Impact of SORL1 Single Nucleotide Polymorphisms on Alzheimer’s Disease Cerebrospinal Fluid Markers

Panagiotis Alexopoulos  Liang-Hao Guo  Martina Kratzer  Christine Westerteicher  Alexander Kurz  Robert Perneczky  
Alzheimer’s Disease Neuroimaging Initiative  
Department of Psychiatry and Psychotherapy, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Key Words
Dementia  Mild cognitive impairment  Healthy aging  Amyloid cascade  Association

Abstract

Background: Recently, genetic variants of the neuronal sortilin-related receptor with A-type repeats (SORL1, also called LR11 or sorLA) have emerged as risk factors for the development of Alzheimer’s disease (AD). Methods: In this study, SORL1 gene polymorphisms, which have been shown to be related to AD, were analyzed for associations with cerebrospinal fluid (CSF) amyloid beta1–42 (Aβ1–42), phosphorylated tau181, and total tau levels in a non-Hispanic Caucasian sample, which encompassed 100 cognitively healthy elderly individuals, 166 patients with mild cognitive impairment, and 87 patients with probable AD. The data were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). Moreover, the impact of gene-gene interactions between SORL1 single nucleotide polymorphisms (SNPs) and the apolipoprotein E (APOE) ε4 allele, the major genetic risk factor for sporadic AD, on Aβ1–42 levels was investigated. Results: Significant associations between CSF Aβ1–42 levels and the SORL1 SNPs 23 (rs3824968) and 24 (rs2282649) were detected in the AD group. The latter association became marginally statistically insignificant after Bonferroni correction for multiple comparisons. Carriers of the SORL1 SNP24 T allele and the SNP23 A allele both had lower CSF Aβ1–42 concentrations than non-carriers of these alleles. The analysis of the impact of interactions between APOE ε4 allele and SORL1 SNPs on CSF Aβ1–42 levels unraveled significant influences of APOE. Conclusions: Our findings provide further support for the notion that SORL1 genetic variants are related to AD pathology, probably by regulating the amyloid cascade.

Copyright © 2011 S. Karger AG, Basel

Accepted: August 3, 2011  
Published online: October 13, 2011

P.A. and L.-H.G. contributed equally to this work.  
Data used in preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data, but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.ucla.edu/research/active-investigators.
Introduction

The causes of late-onset Alzheimer’s disease (AD) are multifactorial and complex [1]. Twin studies suggest that around 37–78% of the variance in age at onset of clinical AD can be explained by additive genetic effects [1]. In recent years, the gene encoding the neuronal sortilin-related receptor with A-type repeats (SORL1, also called LR11 or sorLA) has emerged among others as a candidate genetic risk factor for AD [2]. It is located on chromosome 11q23.2–q24.2 and encodes a membrane protein which is specifically expressed in neurons. Several studies have replicated the initial observation of the genetic association between SORL1 and AD [3–13]. Nonetheless, no general consensus on the role of SORL1 genetic variants as risk factors for AD exists, since other investigations only found weak or no associations between SORL1 genetic variants and AD [14–19]. Furthermore, the detected allelic associations varied across studies and the impact on AD risk were only modest with odds ratios ranging from 1.4 to 2.2 [1]. However, a recent meta-analysis of all available data derived from studies including individuals of Caucasian or Asian origin confirmed that variants in the SORL1 gene are related to risk for AD [20].

SORL1 is a member of the apolipoprotein E (APOE) and low-density lipoprotein receptor family; it is diffusely expressed throughout the brain and acts as an intracellular sorting receptor that engages in the Golgi apparatus-endosome transport [21]. SORL1 is thought to be crucially involved in the sorting of amyloid precursor protein (APP) and in its interactions with secretases [22, 23]. Low levels of SORL1 lead to overproduction of amyloid beta (Aβ) [2]. Interestingly, it has been reported that in patients with AD the expression of SORL1 is decreased in neurons [24, 25]. Attempting to unravel possible associations between SORL1 gene variants and biomarkers [26] of AD is a challenging task that may offer a meaningful contribution to our understanding of AD pathogenesis. Due to the role of SORL1 in the processing of APP, we explored possible associations between sequence variations within SORL1 and established cerebrospinal fluid (CSF) markers of amyloid pathology (Aβ1–42) and axonal degeneration (total tau, tTau; tau phosphorylated at threonine 181, pTαu181) in a large sample of patients with probable AD, mild cognitive impairment (MCI), and cognitively healthy control subjects. Additionally, the impact of sequence variations within SORL1 on Aβ1–42 levels in CSF was investigated in association with the presence of an APOE e4 allele, since APOE e4 constitutes the major genetic predisposition factor for the development of late-onset AD [27] and since SORL1 levels in CSF are particularly increased in patients with AD carrying the APOE e4 allele [28].

