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A Genetic Variant of the Sortilin 1 Gene is Associated with Reduced Risk of Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is a neurodegenerative disorder represented by the accumulation of intracellular tau protein and extracellular deposits of amyloid-β (Aβ) in the brain. The gene sortilin 1 (*SORT1*) has previously been associated with cardiovascular disease in gene association studies. It has also been proposed to be involved in AD pathogenesis through facilitating Aβ clearance by binding apoE/Aβ complexes prior to cellular uptake. However, the neuropathological role of *SORT1* in AD is not fully understood. To evaluate the associations between gene variants of *SORT1* and risk of AD, we performed genetic analyses in a Swedish case-control cohort. Ten single nucleotide polymorphisms (SNPs), covering the whole *SORT1* gene, were selected and genotyped in 620 AD patients and 1107 controls. The SNP rs17646665, located in a non-coding region of the *SORT1* gene, remained significantly associated with decreased risk of AD after multiple testing ($p_c = 0.0061$). In addition, other SNPs were found to be nominally associated with risk of AD, as well as altered cognitive function and the CSF biomarker $A\beta_{42}$, but these associations did not survive correction for multiple testing. The fact that *SORT1* has been strongly associated with risk of cardiovascular disease is intriguing as cardiovascular disease is also regarded as a risk factor for AD. Finally, increased knowledge about *SORT1* function has a potential to increase our understanding of *APOE*, the strongest risk factor for AD.

Keywords: Amyloid beta-peptides, apolipoprotein E, biomarkers, genetic association studies, genotype, neuropsychological tests, risk factors, single nucleotide polymorphism, tau proteins, vesicular transport adaptor proteins

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder and the most predominant form of dementia in the elderly population, with a prevalence of 10% in adults older than 65 years of age [1]. The disease is characterized by a gradual synaptic and neuronal loss that causes cognitive impairment with gradual progression into severe dementia with a terminal outcome. The histopathological hallmarks of AD include the accumulation of intracellular neurofibrillary tangles composed of hyperphosphorylated protein tau (P-tau) and extracellular deposits of amyloid-β (Aβ) in the brain of AD patients [1]. Today, a genetic background can partly explain the incidence of early-onset AD, also called familial AD (fAD), where the genes involved in Aβ production and processing contain mutations, namely amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) [2].

For late-onset, sporadic AD (sAD), which is the predominant form of AD, the *APOE ε4* allele has been shown to be the major genetic risk factor [3, 4]. ApoE is mainly produced by astrocytes and microglia in the central nervous system [5] where it delivers peptides to neurons for synaptic maintenance [6]. With regard to AD, the role of apoE is not fully understood. Although some evidence shows that apoE can bind to Aβ and facilitate cellular uptake of the peptide from the extracellular space into endosomes for catabolism [7, 8], the underlying molecular mechanisms are still unclear.

Sortilin 1 (referred to as sortilin here), is a transmembrane sorting receptor encoded by the *SORT1* gene. Sortilin is expressed both in neurons and non-neuronal cells and was first discovered in a screen for endocytic receptors [9]. In the brain, high levels of sortilin expression and immunoreactivity have been found in neuronal cell bodies and dendrites of allocortical areas such as the piriform cortex and hippocampus [10]. Sortilin was initially recognized as an intracellular transporter located in the trans-Golgi network [11], showing high similarities to the sorting receptor VPS10P in *Saccharomyces cerevisiae* [12]. The VPS10P domain of sortilin encompasses the extracellular part of the protein [13] and is shared among receptors in the VPS10P domain receptor family [14]. Sortilin is involved in complex trafficking patterns in which it can relocate from the cell surface and bind unrelated ligands, mediating transport to various cellular compartments including endosomes and lysosomes [15].

With regard to AD, sortilin has been proposed to be involved in the pathogenesis through several, sometimes contradictory, mechanisms. In cell studies, sortilin facilitates retrograde trafficking of the AβPP-cleaving enzyme beta secretase-1 (BACE-1) to Golgi and increases the cleavage of AβPP, stimulating Aβ production [16, 17]. Also, oligomerized Aβ has been shown to act as a ligand for sortilin, inducing endocytosis of Aβ and apoptosis [18]. Additionally, other experiments have revealed that sortilin also binds to and targets AβPP for lysosomal degradation, as well as promotes α-secretase cleavage of AβPP [19]. Studies in rodents demonstrate that sortilin is capable of binding extracellular apoE/Aβ complexes delivering them to lysosomes for degradation. Hence, rodents lacking the sortilin gene displayed increased apoE levels and Aβ plaque burden [20]. Furthermore, a significant increase in sortilin protein levels has been observed in postmortem brains has of patients with AD [21].

