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The influence of genetic variants in *SORL1* gene on the manifestation of Alzheimer's disease

Eva Louwersheimer [a,*], Alfredo Ramirez [b,c], Carlos Cruchaga [d], Tim Becker [e,f], Johannes Kornhuber [g], Oliver Peters [h], Stefanie Heilmann [c,i], Jens Wiltfang [j], Frank Jessen [b,e], Pieter Jelle Visser [a,k], Philip Scheltens [a], Yolande A.L. Pijnenburg [a], Charlotte E. Teunissen [l], Frederik Barkhof [m], John C. van Swieten [n,o], Henne Holstege [a,o], Wiesje M. Van der Flier [a,p], for the Alzheimer's Disease Neuroimaging Initiative and Dementia Competence Network[1]

[a] *Alzheimer Center and Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, the Netherlands*
[b] *Department of Psychiatry and Psychotherapy, University of Bonn, Bonn, Germany*
[c] *Institute of Human Genetics, University of Bonn, Bonn, Germany*
[d] *Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA*
[e] *German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany*
[f] *Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany*
[g] *Department of Psychiatry and Psychotherapy, Friedrich-Alexander University of Erlangen, Nuremberg, Germany*
[h] *Department of Psychiatry, Charite, Berlin, Germany*
[i] *Department of Genomics, Life and Brain Center, University of Bonn, Bonn, Germany*
[j] *Department of Psychiatry and Psychotherapy, University Medical Center (UMG), Georg-August-University, Göttingen, Germany*
[k] *Department of Psychiatry and Neuropsychology, Alzheimer Centre Limburg, School for Mental Health and Neuroscience, Maastricht University Medical Centre, Maastricht, the Netherlands*
[l] *Neurochemistry Laboratory and Biobank, Department of Clinical Chemistry, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, the Netherlands*
[m] *Department of Radiology, VU University Medical Center, Amsterdam, the Netherlands*
[n] *Department of Neurology, Erasmus Medical Center, Rotterdam, the Netherlands*
[o] *Department of Clinical Genetics, VU University Medical Center, Amsterdam, the Netherlands*
[p] *Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, the Netherlands*

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ABSTRACT

We studied the association of *SORL1* single-nucleotide polymorphisms genotypes with measures of pathology in patients with probable Alzheimer's disease (AD) using an endophenotype approach. We included (1) 133 patients from the German Dementia Competence Network (71 ± 8 years; 50% females; Mini Mental State Examination [MMSE], 24 ± 3); (2) 83 patients from the Alzheimer's Disease Neuroimaging Initiative (75 ± 8 years; 45% females; MMSE, 24 ± 2); and (3) 452 patients from the Amsterdam Dementia Cohort 66 ± 8 years; 47% females; MMSE, 20 ± 5). As endophenotype markers we used cognitive tests, cerebrospinal fluid (CSF) biomarkers amyloid-beta, total tau (tau), tau phosphorylated at threonine 181, and hippocampal atrophy. We measured 19 *SORL1* SNP alleles. Genotype-endophenotype associations were determined by linear regression analyses. There was an association between rs2070045-G allele and increased CSF-tau and more hippocampal atrophy. Additionally, haplotype-based analyses revealed an association between haplotype rs11218340-A/rs3824966-G/rs3824968-A and higher CSF-tau and CSF-tau phosphorylated at threonine 181. In conclusion, we found that *SORL1* SNP rs2070045-G allele was related to CSF-tau and hippocampal

* Corresponding author at: Alzheimer Center and Department of Neurology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, the Netherlands. Tel.: +31 204440685; fax: +31 204448529.
E-mail address: e.louwersheimer@vumc.nl (E. Louwersheimer).

[1] Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and provided data, or they solely provided data. But the investigators within the ADNI did not participate in analysis or writing this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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atrophy, 2 endophenotype markers of AD, suggesting that *SORL1* may be implicated in the downstream pathology in AD.

1. Introduction

Sporadic Alzheimer's disease (AD) is a complex neurodegenerative disease, caused by a combination of genetic, epigenetic, and environmental influences. APOE e4 allele is the strongest known genetic risk factor, with a dose-effect relationship (Farrer et al., 1997). In addition to conferring an increased risk for AD, possession of the e4 allele has also been suggested to account for some of the variability in clinical manifestation of AD (van der Flier et al., 2006, 2011). Patients that carry at least 1 APOE e4 allele, perform worse on cognitive (memory) testing (van der Flier et al., 2006), they have decreased concentrations of amyloid-beta1-42 (abeta) in cerebrospinal fluid (CSF) (Prince et al., 2004), more hippocampal atrophy (Pievani et al., 2011) and more metabolic impairment in the posterior part of the cortex (Ossenkoppele et al., 2013). By contrast, APOE e4 noncarriers, especially when they develop the disease at an early age, have been suggested to have a faster rate of cognitive deterioration and more generalized atrophy with faster atrophy rates (Sluimer et al., 2008; van der Vlies et al., 2009).