Materials and Methods

The data used in this study were obtained on September 9, 2010, from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). 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 non-profit organizations as a USD 60 million 5-year public-private partnership. The primary goal of ADNI has been to explore whether serial MRI, PET, other biological markers, and clinical and neuropsychological data can be combined to assess the progression of MCI and early AD. The determination of sensitive and specific markers of very early AD progression is intended to support researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and costs of clinical trials. The principal investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California San Francisco, USA. ADNI is the result of a broad collaboration of academic institutions and private corporations. Subjects have been recruited from over 50 sites across the USA and Canada. The initial goal of ADNI was to recruit 800 adults aged 55–90 years to participate in the research: approximately 200 cognitively normal older individuals to be followed for 3 years; 400 people with MCI to be followed for 3 years; and 200 people with early AD to be followed for 2 years. Detailed information on ADNI can be found in previous publications and at www.adni-info.org. The study was approved by the institutional review boards of all participating centers and written informed consent was obtained from all participants or authorized representatives after extensive description of ADNI.

Baseline CSF samples were obtained from 416 ADNI subjects and analyzed at the ADNI biomarker core laboratory at University of Pennsylvania; the detailed sampling methods have been described previously [29]. The CSF concentrations of Aβ1–42, tTau, and pTαu181 were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, Tex., USA) with Innogenetics immunoassay kit-based reagents (INNO-BIA AlzBio 3; Ghent, Belgium; for research use-only reagents). From 416 samples, 410 passed quality control and an additional subject later failed ADNI screening, resulting in 409 valid CSF samples. This sub-sample is comparable to the entire ADNI cohort regarding demographic, clinical, and APOE genotyping results.

Single nucleotide polymorphism (SNP) genotyping for more than 620,000 target SNPs was performed on all ADNI participants according to published protocols [29]. Genomic DNA samples were analyzed using the Human 610-Quad BeadChip (Illumina Inc., San Diego, Calif., USA) according to the manufacturer’s instructions (Infinium HD Assay; Super Protocol Guide; rev. A, May 2008). SNP genotypes were generated in Illumina BeadStudio software v3.2 from bead intensity data. The previously reported most significant SORL1 SNPs for AD were selected from the literature [1, 20]. These markers included rs661057 (SNP4), rs668387 (SNP8), rs689021 (SNP9), rs641120 (SNP10), rs2070045 (SNP19), rs2070045 (SNP19), and rs668387 (SNP8) were analyzed using the Human 610-Quad BeadChip (Illumina Inc., San Diego, Calif., USA) according to the manufacturer’s instructions (Infinium HD Assay; Super Protocol Guide; rev. A, May 2008). SNP genotypes were generated in Illumina BeadStudio software v3.2 from bead intensity data. The previously reported most significant SORL1 SNPs for AD were selected from the literature [1, 20]. These markers included rs661057 (SNP4), rs668387 (SNP8), rs689021 (SNP9), rs641120 (SNP10), rs2070045 (SNP19),
rs1699102 (SNP22) and rs3824968 (SNP23), rs2282649 (SNP24) and rs1010159 (SNP25). SNP23 and SNP24 are not available in the ADNI database. Therefore, they were genotyped at Washington University St. Louis as part of genome-wide association studies [30]. The present analysis was restricted to non-Hispanic Caucasians, who were identified in the clinical database and whose genotype data of 

