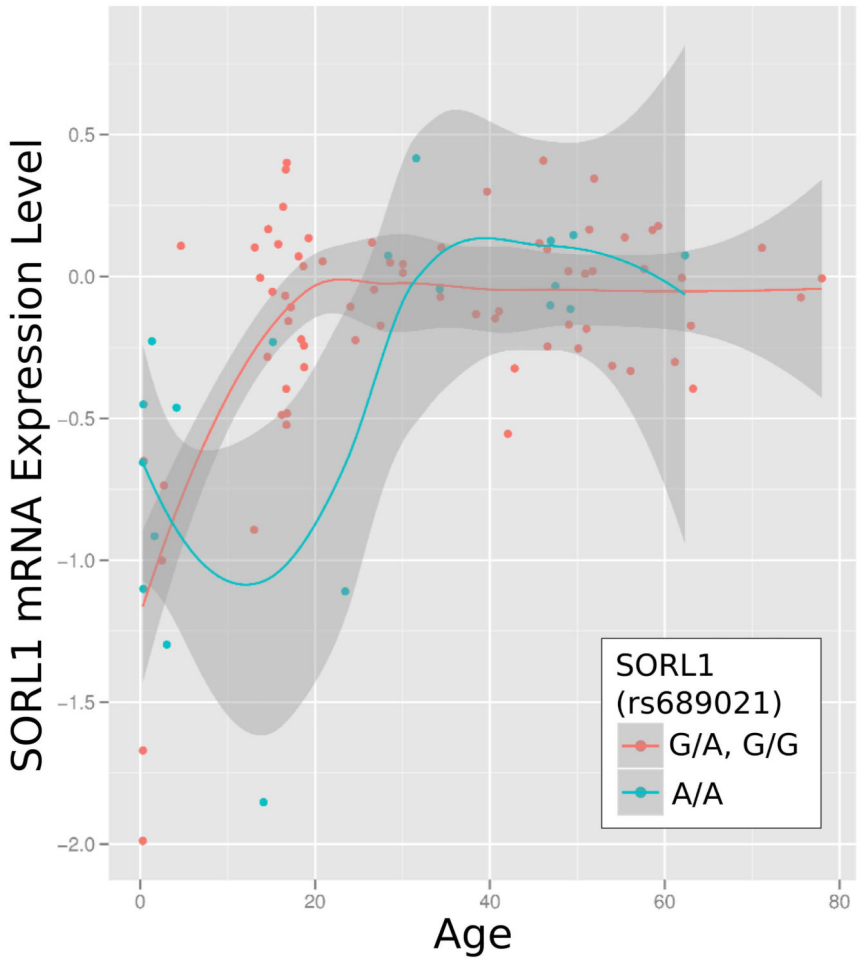

Figure 2.

SORL1 mRNA expression in the prefrontal cortex plotted against age, according to SNP 9 (rs689021) genotype in the BrainCloud postmortem sample. Raw expression data are shown fit with loess smoothing curves for each genotype. Ordinary least squares regression model shows a non-linear genotype by age interaction (interaction effect: $F_{11,78}$=7.03, two-tailed p=0.0003).

Table 1

Demographic Summary Statistics by SORL1 rs689021 Genotype, Recessive Model

	CAMH Sample (n=118)			Zucker Hillside Sample (n=68)		
	SORL1 rs689021	Genotypic Groups	Diff	SORL1 rs689021 G	enotypic Groups	Diff
Demographic	**G-carriers (n=95)**	**A/A (n=23)**	**p**	**G-carriers (n=53)**	**A/A (n=15)**	**p**
Both Samples						
Age, Y(SD)	45(19)	44(19)	0.87	22(7)	21(8)	0.68
Education, Y(SD)	15(2)	15(2)	0.69	13(4)	11(4)	0.15
IQ (SD)	118(8)	118(9)	0.92	107(10)	106(8)	0.89
Sex	52 M, 43 F	15 M, 8 F	0.48	25 M, 28 F	8 M, 7 F	0.77
Handedness	86 R, 6 L, 3 A	23 R	0.64	59 R, 4 L	13 R, 2 L	0.59
Ethnicity	84 Cau, 7 As, 4 O	18 Cau, 3 As, 2 O	0.32	53 Cau	15 Cau	-
APOE ε4, N(%)	24(25)	6(26)	1	9(17)	2(13)	1
CAMH Only						
MMSE (SD)	29(1)	29(1)	0.76	-	-	-
BMI (SD)	25(5)	26(4)	0.48	-	-	-
Systolic BP (SD)	124(16)	125(16)	0.80	-	-	-
Diastolic BP (SD)	76(11)	74(8)	0.25	-	-	-

Note: IQ measured using standardized scores of the Weschler Test of Adult Reading (WTAR) for the CAMH sample and the Wide Range Achievement Test 3 (WRAT3) for the Zucker Hillside sample. Continuous variables (age, education, BMI, IQ, MMSE, B P, and CIRS-G) were analyzed for genotypic group differences using a student's t-test (two-tailed). Factor variables (sex, handedness, ethnicity, and APOE ε4 status) were analyzed using Fisher's exact test (two-tailed). Y = years; M = male; F = female; R = right; L = left; A = ambidextrous; Cau = Caucasian; As = Asian; O = other; MMSE = Mini Mental Status Exam; BMI = body mass index (height(cm)/weight(kg)^2); BP = blood pressure; CIRS-G = Cumulative Illness Rating Scale – Geriatrics.