Single nucleotide polymorphisms (SNPs) within and in the vicinity of the *SORT1* gene have previously been associated with cardiovascular diseases (CVD) [22–27] and serum LDL levels [25, 28–33]. Also, altered *SORT1* mRNA expression [34] and increased serum levels of sortilin has been found in patients suffering from depression, where two SNPs within *SORT1* were significantly associated with increased serum levels of the protein [35].

Gene association studies have highlighted sortilin-related receptor 1 (*SORL1*), another member of the VPS10P domain receptor family, as a candidate gene for AD [36]. According to AlzGene [37], a database collecting published genetic association data in AD patients, no studies so far have shown any significant associations between gene variants of *SORT1* and the risk of developing AD. However, since preclinical findings indicate promising functional roles for sortilin in AD pathogenesis, we were intrigued to test whether *SORT1* was associated with AD in a clinical AD material. In the present study we report a case-control association study of *SORT1* in a Swedish cohort of AD patients and controls of Caucasian descent. The genetic variation in *SORT1* was tested against diagnosis, mini-mental state examination (MMSE) and characteristic cerebrospinal fluid (CSF) biomarkers for AD, i.e., total tau (T-tau), P-tau, and $A\beta_{42}$.

MATERIALS AND METHODS

Study cohort

The Swedish case-control study population consisted of 620 AD patients and 1,107 controls of Caucasian origin. AD patients were recruited from Piteå ($n = 182$) and Malmö ($n = 438$), whereas control patients were recruited from Malmö ($n = 433$) and Gothenburg (Mölndal) ($n = 674$). Demographics for the participants are found in Table 1. All diagnoses were set according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) criteria [38] following detailed clinical investigation including medical history, physical, neurological and psychiatric examination, screening laboratory tests, ECG, chest X-rays, EEG, and computerized tomography (CT) of the brain. No patient had a family history raising suspicion of familial AD, but *PSEN1*, *PSEN2*, and *APP* genes were not sequenced. Controls had no dementia and showed no signs of other psychiatric or neurological disorders including stroke, malignant disorders or infectious diseases. Controls with a Mini-Mental State Examination (MMSE) score lower than 28 were excluded from the study to reduce the risk of including controls with potential of developing AD. MMSE score data were available for 568 AD patients and 837 controls and were tested according to Folstein et al. [39]. The *APOE ε4* carrier status is presented in Table 1. Quantifications of AD-related CSF biomarkers (T-tau, P-tau, and $A\beta_{42}$) were available for a subset of the AD patients (T-tau $n = 493$, P-tau $n = 266$, and $A\beta_{42}$ $n = 481$). The diagnosis was set without prior knowledge of genetic background or biochemical analysis and vice versa.

Genotyping and DNA preparation

The sequence of *SORT1* (Gene ID: 6272) was obtained from the UCSC Genome Browser, assembly GRCh38/hg38 [40] and the HapMap project [41]. The software Haploview 4.2 was used to create a linkage disequilibrium (LD) block and to identify genetic variation (tag SNPs) in the gene. The TAGGER function in Haploview was used to select tag SNPs with the criteria of a minor allele frequency (MAF) ≥ 0.05 and a pairwise tagging was set to $r^2 = 0.8$.

Table 1
Demographics for Alzheimer's disease (AD) cases and controls

Parameter		AD	Control	*p*
Number of subjects	*n*	620	1107	
Age[1]	years	75.4 ± 7.3	71.8 ± 5.6	<0.001
Sex	male	243 (39.5)	372 (33.7)	<0.05
	female	372 (60.5)	733 (66.3)	
MMSE[2]		20 ± 5.6	29 ± 0.8	<0.001
Number of	0	186 (30.3)	774 (70.2)	
APOE ε4	1	317 (51.6)	298 (27.0)	<0.001
alleles	2	111 (18.1)	31 (2.8)	

Data are presented as absolute numbers with percentages in parentheses or as mean ± SD. *P*-values for categorical variables were calculated using chi-square statistical test and *p*-values for continuous variables were calculated using *t*-test. [1]$n_{AD} = 614$; $n_{Control} = 1104$. [2]$n_{AD} = 568$; $n_{Control} = 837$.