In the past years, by genome-wide association studies (GWAS) more than 20 loci were found to be associated with AD (Beecham et al., 2009; Lambert et al., 2009; Laumet et al., 2010; Meng et al., 2007; Seshadri et al., 2010). In a recent GWAS, *Sortilin-related receptor (SORL1)* was one of these risk genes found to be associated with an increased risk for AD (Lambert et al., 2013). Additionally, in candidate gene studies, associations between *SORL1* single-nucleotide polymorphisms (SNPs) and AD risk have repeatedly been shown, including associations with selected biomarkers of AD, which makes this a very interesting gene (Alexopoulos et al., 2011; Cuenco et al., 2008; Kimura et al., 2009; Lee et al., 2008; Ning et al., 2010; Rogaeva et al., 2007; Tan et al., 2009).

SORL1 gene is located on chromosome 11 and it encodes a protein, sorLA or LR11 (Rogaeva et al., 2007). LR11 has many functional domains with different functions, including cargo transport, chaperone-like activity, signaling, and intracellular sorting (Fig. 1) (Jacobsen et al., 2001). By acting as a sorting receptor, LR11 protects APP from being directed to the endosome where it would be cleaved by beta-secretase producing amyloid-beta, an important component in the pathophysiological pathway of AD. Indeed, downregulation or dysfunction of LR11 has been shown to lead to amyloidogenesis in mice (Dodson et al., 2008). In addition, LR11 is a member of the low-density lipoprotein receptor family implicated in cholesterol metabolism (Dodson et al., 2006; van Vliet, 2012).

To further investigate this association between *SORL1* SNP risk alleles and the pathophysiology of AD, we took an endophenotype approach: we included solely AD patients and looked at associations with a number of markers of Alzheimer pathology (endophenotypes). These endophenotype markers may be modulated by a putative differential effect of *SORL1* SNP genotypes on pathophysiology (Reitz and Mayeux, 2009). We aimed to investigate associations between *SORL1* SNPs and 6 endophenotype markers (Jack et al., 2013): we used CSF abeta as marker for amyloidosis, CSF total tau (tau), CSF-tau phosphorylated at threonine-181 (ptau), and hippocampal atrophy as markers for neuronal injury and neurodegeneration, and we used neuropsychological tests as markers for cognitive impairment.

2. Methods

2.1. Participants

The inclusion criteria applied were as follows: (1) diagnosis of AD made according to the NINCDS-ADRDA criteria for probable AD (McKhann et al., 1984, 2011); (2) availability of measurements of cognitive performance, CSF, and hippocampal atrophy on magnetic resonance imaging (MRI); (3) availability of genotyped data of