**Table 1. Characteristics of the study sample**

<table>
<thead>
<tr>
<th>Patients, n</th>
<th>Control group</th>
<th>MCI group</th>
<th>AD group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>75.75 ± 5.32</td>
<td>74.98 ± 7.41</td>
<td>74.84 ± 7.52</td>
</tr>
<tr>
<td>Men:women</td>
<td>50:50</td>
<td>114:52</td>
<td>50:37</td>
</tr>
<tr>
<td>MMSE score</td>
<td>29.04 ± 1.06</td>
<td>26.93 ± 1.81</td>
<td>23.49 ± 1.93</td>
</tr>
<tr>
<td>APOE e4 carriers, n</td>
<td>5</td>
<td>92</td>
<td>58</td>
</tr>
<tr>
<td>CSF Aβ42, ng/l</td>
<td>205.46 ± 55.76</td>
<td>162.45 ± 54.38</td>
<td>144.34 ± 2.90</td>
</tr>
<tr>
<td>pTau181, ng/l</td>
<td>25.27 ± 15.21</td>
<td>36.20 ± 18.19</td>
<td>42.46 ± 20.54</td>
</tr>
<tr>
<td>tTau, ng/l</td>
<td>69.82 ± 31.00</td>
<td>104.39 ± 59.78</td>
<td>123.01 ± 58.89</td>
</tr>
<tr>
<td>SNP4 (rs661057) TT/CT/CC</td>
<td>32/48/20</td>
<td>62/75/29</td>
<td>36/36/15</td>
</tr>
<tr>
<td>SNP8 (rs668389) CC/CT/TT</td>
<td>27/46/27</td>
<td>60/78/28</td>
<td>40/35/12</td>
</tr>
<tr>
<td>SNP9 (rs689021) GG/AG/AA</td>
<td>26/45/29</td>
<td>59/79/28</td>
<td>37/39/11</td>
</tr>
<tr>
<td>SNP10 (rs641120) TT/CT/CC</td>
<td>27/42/31</td>
<td>70/76/26</td>
<td>47/53/13</td>
</tr>
<tr>
<td>SNP19 (rs2070045) TT/AT/AA</td>
<td>61/34/5</td>
<td>110/48/8</td>
<td>59/26/2</td>
</tr>
<tr>
<td>SNP22 (rs1699102) TT/CT/CC</td>
<td>32/48/20</td>
<td>70/78/28</td>
<td>40/35/12</td>
</tr>
<tr>
<td>SNP23 (rs3824968) CC/CT/TT</td>
<td>49/41/9</td>
<td>17/80/69</td>
<td>49/22/5</td>
</tr>
<tr>
<td>SNP25 (rs1010159) TT/CT/CC</td>
<td>43/43/14</td>
<td>69/80/17</td>
<td>43/39/5</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD, unless otherwise indicated.

Regarding the statistical analysis, a stepwise discriminant analysis, employing multiple linear regression models in PASW software v17 (SPSS Inc., Chicago, Ill., USA), was used to identify potential significant covariates for CSF (tTau, pTau181, and Aβ1–42) levels. The potential confounding variables that were tested were age, gender distribution, Mini Mental State Examination (MMSE) scores and the presence of the APOE e4 allele (dichotomized into carriers and non-carriers of the allele). Subsequently, separate linear regression analysis models with the CSF parameters as dependent variables were built to assess the impact of SORL1 SNPs on the neurodegeneration parameter concentrations after adjustment for the appropriate covariates. In order to unravel the influence of possible gene-gene interactions between the aforementioned SORL1 SNPs and the APOE e4 allele on Aβ1–42 concentrations, the interaction parameter SORL1 SNP genotype × APOE e4 carriers/non-carriers was fed as the independent factor together with the significant covariates detected in the first step of the analysis into a linear regression analysis model with Aβ1–42 as the dependent factor. A Bonferroni correction for multiple comparisons was applied to the significance threshold of p < 0.05; this yielded a Bonferroni corrected p < 0.006. To compare the distributions of the dependent variables with the normal distribution, normal p-p plots of regression standardized residuals were generated, which plot the cumulative proportions of standardized residuals of the dependent variable against the cumulative proportions of the respective normal distribution. The normality assumption was supported by these plots (results not shown).

### Results

Characteristics and SNP distributions of the sample are given in table 1. In the AD group, APOE (p < 0.001, n = 87), age (p = 0.02, n = 87), and gender (p = 0.04, n = 87) were associated with Aβ1–42, and age with pTau181 (p < 0.01, n = 87). In the MCI group, there was an association between APOE and pTau181 (p < 0.01, n = 166), APOE and Aβ1–42 (p < 0.01, n = 166), as well as APOE (p < 0.01, n = 166) and gender (p = 0.02, n = 166) with tTau. In the control group, APOE was correlated with Aβ1–42 (p < 0.001, n = 100) and tTau (p = 0.02, n = 100), as well as APOE (p < 0.01, n = 100) and age (p = 0.02, n = 100) with pTau181. The separate multivariate variance analyses yielded, after Bonferroni correction for multiple comparisons, a significant association between CSF Aβ1–42 and the A allele of the SORL1 SNP23 (p = 0.003, n = 87) in the AD group. SORL1 SNP23 A allele carriers had lower CSF Aβ1–42 concentrations than non-carriers (carriers vs. non-carriers: mean ± SD, 131.77 ± 35.65 vs. 154.56 ± 45.85 ng/l; fig. 1). Interestingly, the presence of
the SORL1 SNP24 T allele was also significantly associated with CSF Aβ1–42 levels in patients with AD (p = 0.007, n = 87). However, this association marginally failed to survive the Bonferroni correction. In carriers of the SORL1 SNP24 T allele, lower CSF Aβ1–42 concentrations were detected (carriers vs. non-carriers: 127.76 ± 25.74 vs. 157.20 ± 49.00 ng/l; fig. 1). Furthermore, SORL1 SNP8 genotypes (p = 0.04, n = 87) and SNP25 genotypes (p = 0.03, n = 87) were associated with CSF Aβ1–42 levels. Nonetheless, these associations did not remain statistically significant after Bonferroni correction. Unexpectedly, such a trend was also observed in the group of patients with MCI between pTau181 and SORL1 SNP24 genotypes (p = 0.03, n = 166), which did not reach statistical significance after Bonferroni correction. No further associations were detected between SORL1 SNPs and CSF protein concentrations in any of the three study groups.