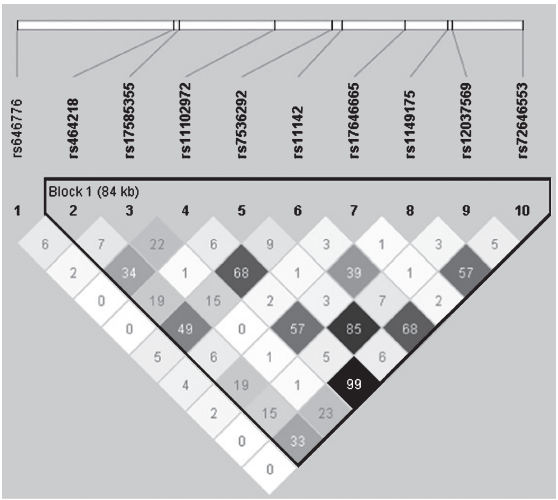

Fig. 1. Linkage disequilibrium (LD) plot for the ten selected SNPs within and in the vicinity of the *SORT1* gene. The correlation between SNPs is shown by their pairwise r^2 values indicated by numbers and greyscale. The plot was generated using Haploview software from the genotyping data in the cohort.

A regional linkage disequilibrium (LD) plot was created for the SNP rs17646665 using SNAP software [42], to localize SNPs in the vicinity of rs176 46665 which are in LD. The plot was created using the parameters: 1000 Genomes Pilot 1 dataset, CEU population panel, r^2 threshold set to 0.8 with a distance limit of 500.

In total, nine tag SNPs were chosen to fully cover the *SORT1* gene, forming one LD block (Fig. 1). In addition, the SNP rs646776 was also included in the study since it has been shown to regulate the expression of *SORT1* [28, 43] and has previously

been associated with LDL serum levels as well as cardiovascular implications [28, 43].

Genomic DNA was extracted from whole blood using GenoPrep™ DNA Blood and DNA Mag-Attract kit (Qiagen, Germany), together with the GenoMTM-48 Robotic Workstation (GenoVision, Norway) and stored in –20°C until it was used for analysis. Samples were sent to LGC Genomics [44] for sequencing using a KASP™ genotyping assay.

Cerebrospinal fluid analysis

Studies have shown that AD patients have decreased levels of $A\beta_{42}$ and increased levels of T-tau and P-tau in CSF compared with controls [45]. Levels of T-tau, P-tau, and $A\beta_{42}$ were therefore measured and used as biochemical markers in this study [46, 47]. CSF samples were obtained from AD patients by lumbar puncture in the L3/L4 or L4/L5 interspace as previously described [48]. T-tau and P-tau were determined using a sandwich ELISA (Innotest™ hTAU-Ag, Fujirebio, Ghent, Belgium) [46] and $A\beta_{42}$ levels were also measured in a sandwich ELISA (Innotest β-amyloid (1-42), Fujirebio, Ghent, Belgium) specifically constructed for $A\beta_{42}$ as described previously [47].

Statistical analyses

Controls and AD patients were compared using t-test for continuous parameters (MMSE, age, and CSF biomarkers) whereas Pearson chi-square test was used when categorical parameters (*APOE ε4* carrier status and sex) were analyzed. All SNPs were tested for deviation from Hardy-Weinberg equilibrium (HWE). SNP association to diagnosis was performed using logistic regression with an additive genetic model with sex and number of *APOE ε4* alleles as covariates. Single marker associations to continuous parameters (MMSE and CSF biomarkers) were only performed in the AD patient group and analyzed using linear regression with sex, age, and number of *APOE ε4* alleles as covariates. *P*-values <0.05 were considered statistically significant. A permutation test (set to 10 000 permutations) was performed for all SNPs to correct for multiple testing. Haplotype analyses were performed using a window size of 2-3. The statistical analyses in this study were carried out using IBM SPSS Statistics version 20, (New York, NY, USA) and Plink v1.07 [49].

Ethics

The clinical evaluation of all study participants was set without any prior knowledge of genetic background or any other biochemical analysis results, and vice versa. Participants (or their close relatives) gave their consent for participating in the study as well as for future results being published, which was directed in accordance with the provisions of the Helsinki Declaration. This study was approved by the ethical committees in Lund, Gothenburg and Umeå.