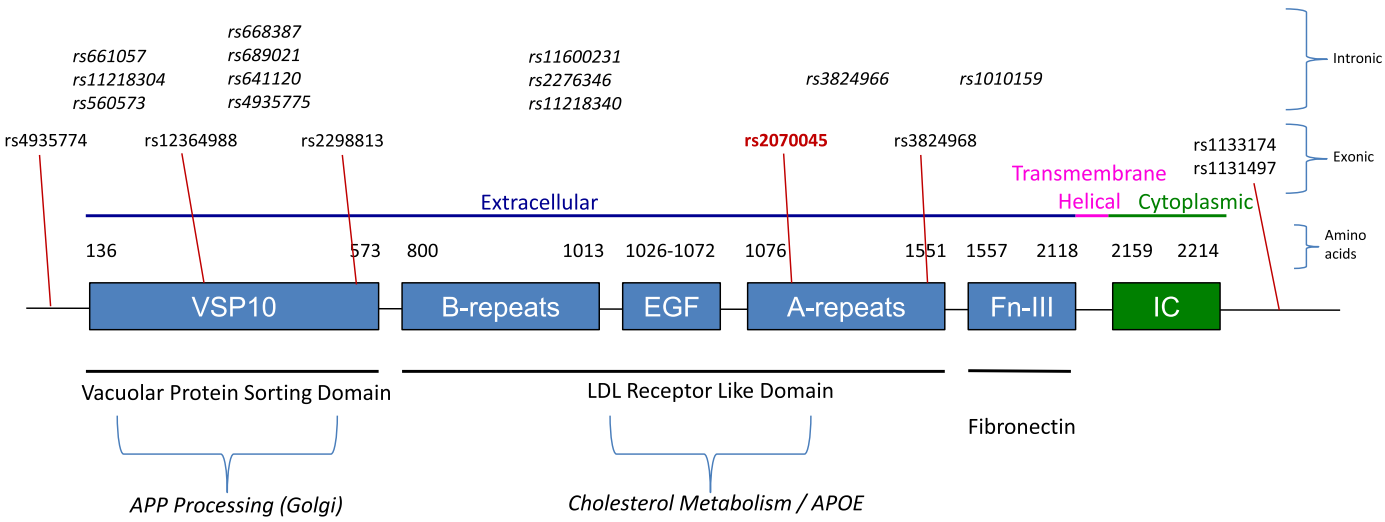

Fig. 1. Sortilin-related receptor gene (*SORL1*). Displayed are the *SORL1* SNPs investigated in the present study: the SNPs displayed on the first level are the intronic SNPs, on the second level the exonic SNPs are displayed. The SNPs are ranked according to chromosome position, with the exonic SNPs linked to the position of the amino acid number. The significantly associated SNP rs2070045 is depicted in red. The lower part of the figure displays the function of the different parts of the gene: the vacuolar protein sorting domain (which is involved in APP processing) and the LDL receptor-like domain (which is involved in cholesterol metabolism). Abbreviations: LDL, low-density lipoprotein; SNPs, single-nucleotide polymorphisms.

SORL1 SNPs. By applying these inclusion criteria on 3 different data sets, we included 678 patients in total (Table 1).

The first sample consisted of 133 German AD patients (71 ± 8 years of age; 67 [50%] females; mean Mini Mental State Examination [MMSE], 24 ± 3) recruited for the German Dementia Competence Network (DCN). DCN is a research platform of 14 German specialized memory clinics of university hospitals (Kornhuber et al., 2009). Participating subjects were uniformly evaluated using a defined set of clinical, neuropsychological, biochemical, and imaging tests and followed up longitudinally. The network was approved by the ethics committee, and written informed consent was given by the patients or their legal guardians. Further details are available at the DCN website (www.kompetenznetz-demenzen.de).

The second set of 83 patients (75 ± 8 years of age; 37 [45%] females; MMSE, 24 ± 2) was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies and nonprofit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment and early AD. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California–San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the United States and Canada. For up-to-date information, see www.adni-info.org. For ADNI, we included only patients who were registered as non-hispanic or non-latino in the ADNI database.

The third set of 452 patients(66 ± 8 years of age; 219 [47%] females; MMSE, 20 ± 5) was included from the Amsterdam Dementia Cohort (ADC) of the Alzheimer Center at the VU University Medical Center, Amsterdam, the Netherlands (van der Flier et al., 2014). Patients enrolled in the ADC underwent an extensive standardized dementia assessment, including medical history, informant-based history, a physical examination, routine blood and CSF laboratory tests, neuropsychological testing, electroencephalogram, and MRI of the brain (van der Flier et al., 2014). Diagnoses were given in a multidisciplinary consensus meeting. All patients gave informed consent.

Table 1
Clinical characteristics of the AD patients in 3 different sample sets

	DCN	ADNI	ADC
n	134	83	452
Female (n, %)	67 (50)	37 (45)	219 (47)
Age (y)	71 ± 8	75 ± 8	66 ± 8
MMSE	24 ± 3[a]	24 ± 2	20 ± 5
ADAS (DCN and ADNI)	18 ± 6[b]	18 ± 6	—
CAMCOG (ADC)	—	—	67 ± 15[c]
APOE e4 carriers (n, %)	75 (56)[d]	58 (70)	313 (69)
Abeta level in CSF (ng/L)[e]	560 ± 237	143 ± 42	456 ± 108
Tau level in CSF (ng/L)[e]	596 ± 267[f]	121 ± 56	779 ± 416
p-Tau level in CSF (ng/L)[e]	79 ± 37	41 ± 18	94 ± 39
Total hippocampal volume (mm^3)[g]	3391 ± 943.6[h]	5715 ± 1083	MTA: 1.4 ± 0.9[i]