In line with the literature, the presence of the APOE ε4 allele was associated with lower CSF Aβ1–42 concentrations in all three study groups (p < 0.001 for all groups). The interactions between the APOE ε4 allele and SORL1 SNP23 genotypes (p = 0.001, n = 87), SNP24 genotypes (p = 0.004, n = 84), SNP25 genotypes (p = 0.009, n = 87), SNP8 genotypes (p = 0.03, n = 87), and SNP9 genotypes (p = 0.04, n = 87) were found to exert significant influences on CSF Aβ1–42 concentrations in patients suffering from AD. The influence of the former two interaction factors on Aβ1–42 remained statistically significant after Bonferroni correction. No further significant associations were observed.

Discussion

SORL1 is listed among the top 10 AD risk genes in the Alzgene.org database (accessed on February 6, 2011) [31]. In the present study, associations between variants of the SORL1 gene and established CSF biomarkers of AD pathology were investigated in patients with probable AD and MCI, as well as healthy elderly controls. The main finding of our study is that patients with probable AD carrying the SORL1 SNP24 A allele had lower levels of Aβ1–42 compared with non-carriers. Moreover, a marginal association was also detected between the presence of the SORL1 SNP24 T allele and Aβ1–42 in patients with probable AD. Other studied SORL1 SNPs tended to relate to altered levels of Aβ1–42 or pTau181. However, these associations did not survive Bonferroni correction.

A number of studies have tried to dig up biological evidence for a role of SORL1 in AD, suggesting an influence of SORL1 gene variants on AD endophenotypes. In contrast to our results, a study which derived its sample from the population-based Swedish Twin Registry [32] and an investigation partly using ADNI data [30, 33] both failed to detect associations between SORL1 SNPs and CSF biomarkers of AD. Three possible reasons might be responsible for this inconsistence. Firstly, the former study significantly differed from our study in terms of gender distribution within the AD group (χ² test, p < 0.001). Our analysis revealed that gender influenced the levels of Aβ1–42 in the AD group. This finding is in line with the previously reported association between SORL1 gene variants and gender [13] and with reports from AD transgenic animal models indicating an impact of gender on amyloid pathology [34]. Secondly, our study was restricted to individuals with a non-Hispanic Caucasian ancestry, whereas the Swedish Twin Registry Study comprised individuals drawn from the multiethnic Swedish society regardless of their origin. A recent meta-analysis on the association between variants in SORL1 and AD showed clear deviations in the AD associated SORL1 SNPs in the different ethnic groups [20]. Thirdly, in the referenced ADNI study [30], patients with probable AD and MCI as well as healthy controls were treated as a single group, and no separate analyses were performed in each of the three groups. As a consequence it is possible that the effect of SORL1 variants on Aβ1–42 in the group of patients with AD was masked by the absence of such effects in the rest of the sample. A German multicenter study, which was not restricted to non-Hispanic Caucasians, identified an association between Aβ1–42 and SORL1 SNP21 in 153 pa-

Fig. 1. CSF Aβ1–42 concentrations in relation to SORL1 SNPs 23 and 24 in the AS group (mean value indicated by horizontal line).
patients with AD [35]. Such an association could not be replicated in our study sample. In addition, it should be underscored that linking gene variants with discrete variations in biological markers is a challenging task. It is possible that the investigated genetic variants exert a direct influence on the biomarker levels, but it is also plausible that the genetic variation mediates an effect through other downstream functional change or through the regulation of other genes [36]. These caveats must be borne in mind when the observed influence of SORL1 genetic variants on Aβ1–42 is considered or deviations in study observations are interpreted.