RESULTS

In this study, a total of 1727 samples were analyzed, including 620 AD patients and 1107 controls of Swedish, Caucasian descent (Table 1). When background parameters were compared, *APOE ε4* allele frequencies, sex, age, and MMSE were shown to differ between AD cases and controls. In the case of *APOE ε4* and MMSE, our results are expected and in line with prior knowledge that AD patients carry the *APOE ε4* allele more frequently and show lower MMSE scores. Regarding distribution differences in sex, a portion of the controls were recruited from a longitudinal population study of female participants which explains the skewed ratio.

We selected nine SNPs to cover the full *SORT1* gene, and also included a SNP located downstream of *SORT1* (Table 2 and Fig. 1), which has shown a strong association with LDL serum levels [25, 28, 30, 31, 33]. The genotyping of *SORT1* generated an average call rate of 98.4% for all selected SNPs in

Table 2
Summary of *SORT1* SNPs studied

SNP	Genomic position	Alleles	MAF (%)	SNP location
SORT1	Chromosome 1	minor:major		
rs646776	109275908	C:T	23.6	Near gene 3'
rs464218	109313684	G:A	46.5	3'UTR
rs17585355	109315193	C:A	6.3	Intron
rs11102972	109338099	C:T	23.0	Intron
rs7536292	109352071	C:T	17.4	Intron
rs11142	109354481	A:G	30.2	Coding syn.
rs17646665	109369429	G:A	6.9	Intron
rs1149175	109379755	A:G	14.5	Intron
rs12037569	109381055	T:G	15.2	Intron
rs72646553	109397881	C:G	22.4	Coding syn.

Presented are single-nucleotide polymorphisms (SNPs) numbered according to the gene location. Genome positions were obtained from the NCBI genome database. MAF, minor allele frequency, presented as percentages for each SNP. UTR, untranslated region. Coding syn., Coding synonymous.

this study. Genotype frequencies were in agreement with HWE for both AD patients and controls. The MAFs ranged from 0.063 to 0.47 which was in the same range as the published HapMap data for the same SNPs in other Caucasian cohorts [41].

Next, we used the genotyping data to investigate possible associations between *SORT1* and the risk of AD (Table 3). When only the SNPs in *SORT1* were used in the calculations for association to AD, i.e., without any covariates in the analysis, the SNPs rs17646665 ($p = 0.0034$) and rs72646553 ($p = 0.014$) were both nominally associated with a reduced risk for AD. When the identified covariates for this population (sex and number of *APOE ε4* alleles) were included in the analysis, rs72646553 was no longer significantly associated with diagnosis ($p_{adj} = 0.052$) (Table 3). However, rs17646665 remained strongly significantly ($p_{adj} = 0.00063$) associated with risk of AD (Table 3). The association also survived a multiple testing correction ($p_c = 0.0061$).

Since the patients originated from different regions in Sweden, there is a risk that the association would be the result of regional genetic differences. To evaluate this possibility, we performed an analysis of rs17646665 in the Malmö/Lund population only where both controls and AD patients were available. This analysis showed the same association with risk of AD (OR_{adj} (95% CI) = 0.5 (0.3–0.8); $p_{adj} = 0.0015$) as our analysis of the whole population. This indicates that the association was not due to regional genetic differences in the populations studied. A haplotype analysis was also performed although the significant associations found did not add anything beyond the association of the SNP associated with the disease, rs17646665.

To further investigate genes that could be related to our gene variant of *SORT1*, we created a regional LD plot from available CEU population panel data for rs17646665 to localize SNPs in its vicinity, which are in LD (Fig. 2). According to the regional LD plot, it is clear that no other SNP within the *SORT1* gene are in strong LD with rs17646665. In total, six SNPs were identified ($r^2 > 0.8$) and localized in or near the genes synaptophysin-like 2 (*SYPL2*; rs2272272), adhesion molecule with Ig-like domain 1 (*AMIGO1*; rs17575427), 5' of G protein-coupled receptor 61 (*GPR61*; rs552101), between *AMIGO1* and *GPR61* (rs56018934), guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3 (*GNAI3*; rs1279195) and glutathione S-transferase mu 4 (*GSTM4*; rs650985). This indicates that these SNPs are also likely to be associated with risk of AD.

Finally, all SNPs in this study were also analyzed in relation to MMSE scores and levels of CSF biomarkers T-tau, P-tau, and $A\beta_{42}$ in the AD patients. For MMSE, the minor allele of rs7536292 (adjusted slope (β_{adj}) = 0.85, $p_{adj} = 0.044$) was nominally significantly associated with a higher MMSE score, although the results did not survive a multiple testing correction ($p_c = 0.26$).