Values are presented in mean ± standard deviation.
Key: abeta, amyloid-beta1-42; AD, Alzheimer's disease; ADAS, Alzheimer's Disease Assesment Scale; ADC, Amsterdam Dementia Cohort; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; DCN, Dementia Competence Network; ELISA, enzyme-linked immunosorbent assay; MMSE, Mini Mental State Examination; ptau, tau phosphorylated at threonine 181; tau, total tau.
[a] n = 132.
[b] n = 126.
[c] n = 260.
[d] n = 130.
[e] Note that CSF values in ADC and DCN samples were measured on an ELISA platform (reference values: abeta >550 ng/L, tau <375 ng/L, ptau >52 ng/L). ADNI samples were measured on a Luminex platform (reference values: abeta >192 ng/L, tau <93 ng/L, ptau >23 ng/L). Hence, different average values for CSF biomarkers are displayed in this table. Note that raw levels are presented, although statistical analyses were performed using log-transformed variables.
[f] n = 131.
[g] Note that hippocampal atrophy in the ADC samples is displayed by using the Scheltens scale from 0 (absent atrophy) till 4 (severe atrophy). For DCN manual volumentry with DISPLAY was performed. Total hippocampal volume of ADNI samples were analyzed by making use of FreeSurfer.
[h] n = 72.
[i] n = 446.

2.2. Genotyping

We based our first selection of 19 *SORL1* SNPs on the original article describing the association between *SORL1* and AD, in which a basic list of 29 *SORL1* SNPs was selected for analysis (Rogaeva et al., 2007). For the ADNI data set, we had the availability over 8 originally genotyped and 10 imputed SNPs of this list of 29 *SORL1* SNPs. These 19 SNPs included 9 SNPs (rs661057; rs11218304; rs668387; rs689021; rs641120; rs2070045; rs3824966; rs3824968; rs1010159) covering the 5′ and 3′ end, and belonging to different linkage disequilibrium (LD) blocks. In a recent meta-analysis by Reitz et al. (2011), these 9 SNPs were described to be significantly associated with an increased risk on AD. Additionally, we used the other 10 available SNPs for a more complete coverage of the genome, and because we did not want to limit ourselves to these already well-studied SNPs. We took the ADNI data set as our starting point for SNP selection, for the DCN data set the same 19 *SORL1* SNPs were available for analysis, and for the ADC data set we designed an assay.

Individuals from DCN were genotyped either on the Illumina 610-quad chip or on the Illumina Omni 1M-quad chip (Cruchaga et al., 2013; Harold et al., 2013; Saykin et al., 2010). For ADNI, as mentioned earlier, the original genotyped data included 8 *SORL1* SNPs and through imputation, as described elsewhere (Cruchaga et al., 2013), we added 10 other SNPs. For ADC, the primers for the multiplex reaction were designed using the Assay Design Suite tool (www.mysequenom.com, Sequenom, San Diego, CA, USA). Primer sequences and assay conditions for the genotyped *SORL1* SNPs are described in more detail in Supplementary Tables 1–4. Assay designs were successful for 17 of the 19 selected variants; SNPs rs2070045 and rs1133174 were rejected during this phase because of technical problems. For as not enough genotyped data were available, imputation of rs2070045 and rs1133174 could not be performed. Instead, we computed pairwise LD (r^2 measure) from the 1000 Genomes reference data (Abecasis et al., 2012) (www.1000Genomes.org) using the software package INTERSNP version 1.14, (Herold et al., 2009). Patients from the ADC were genotyped for 17 SNPs using Sequenom's Mass Array System (Sequenom) and iPlex Gold reagents in accordance with the manufacturer's instructions.

In all data sets, quality control was performed using PLINK software (pngu.mgh.harvard.edu/~purcell/plink/). SNPs and patients were included when meeting the following criteria: (1) SNP inclusion by call rate per SNP: SNP genotype information was present for >98% of patients; (2) inclusion by call rate per patient: >98% of all SNP genotypes were available for each patient; (3) Hardy-Weinberg equilibrium test for deviation of the Hardy-Weinberg equilibrium, $p < 0.0001$. In both the ADC and the DCN data set 1 individual had to be excluded based on low SNP call rate.

Alternate and reference allele genotypes were annotated to comply with the dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP/), build 138 (Table 2).

2.3. Outcome measures

As outcome measures we used 6 endophenotype markers of AD.

Two markers were tests of cognition: MMSE and Alzheimer's Disease Assessment Scale Cognition (ADAS) for ADNI and DCN or MMSE and CAMCOG for ADC (Derix et al., 1991; Folstein et al., 1975; Rosen et al., 1984).