The detected significant influence of SORL1 SNP23 A allele and SNP24 T allele on Aβ1–42 was restricted to patients suffering from AD and no association between SORL1 polymorphisms and CSF Aβ1–42 concentrations was observed in patients with MCI. Although the clinical entity of MCI represents in many cases a prodromal phase of AD, it is not exclusively caused by AD and it has a variable prognosis [37–38]. Since the diagnosis of MCI in our study was based on clinical criteria, the MCI group probably did not exclusively encompass patients with incipient AD in whom an association between SORL1 SNPs and Aβ1–42 could be expected. As a consequence it can be reckoned that the presence of the aforementioned alleles may foster alterations, for instance in SORL1 shedding or intracellular concentrations [22, 28, 39], which exclusively occur in patients with AD pathology.

Decreased CSF Aβ1–42 levels are generally found in AD and it has been reported that Aβ1–42 concentrations decrease with disease progression [35, 40], although not in all published studies [41]. Thus, it might be argued that reduced levels of Aβ1–42 in patients with AD possessing the SORL1 SNP24 T allele or the SORL1 SNP23 A allele are attributable to differences in the severity of amyloid pathology. However, in line with previous observations [35] no impact of MMSE scores, mirroring clinical disease severity, on CSF concentrations of Aβ1–42 was observed in our sample.

The revealed impact of gene-gene interactions between SORL1 genetic variants and the presence of the APOE e4 allele on Aβ1–42 provides further evidence for possible interactions between APOE and SORL1, which may affect the pathogenesis of AD. SORL1 binds multiple ligands including APOE and induces the endocytosis of APOE-containing lipoproteins [42]. Interactions between SORL1 and APOE might interfere with the formation of the APOE-Aβ complex, which has been detected in the CSF, and this process may foster the deposition of Aβ in brain by increasing unbound Aβ species [28].

The trend of SORL1 SNP24 to affect the levels of pTau181 in patients with MCI was unexpected since SORL1 has been shown to be implicated in the sorting of APP and in its interactions with the secretases [22] and not in the processes of hyperphosphorylation of tau. Though it cannot be ruled out with final certainty that this observation is due to a type I error, this finding is intriguing especially in the light of the absence of such an association in patients with AD. Further investigations are warranted, since SORL1 SNP24 may be involved in the interrelation between the amyloid cascade and the hyperphosphorylation processes of tau [43] or hypothetically through gene-gene interactions in the molecular mechanisms inducing tau hyperphosphorylation in patients suffering from pathologies other than AD (e.g. frontotemporal lobar degeneration, Lewy-body pathology), which also lead to the clinical entity of MCI.

Though relatively large for a CSF investigation, it can be claimed that the present study sample is of limited size. However, our findings are in line with previous publications, which reported that SORL1 exerts a relevant influence on amyloid metabolism and thus on AD risk and pathology [20–23]. Nonetheless, replication studies with independent larger samples are warranted.

To conclude, our findings show that SORL1 variants have a significant influence on brain amyloid pathology within the framework of AD. Therefore, our results provide further in vivo validation of SORL1 as a risk gene for AD and stress the need for subsequent studies to unveil its pathogenic and clinical relevance [44].

Acknowledgments

The study was supported by the Kommission für Klinische Forschung of the Klinikum rechts der Isar München (grant No. B06-09, B08-10). Data collection and sharing for this project was funded by the ADNI (National Institutes of Health grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Abbott, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corporation, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson and Johnson, Eli Lilly and Co., Medpace, Inc., Merck and Co., Inc., Novartis AG, Pfizer Inc., F. Hoffman-La Roche, Schering-Plough, Synarc, Inc., as well as non-profit partners including the Alzheimer’s Association and Alzheimer’s Drug Discovery Foundation, with participation from the US Food and Drug Administration. Private sector contributions to ADNI are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is co-

168 Dement Geriatr Cogn Disord 2011;32:164–170

Alexopoulos et al.
ordained by the Alzheimer’s Disease Cooperative Study at University of California San Diego. ADNI data are disseminated by the Laboratory for Neuroimaging at University of California Los Angeles. This research was also supported by NIH grants P30AG010129, K01 AG030514, and the Dana Foundation. The sponsors did not have 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.

The authors wish to thank John S.K. Kauwe, PhD, and Carlos Cruchaga, PhD, from the Department of Biology at Brigham Young University; Provo, Utah, and Alison M. Goate, DPhil, from the Department of Psychiatry at Washington University School of Medicine, St. Louis, Mo., for sharing some of the genotyping information used for this study, and for valuable discussion on the manuscript.

References


Impact of SORL1 SNPs on AD CSF Markers

Dement Geriatr Cogn Disord 2011;32:164–170