In the case of $A\beta_{42}$, the two SNPs rs646776 ($\beta_{adj} = 26.60$, $p_{adj} = 0.031$) and rs72646553 ($\beta_{adj} = -33.07$, $p_{adj} = 0.010$) were both nominally associated with altered levels of this biomarker. The minor allele of rs646776 was associated with higher levels of $A\beta_{42}$, whereas rs72646553 showed a reverse trend, with a minor allele associated with lower $A\beta_{42}$ levels. These associations were however not significant after a correction (rs646776 $p_c = 0.20$; rs72646553 $p_c = 0.082$). Regarding T-tau and P-tau, no SNPs were found to be associated with levels of the two biomarkers. A haplotype analysis was also performed, but no significant associations were found.

DISCUSSION

The main objective of this study was to investigate possible associations between variants of the *SORT1* gene and the risk of AD. In addition, in the AD cohort, the SNPs were also tested for associations to the AD CSF biomarker levels, as well as MMSE scores, with the latter serving as a measure for cognitive function. To this end, SNPs selected in this study consisted of both new tag SNPs as well as SNPs previously investigated for association to AD or other diseases (Table 2). Our results showed that the minor allele of rs17646665 was strongly associated ($p_{adj} = 0.00063$; $p_c = 0.0061$) with a reduced risk of AD. The association thus remained significant after adjusting for relevant covariates (sex and number of *APOE ε4* alleles) and correcting for multiple testing. To evaluate this association further, future replications in Swedish and other populations are warranted.

As the SNP rs17646665 has not previously been associated with any disease, we were interested in studying it further. According to the regional LD plot created for rs17646665 (Fig. 2), it is clear that no other SNPs within the *SORT1* gene are in LD with rs17646665. The six SNPs in LD with rs17646665 were localized in or near the genes *SYPL2* (rs2272272), *AMIGO1* (rs17575427), 5' of *GPR61* (rs552101), between *AMIGO1* and *GPR61* (rs56018934), *GNAI3* (rs1279195) and *GSTM4*

Table 3
SORT1 single marker (SNP) frequencies and associations with risk of Alzheimer's disease

SNP	Genotype	AD (%)	Control (%)	OR_{adj} (95% CI)	p_{adj}
rs646776	CC	27 (4.4)	70 (6.4)		
	CT	221 (36.2)	388 (35.5)	1.0 (0.8–1.2)	0.81
	TT	362 (59.3)	636 (58.1)		
rs464218	GG	124 (20.3)	254 (23.1)		
	GA	308 (50.5)	527 (47.9)	1.0 (0.8–1.2)	0.88
	AA	178 (29.2)	319 (29.0)		
rs17585355	CC	2 (0.3)	3 (0.3)		
	CA	64 (10.4)	142 (12.9)	0.8 (0.6–1.2)	0.27
	AA	552 (89.3)	952 (86.8)		
rs11102972	CC	33 (5.5)	59 (5.4)		
	CT	192 (31.7)	405 (37.2)	0.9 (0.7–1.1)	0.15
	TT	380 (62.8)	626 (57.4)		
rs7536292	CC	23 (3.8)	30 (2.7)		
	CT	177 (29.0)	311 (28.3)	1.2 (0.9–1.4)	0.17
	TT	410 (67.2)	757 (68.9)		
rs11142	AA	50 (8.3)	99 (9.3)		
	AG	239 (39.8)	466 (44.0)	0.9 (0.7–1.1)	0.17
	GG	311 (51.8)	494 (46.6)		
rs17646665	GG	0 (0.0)	7 (0.6)		
	GA	64 (10.5)	159 (14.5)	0.6 (0.4–0.8)	**0.00063**
	AA	547 (89.5)	930 (84.9)		
rs1149175	AA	17 (2.8)	25 (2.3)		
	AG	133 (21.6)	279 (25.4)	0.9 (0.7–1.1)	0.35
	GG	466 (75.6)	796 (72.4)		
rs12037569	TT	14 (2.3)	24 (2.2)		
	TG	158 (25.9)	285 (26.0)	1.0 (0.8–1.3)	0.75
	GG	437 (71.8)	786 (71.8)		
rs72646553	CC	33 (5.6)	58 (5.4)		
	CG	169 (28.7)	398 (36.7)	0.8 (0.7–1.0)	0.05
	GG	386 (65.6)	628 (57.9)		

Genotype data are presented as absolute numbers (percentages). OR, odds ratio per minor allele; CI, confidence interval; OR_{adj}, adjusted odds ratio; p_{adj}, adjusted *p*-value using covariates. *P*-values were calculated using a logistic regression model adjusted for sex and number of *APOE ε4* alleles. Bold numbers indicate *p*-values <0.05.