Three markers were CSF biomarkers of AD: abeta, tau, and ptau. For the sample sets of DCN and ADC, CSF abeta, tau, and ptau were determined using commercially available sandwich enzyme-linked immunosorbent assay (Innogenetics, Gent, Belgium) (Mulder et al., 2010; Popp et al., 2010). For ADNI, abeta, tau, and ptau were analyzed using a multiplex xMAP Luminex platform (Luminex Corp) with immunoassay kit-based reagents (INNO-BIA Alzbio3; Innogenetics) as described elsewhere (Shaw et al., 2009).

The sixth marker was hippocampal atrophy. Imaging data for DCN were obtained on 1.5-Tesla MRI scanners (Kornhuber et al., 2009). For standardization of MRI acquisition across centers, acquisition parameters were provided to all centers as a guideline (Ewers et al., 2006). Hippocampal volumes were determined using the interactive software package DISPLAY developed at the McConnell Brain Imaging Centre at the Montreal Neurological Institute (Teipel et al., 2006). For ADNI, a structural MRI 1.5T scan was acquired during screening or baseline visit (Jack. et al., 2008). These imaging data have been processed with cortical reconstruction and volumetric segmentation with the FreeSurfer 4.3 image analysis suite (surfer.nmr.mgh.harvard.edu/) (Fischl, 2012). The processing results using Freesurfer are published on the ADNI website, ida.loni.usc.edu/ (Shen et al., 2010). For ADC, MRI scans were made during screening on a 1.5T or 3T scan. On 3D T1 images, the coronal coupes were visually rated (scale 0–4 points) for medial temporal lobe atrophy by experienced neuroradiologists (Scheltens et al., 1995).

Table 2
The 19 *SORL1* SNPs used for analysis

	Marker number	SNP position (NCBI)	Amino acid	Alleles (NCBI)	Reference allele (NCBI)
rs4935774	1	5'prime UTR		T/C	T
rs661057	4	Intron		T/C	T
rs11218304	5	Intron		A/G	A
rs560573	6	Intron		T/A	T
rs12364988	7	Exon	His269His	C/T	C
rs668387	8	Intron		G/A	G
rs689021	9	Intron		C/T	C
rs641120	10	Intron		C/T	C
rs4935775	11	Intron		T/G	T
rs2298813	13	Exon	Ala528Thr	G/A	G
rs11600231	14	Intron		T/C	T
rs2276346	15	Intron		G/T	G
rs11218340	18	Intron		A/T	A
rs2070045[a]	19	Exon	Ser1187Ser	T/G	T
rs3824966	20	Intron		C/G	C
rs3824968	23	Exon	Ala1548Ala	T/A	T
rs1010159	25	Intron		A/G	A
rs1133174[a]	28	3′prime UTR		G/A	G
rs1131497	29	3′prime UTR		C/G	C

A total of 19 *SORL1* SNPs were genotyped for all 3 samples.
SNPs are numbered following their sequential order on the physical map and are displayed this way in other *SORL1* studies (Rogaeva et al., 2007).
The genotypes are annotated corresponding dbSNPs database of NCBI (www.ncbi.nlm.nih.gov/projects/SNP).
Key: ADNI, Alzheimer's Disease Neuroimaging Initiative; Ala, alanine; DCN, Dementia Competence Network; His, histidine; Ser, serine; SNPs, single-nucleotide polymorphisms; Thr, threonine.
[a] rs2070045 and rs1133174 were only available for ADNI and DCN.

2.4. Statistical analyses

For statistical analysis we used SPSS IBM Statistics version 20 (SPSS Inc, Chicago, IL, USA) and PLINK v1.07 (pngu.mgh.harvard.edu/purcell/plink) (Purcell et al., 2007). First, using SPSS, CSF biomarkers were log-transformed as they were not normally distributed. Because different platforms were used for CSF analyses and hippocampal atrophy assessments, and because different cognitive tests were used, endophenotype values were not directly comparable between centers. Therefore, to normalize measurements, we calculated z-scores for each (log-transformed) endophenotype measurement. We used the linear regression option in PLINK to perform linear regression analyses to test for associations between the *SORL1* SNP risk alleles and the cognitive tests, the 3 CSF values and hippocampal atrophy with sex, age, and the dichotomous APOE e4 status (non e4 vs. at least 1 e4 allele) as covariates.

Subsequently, to increase power, we pooled the data for a combined association study between the *SORL1* SNPs risk alleles and AD markers. As rs2070045 and rs1133174 were not available in ADC data set, for these 2 SNPs we pooled only the data from ADNI and DCN for association to these SNPs with endophenotypes. For the pooled analysis, an indicator variable of study of origin was used as an additional covariate.