Table 2

Details for Analyzed SNPs in SORL1 5′ Haplotype by Study Sample

Sample	SORL1 SNPs Directly Genotyped or Imputed (D/I)						Phenotypes	# Independent Tests Performed
	SNP	#	Location (Chr:Pos)	Orientation/Strand	SNP Type	Alleles (min/maj)		
CAMH	rs668387 (D)	8	11: 121367921	Rev/B	Intronic	T/C	White Matter FA	1 (haplotype in perfect LD, dominant and recessive models)
	rs689021 (D)	9	11: 121371120	Rev/T	Intronic	A/G		
	rs641120 (D)	10	11: 121380965	Fwd/T	Intronic	T/G		
Zucker Hillside	rs668387 (I)	8	11: 121367921	Rev/B	Intronic	T/C	White Matter FA	1 (haplotype in dominant and recessive models)
	rs689021 (I)	9	11: 121371120	Rev/T	Intronic	A/G		
BrainCloud	rs689021 (D)	9	11: 121371120	Rev/T	Intronic	A/G	SORL1 mRNA	1 (recessive model)
ROS/MAP	rs668387 (I)	8	11: 121367921	Rev/B	Intronic	T/C	Aβ Plaques	3 (one in each diagnostic group: HC, MCI, AD)
	rs689021 (I)	9	11: 121371120	Rev/T	Intronic	A/G		
	rs668387 (I)	8	11: 121367921	Rev/B	Intronic	T/C	PHFtau Tangles	3 (one in each diagnostic group: HC, MCI, AD)

Note: SNP locations according to NCBI dbSNP build 37. SNP #s correspond to those assigned by Rogaeva et al. (2007). CAMH = Centre for Addiction and Mental Health; ROS/MAP = Religious Orders Study/Memory and Aging Project; D = directly genotyped; I = imputed (see Methods section); Chr = chromosome; Pos = position; Rev = reverse direction; Fwd = forward direction; B = bottom strand; T = top strand; Ex. = exonic; syn = synonymous; Ala = alanine; min = minor allele; maj = major allele; F A = fractional anisotropy; LD = linkage disequilibrium; mRNA = messenger ribonucleic acid; Aβ = beta-amyloid; PHFtau = paired helical filament tau; HC = healthy controls; MCI = mild cognitive impairment; AD = Alzheimer's disease. Genotype frequencies of compared groups are reported for each sample separately in Tables 1 and 3.

Table 3

ROS/MAP Demographic Summary Statistics by SORL1 rs689021 Genotype, Recessive Model

	ROS/MAP Post mortem Sample (n=705)								
	SORL1 rs689021 Genotypic Groups by Diagnosis								
Demographic	**HC (n=247)**			**MCI (n=180)**			**AD (n=278)**		
	G-car (n=201)	A/A (n=46)	Diff (p)	G-car (n=144)	A/A (n=36)	Diff (p)	G-car (n=232)	A/A (n=46)	Diff (p)
Age, Y(SD)	86(6)	87(5)	0.12	89(6)	89(6)	0.73	91(6)	90(5)	0.45
Education, Y(SD)	17(4)	17(3)	0.91	16(4)	17(3)	0.58	16(3)	16(4)	0.68
Sex	122 F, 79 M	30 F, 16 M	0.62	90 F, 54 M	21 F, 15 M	0.70	159 F, 73 M	30 F, 16 M	0.73
APOE ε4, N(%)	33(17)	5(11)	0.50	33(23)	11(32)	0.39	81(35)	18(39)	0.62
MMSE (SD)	29(2)	28(2)	0.54	28(2)	28(2)	0.48	25(5)	26(4)	0.47
BMI (SD)	27(5)	27(5)	0.48	27(5)	26(5)	0.57	26(5)	25(4)	0.34
Systolic BP (SD)	134(19)	131(16)	0.20	137(18)	137(16)	0.90	137(18)	139(18)	0.58
Diastolic BP (SD)	71(11)	72(10)	0.63	70(14)	72(9)	0.33	71(12)	72(12)	0.69

Note: Continuous variables (age, education, BMI, MMSE, and BP) were analyzed for genotypic group differences using a student's t-test (two-tailed). Factor variables (sex and APOE ε4 status) were analyzed using Fisher's exact test (two-tailed). SD = standard deviation; car = carrier; HC = healthy control; MCI = mild cognitive impairment; AD = Alzheimer's disease; Y = years; M = male; F = female; MMSE = Mini Mental Status Exam; BMI = body mass index (height(cm)/weight(kg)^2); BP = blood pressure. All subjects were of Caucasian ancestry.