(rs650985). This indicates that variants in these genes have the potential to be involved in the pathogenesis of AD. Interestingly, *AMIGO1* has been shown to be involved in both neuronal development and survival, with a potential correlation to $A\beta_{42}$ [50, 51]. In addition, analysis of rs17646665 in Genotype-Tissue Expression (GTEx) databases at the GTEx Portal [52] revealed that this SNP is associated with reduced *SYPL2* expression in many different tissues. Moreover, *SYPL2* (also known as mitsugumin 29) expression has been identified in astrocytes around Aβ plaques in AD brains [53].

For AD patients, genetic variations in *SORT1* were also investigated with regard to characteristic AD CSF biomarkers, namely T-tau, P-tau and $A\beta_{42}$, as well as MMSE scores. While rs17646665, which was associated with risk of AD, showed no significant association to any of the CSF biomarkers, rs646776 and rs72646553 were nominally associated with CSF levels of $A\beta_{42}$. The two SNPs had different effects on $A\beta_{42}$ levels, with the minor allele of rs646776 being associated with increased CSF $A\beta_{42}$ levels, whilst the minor allele of rs72646553 caused an opposing outcome. This is in line with the LD pattern between the two SNPs.

The SNP rs646776 is located in a non-coding DNA region in-between the genes cadherin EGF LAG seven-pass G-type receptor 2 (*CELSR2*) and proline and serine rich coiled-coil 1 (*PSRC1*), where the minor allele has been associated with increase in expression of *SORT1* [43]. This is in agreement with the hypothesis that increased sortilin levels are protective against $A\beta_{42}$ accumulation by facilitating uptake and degradation of apoE/Aβ complexes [20], as increased CSF levels of $A\beta_{42}$ correspond to lower $A\beta_{42}$ aggregation in the brain [47].

The SNP rs72646553 is localized in the first exon of *SORT1* where the genetic variation generates a synonymous mutation. Data from the GTEx portal indicate that the minor allele is associated with a slight increase in expression of *SORT1* that could affect $A\beta_{42}$ metabolism. However, in the present