We performed a correction for multiple testing by using the false discovery rate (FDR), with significance level set at $p < 0.05$. The FDR controls for the expected proportion of false positives among those SNPs declared significant and is less stringent than the Bonferroni testing (Storey and Tibshirani, 2003).

Additionally, we performed haplotype analyses of the pooled data by using a sliding window of 3 contiguous SNPs. Based on literature, we focused on 2 haplotypes at the 5′ and 3′ end: SNPsrs668387; rs689021; rs641120 and SNPsrs11218340; rs3824966; rs3824968.

3. Results

In the pooled data set, we detected a significant association between rs2070045-G allele and higher tau levels in CSF and more hippocampal atrophy, $p = 0.03$ FDR corrected, but not between any SNP and any of the other endophenotype measurements (Table 3). Although rs2070045 was not available in the ADC data set, the SNP is in strong LD with rs3824966 ($r^2 = 0.998$) which is available in all 3

Table 3
Pooled association analyses for 19 *SORL1* SNPs with AD patients from DCN, ADNI and ADC

Marker of AD	SNP	Beta ± SE	*p*-value	FDR
MMSE	rs4935774	0.15 ± 0.06	0.017	0.297
ADAS/CAMCOG	rs2298813	−0.41 ±0.16	**0.009**	0.153
Abeta	—	—	—	—
Ptau	—	—	—	—
Tau	rs11600231	−0.23 ± 0.10	0.021	0.179
	rs11218340	−0.28 ± 0.11	**0.009**	0.152
	rs3824966	0.14 ± 0.07	0.038	0.215
	rs2070045[a]	0.28 ± 0.12	0.016	**0.033**
Hippocampus and/or MTA	rs2070045[a]	−0.32 ± 0.13	0.017	**0.033**

Displayed are beta ± standard error with *p* value <0.05, and in bold the results with *p* value <0.01. FDR below $p<0.05$ was considered as significant (depicted in bold). Log-transformed cerebrospinal fluid values were used. Analyses were performed with z-scores, adjusted for covariates age, gender, APOE status, and center.
Key: AD, Alzheimer's disease; ADAS, Alzheimer's disease assessment scale; ADC, Amsterdam Dementia Cohort; ADNI, Alzheimer's Disease Neuroimaging Initiative; DCN, Dementia Competence Network; FDR, false discovery rate; MMSE, Mini Mental State Examination; ptau, tau phosphorylated at threonine-181; SNPs, single-nucleotide polymorphisms.
[a] Note that for rs2070045 and rs1133174 only patients from ANDI and DCN were available.

data sets and because of this near perfect LD, rs3824966 can be used as a proxy for rs2070045. For rs3824966-G, we found an association with CSF tau (0.14 ± 0.007, $p = 0.038$) in the same direction as we had found for rs2070045-G in the combined analysis with all 3 data sets, but the association did not survive FDR correction (FDR = 0.215), and we found a trend for association with relatively increased CSF ptau (0.125 ± 0.065, $p = 0.053$, after FDR = 0.488).

Haplotype-based analyses revealed the association between SNP risk alleles at the 3'end of the *SORL1* gene (rs11218340-A; rs3824966-G; rs3824968-A) with relatively higher ptau and tau levels in CSF, $p < 0.05$ (Table 4).

The analyses for each cohort separately are shown in Supplementary Table 5. Rs2298813-A allele was associated with increased CSF tau in the DCN cohort and in the ADNI cohort rs11218340-G, rs4935775-G, and rs3824968-T alleles associated with more hippocampal atrophy, $p < 0.05$.

4. Discussion

The main finding of our present study is that *SORL1* influences the clinical manifestation of AD. In particular, the exonic SNP rs2070045-G allele was associated with increased CSF tau and more hippocampal atrophy, both markers of neuronal injury and neurodegeneration. Haplotype-based analyses showed associations between 3 SNP haplotypes at the 3'end of the gene and relatively higher tau and ptau levels in CSF, thereby strengthening the effect of *SORL1* on these markers of neuronal injury and neurodegeneration.

Former studies, investigating the influence of specific *SORL1* alleles on CSF biomarkers in AD patients had conflicting results. Three studies revealed an influence of *SORL1* SNP alleles on abeta in CSF (Alexopoulos et al., 2011; Guo et al., 2012; Kolsch et al., 2008), whereas another study did not find any relationship between *SORL1* SNP variants and abeta levels in CSF (Kauwe et al., 2010). A study with ADNI AD patients reported a significant association between rs668387, rs3824968, rs1010159, and lower abeta level in CSF (Alexopoulos et al., 2011). Our separate analyses of the ADNI data set led to similar results, with an association between the rs3824968-A allele and rs1010159-G allele and lower levels of abeta in CSF, but this did not survive multiple testing correction. Moreover, we were not able to replicate the association in the pooled data set. A previous study in 153 AD patients from DCN detected an association with haplotype rs3824966-rs3824968-rs1010159 and lower abeta (Kolsch et al., 2008). Likewise, we detected the association between rs2070045/rs3824966 and relatively lower abeta in DCN patients, but the association did not survive FDR correction. This in contrast with the results of functional assays performed in *Sorl1* knockout mice, where an association between *SORL1* SNPs and intracerebral increased abeta was found (Dodson et al., 2008). A possible explanation for this difference in outcome could be that we included solely demented AD patients, whereas abeta is known to reach a plateau phase in early stage of AD leading to little meaningful variance in abeta levels between AD patients.

In our present study including 3 different clinical samples and using rigorous statistical testing, we found *SORL1* SNPs to be related to CSF (p)tau and hippocampal atrophy. Associations with markers of neuronal injury and neurodegeneration have been described earlier, for CSF tau in a study with AD patients only (Guo et al., 2012), and for hippocampal atrophy in a study with autopsy confirmed AD patients (Cuenco et al., 2008). The significantly associated SNP rs2070045-G allele has been earlier associated with increased risk of AD in a meta-analysis describing different studies with a case-control design (Reitz et al., 2011). In this meta-analysis, rs2070045-G allele showed in the combined white data sets (11,592 cases; 17,048 control subjects) an increased risk on AD (Odds Ratio (OR) = 1.08 [1.01–1.14]; $p = 0.02$), and also in the combined Asian data sets (872 cases; 881 control subjects) the risk on AD was increased in carriers of the rs2070045-G allele (OR = 1.27 [1.10–1.14; $p = 0.001$). In 3 other studies with a case-control design, which were not included in the earlier mentioned meta-analysis, a same effect of the rs2070045-G allele with an increased risk on AD was found (Kimura et al., 2009; Li et al., 2008; Ning et al., 2010). In conclusion, the observations from the case-control studies are in line with the finding in our endophenotype approach. Taken together, this may suggest that rs2070045-G increases the general risk on AD through a more prominent involvement of high tau.

The SNP rs2070045 is situated in the region coding for A-repeats, part of the low-density lipoprotein receptor-like domain involved in the cholesterol metabolism, (Fig. 1). A relationship between dysfunction of intracellular cholesterol trafficking and tau pathology was earlier described by referring to the presence of neurofibrillary tangles in brains of patients with Niemann-Pick type C disease (NPC) (Nixon, 2004). Patients with NPC have a mutation in the genes NPC1 or NPC2, encoding for proteins involved in the endosomal-lysosomal pathway, and the mutation results in failure of cholesterol trafficking. The suggestion, that cholesterol dysfunction contributes independently of abeta to neurodegeneration, was also proved in another study investigating the relationship between cholesterol and CSF biomarkers of AD (Popp et al., 2013). In this study, in patients with AD, an association between CSF cholesterol, desmosterol, that is, an intermediate of cholesterol synthesis, and CSF ptau was found and not with CSF abeta levels. Just as described earlier for the association between *SORL1* and CSF abeta, also regarding the association between *SORL1* and CSF (p)tau, conflicting result exists. In 2 recent GWAS for CSF tau and ptau levels in AD patients, no associations with *SORL1* were detected (Cruchaga et al., 2013; Ramirez et al., 2014). For the first study, the difference could be explained by the characteristics of the sample, because not only AD patients were included but also patients with mild cognitive impairment and healthy control subjects. Moreover, it seems that the effect of *SORL1* may be too subtle to rise above the noise in a genome-wide analysis. For the second study, in which a GWAS was performed with 363 AD patients from DCN and ADNI in the original sample set and 515 AD patients from ADC in the replication sample set, a substantial overlap between data used in the GWAS and in our present study

Table 4
Haplotype-based analyses with haplotype rs668387-rs689021-rs641120 and haplotype rs11218340-rs3824966-rs3824968

AD marker	Haplotype rs668387-rs689021-rs641120	Beta	Haplotype rs11218340-rs3824966-rs3824968	Beta
MMSE	ATT	0.04	AGA	−0.11
	GCC	−0.03	ACT	0.09
ADAS	ATT	0.04	AGA	−0.13
	GCC	−0.06	ACT	0.07
Abeta	ATT	−0.05	AGA	0.04
	GCC	0.06	ACT	−0.03
Ptau	ATT	0.04	AGA	0.15*
	GCC	−0.05	ACT	−0.06
Tau	ATT	0.07	AGA	0.15*
	GCC	−0.07	ACT	−0.05
Hippocampus	ATT	0.03	AGA	−0.02
	GCC	−0.05	ACT	0.04