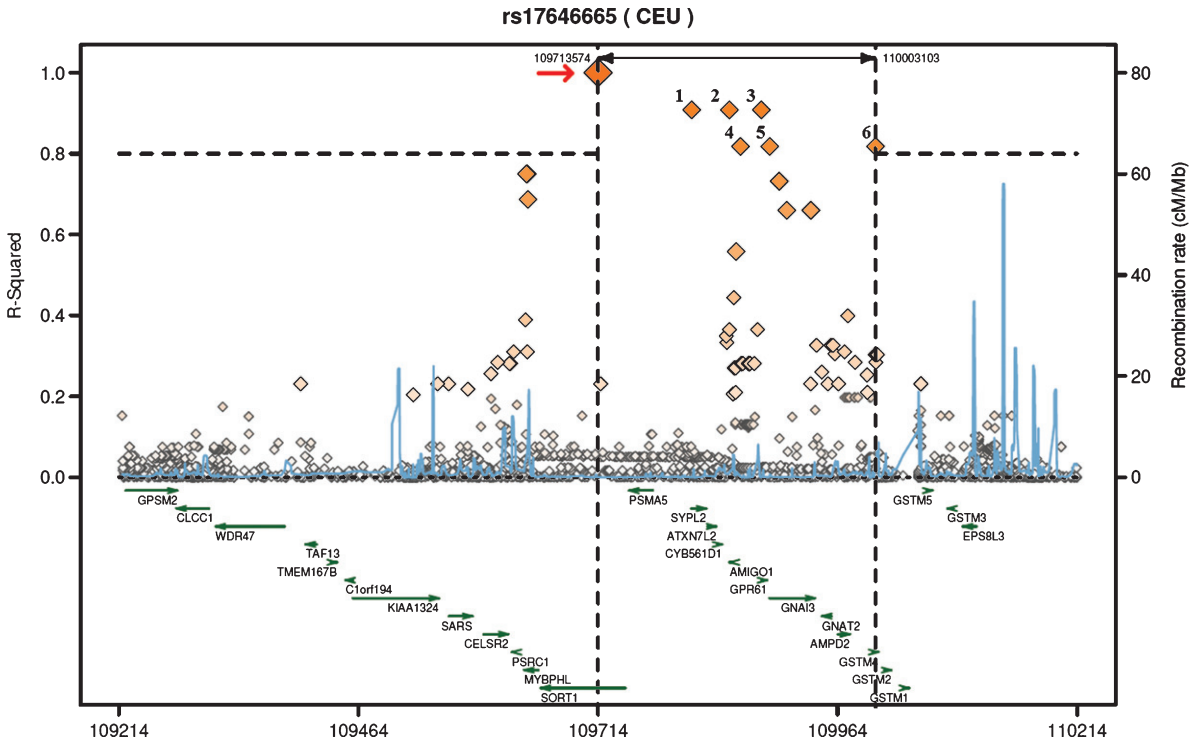

Fig. 2. The regional LD plot was created from a published CEU population panel for rs17646665, located in *SORT1* (marked by the arrow) with 500 kb flanking genomic regions on each side. The r^2 threshold was set to 0.8 and six SNPs were identified with $r^2 > 0.8$ for this region, all of which were located in other genes than *SORT1*. The SNPs are indicated by the numbers over the boxes (1: *SYPL2* (rs2272272), 2: *AMIGO1* (rs17575427), 3: 5' of *GPR61* (rs552101), 4: between *AMIGO1* and *GPR61* (rs56018934), 5: *GNAI3* (rs1279195) and 6: *GSTM4* (rs650985)).

study, rs72646553 was both nominally associated with decreased $A\beta_{42}$ CSF levels, indicative of AD, but it was also nominally associated with reduced risk of AD. It is therefore difficult to draw any conclusions from this finding. Although the results were nominally significant, neither of the SNPs associated with $A\beta_{42}$ levels survived correction for multiple testing.

For MMSE, the minor C allele of another SNP, rs7536292, was nominally associated with higher MMSE scores, but this association did not remain significant after multiple testing correction. None of the SNPs were associated with T-tau or P-tau, neither before nor after correction. This would be logical as unaltered P-tau levels has been reported in *Sort1(-/-)* mice crossed with an AD model strain [54].

When comparing with previous studies, all SNPs have previously been analyzed in different AD populations, in relation to sAD [55–59], fAD [36], or dementia [60]. In the latter study, Reynolds et al. found rs17585335 to be nominally associated with risk of dementia, but the authors concluded that the association was not convincing enough [60]. Zeng et al. investigated a possible association of *SORT1* and AD in a Han Chinese population where none of the tested SNPs showed any significant result [56]. Indeed, two of the SNPs in the study (rs646776 and rs464218) were also included in our study, confirming no associations with AD. On the other hand, the SNP rs17646665 that our study highlighted as strongly associated with a lower risk of AD, was not included in theirs and this SNP shows a very low LD to the tested SNPs in the Han Chinese study, which might explain the negative outcome. Additionally, we assume that a genetic variation may exist between cohorts from different regions, i.e., when comparing Han Chinese and European Caucasians. Moreover, rs17646665 has previously been included in a large scale study where several members of the VPS10P domain receptors family were tested for association with AD [55], but no associations were found for rs17646665 in this study or in previous GWAS analyses [57–59]. Due to differences in reported studies and our material, it is possible that the association in our cohort could be restricted to a Swedish/Scandinavian population.

CVD and serum lipid levels are two of the major risk factors for developing AD [61], and as previously described, *SORT1* has been shown to be associated with both serum lipid levels and CVD. In other words, *SORT1* might play an important role in explaining the underlying relation between CVD, serum lipid levels, and AD. In the present study, we have indeed found an association between *SORT1* and the risk of AD. To expand our knowledge in this field, we need to further investigate how *SORT1* is linked to the disease by exploring underlying mechanisms. We suggest that future research will explore measurements of AD CSF biomarkers in combination with biomarkers for CVD and serum lipid levels, since this combined may generate useful information and knowledge regarding the link between CVD and AD, which would be beneficial for future drug development. We also suggest that pharmacological tools that modulate the uptake and clearance of apoE-Aβ-sortilin complexes could serve as future therapeutics for AD.

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