For haplotype rs668387-rs689021-rs641120, the haplotypes with frequencies above 10% are displayed: ATT = 45% and GCC = 54%.
For haplotype rs11218340-rs3824966-rs3824968, the haplotypes with frequencies above 10% are displayed: AGA = 23% and ACT = 66%.
The haplotype significantly associated with the endophenotype marker is marked with $^*p > 0.05$.
Key: AD, Alzheimer's disease; ADAS, Alzheimer's disease assessment scale; MMSE, Mini Mental State Examination; ptau, tau phosphorylated at threonine-181.

exists. We checked the results of this GWAS, and we found a trend of association for both rs2070045-G and rs3824966-G with CSF tau ($p = 0.06$) in the same direction as the associations found in present study between rs2070045-G and CSF tau and between the 3 SNP haplotype and CSF ptau.

Our study specifically designed to study the effect of *SORL1* on endophenotype markers suggests a role for *SORL1* in the more downstream pathways of AD pathology. This was further strengthened by the haplotype analysis, as the association between the 3 SNP haplotypes at the 3'end of the gene and CSF tau and ptau could be a reflection (and indirect a confirmation) of the effect of rs2070045, resulting from strong LD (D' = 1 and $r^2 = 1$) between rs2070045 and rs3824966.

Because most SNPs were situated in the intronic regions or synonymous variants (Table 2), the exact effect of the risk variants on clinical phenotype is more difficult to interpret. The significantly associated SNP rs2070045-G allele is a synonymous SNP. In a study with brain autopsy material from 88 confirmed AD patients, rs2070045-G allele was associated with poor receptor expression in the brain (Caglayan et al., 2012). Furthermore, the authors described an altered effect on transcript sequence with frequent to rare codon usage for serine. In addition, they are found in Chinese hamster ovary cell cultures, a less efficient translation of the minor receptor transcripts into proteins, resulting in decreased SORLA protein levels. So, although it is difficult to completely understand the effect of a synonymous SNP mutation, using functional assays more knowledge can be achieved on these variants.

4.1. Strength and limitations

Strength of our study is that we took an endophenotype approach, evaluating 6 different outcome measurements relevant for AD. Endophenotype markers reflect variation in expression of disease. By lying more closely to the effects of genetic variation, endophenotypes teach us more about the pathologic effect of the *SORL1* SNPs than a case-control design would. We included AD patients only, and as a result the observed relationships can reflect the influence of *SORL1* on heterogeneity in manifestation of disease only, rather than risk of disease. The selected SNPs were available from 2 distinctive haplotype blocks in the 5′ and 3′ gene regions found to be associated with the risk of AD in earlier studies (Reitz et al., 2011; Rogaeva et al., 2007). We analyzed 3 independent cohorts with totally 674 AD patients and controlled for the effect of multiple testing by using the FDR.

Among the limitations of our study is the use of different platforms for measurement of the endophenotype markers, which we attempted to solve by calculating z-scores to enable pooled analysis. There was considerable variation in observed associations between the 3 data sets, which could be the result of allelic heterogeneity, that is, multiple different risk alleles in the same gene can cause a similar genotype (Scriver, 2005). These findings are in line with 2 meta-analyses, which provided evidence that multiple *SORL1* alleles in distinct LD blocks are associated with AD risk but also conclude that the *SORL1* SNPs only account for a modest degree of the genetic variance of AD, with difficulty in replication (Reitz et al., 2011; Reynolds et al., 2010). Two other limitations were in particular, the failure of genotyping rs2070045 and rs1133174 in the ADC sample, and in general, the availability of a relatively small amount of exonic SNPs with nonsynonymous effect, making it more difficult to predict functional effect of the risk variants. For future studies, we intend to investigate the influence of more exonic and rare *SORL1* SNPs on Alzheimer's disease pathology in AD patients.

Our study confirms the relationship between *SORL1* and AD endophenotypes. In particular, we found that in the spectrum of AD dementia, SORL1 seems to influence neuronal injury (increased CSF tau) and neurodegeneration (more hippocampal atrophy), suggesting that *SORL1* SNPs mainly influence the more downstream aspects of the cascade of events leading to Alzheimer dementia.

Disclosure statement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2014.12.007.

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