Table 1
Characteristics of the study sample. Data presented as mean (SD)

| | MCI | AD | FTD |
|---|---|---|---|
| *n* | 76 | 61 | 17 |
| Age (in years) | 65.5 (9.4) | 66.9 (9.5) | 62.9 (6.2) |
| Gender (men/women) | 38/38 | 32/29 | 7-Oct |
| MMSE (standard deviation) [range] | 26.89 (2.08) [22–30] | 22.54 (2.86) [16–27]* | 24.18 (3.58) [17–29]# |
| Tau (ng/ml) | 405.18 (270.43) | 599.93 (360.25)* | 214.35 (103.00)#‡ |
| Soluble AβPPβ (ng/ml) | 1059.94 (479.68) | 836.27 (383.71)* | 203.71 (103.94)#‡ |
| SORL1 (ng/ml) | (*n* = 57), 11.92 (4.28) | (*n* = 42), 11.89 (4.74) | (*n* = 17) 10.38 (3.35) |
| Amyloid-$\beta_{1-42}$ (ng/ml) | 737.46 (333.37) | 536.49 (235.43)* | 934.12 (345.24)#‡ |

MCI: Mild cognitive impairment, AD: Alzheimer's disease, FTD: Frontotemporal dementia, MMSE: Mini- mental state examination, SORL1: Sortilin-related receptor with A-type repeats; *Statistically significant differences between the MCI and AD groups, $p<0.05$; #Statistically significant differences between the MCI and FTD groups, $p<0.05$; ‡Statistically significant differences between the AD and FTD groups, $p<0.05$.

possible differences in the relationship between tau and $A\beta_{42}$ and sAβPPβ between patients with MCI, developing on a neurodegenerative basis (>253 ng/L)

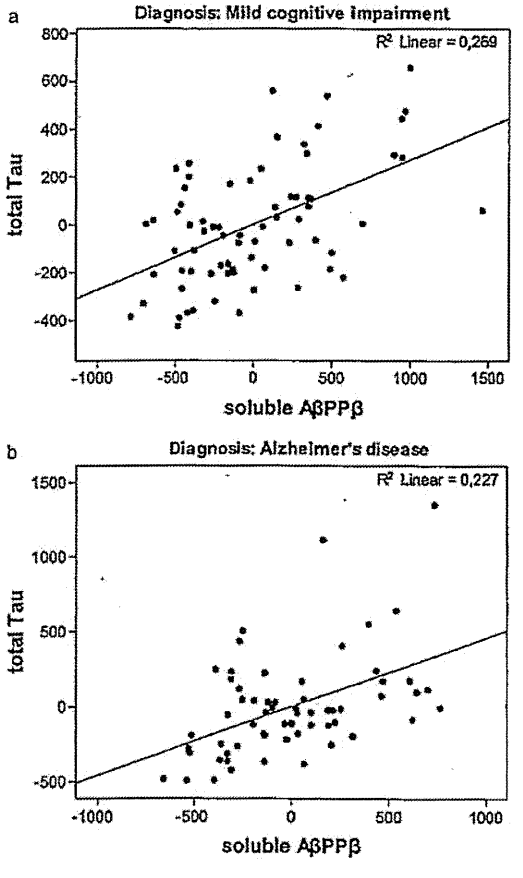


Fig. 1. Partial regression diagrams of total tau and soluble AβPPβ concentrations in cerebrospinal fluid in (a) patients with mild cognitive impairment and in (b) patients with Alzheimer's disease. Values are standardized and at zero centered.

[39] and those with non-degenerative MCI. The regression analysis with tau as dependent variable included sAβPPβ, $A\beta_{42}$, age, and gender as explanatory factors. The relations between CSF sAβPPβ and $A\beta_{42}$ and SORL1 concentrations were studied with linear regression models which included sAβPPβ or $A\beta_{42}$ as dependent factor and SORL1, age and gender as independent parameters. *P* values of less than 0.05 were considered statistically significant.

## RESULTS

Statistically significant differences across diagnostic groups regarding MMSE scores and CSF parameters were detected and are presented in Table 1. The linear regression analysis in the MCI group revealed statistically significant correlations of both sAβPPβ (standardized coefficient B = 0.486; $p<0.001$) (Fig. 1) and $A\beta_{42}$ (standardized coefficient B = −0.465; $p<0.001$) (Fig. 2) with tau, whereas neither age (standardized coefficient B = 0.083, $p=0.421$) nor gender (standardized coefficient B = −0.045; $p=0.648$) were associated with tau levels in CSF. Moreover, demographic, clinical, and biomarker data of the degenerative and non-degenerative MCI subgroups are presented in Table 2. The regression analysis with tau as dependent variable and sAβPPβ, $A\beta_{42}$, age, and gender as explanatory factors showed that tau correlated significantly in the degenerative MCI subsample with both $A\beta_{42}$ (standardized coefficient B = −0.506; $p=0.001$) and sAβPPβ (standardized coefficient B = 0.370; $p=0.008$). Unexpectedly, in the non-degenerative MCI subsample tau did positively correlate with CSF $A\beta_{42}$ (standardized coefficient B = 0.617; $p=0.004$), while sAβPPβ did not (standardized coefficient B = 0.201; $p=0.34$). In patients with AD, sAβPPβ (standardized coefficient B = 0.487; $p<0.001$) (Fig. 1) was

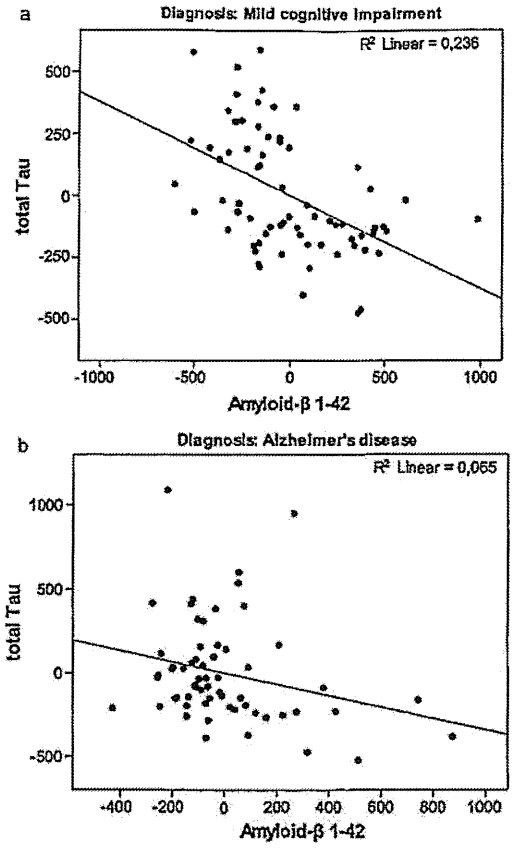


Fig. 2. Partial regression diagrams of total tau and amyloid-β 1-42 concentrations in cerebrospinal fluid in (a) patients with mild cognitive impairment and in (b) patients with Alzheimer's disease. Values are standardized and at zero centered.

significantly associated with tau. The association between $A\beta_{42}$ and tau strongly tended to be statistically significant (standardized coefficient B = −0.221, $p = 0.05$) (Fig. 2), whereas age (standardized coefficient B = −0.096; $p = 0.409$) and gender (standardized coefficient B = 0.080; $p = 0.51$) were not related to tau. In the FTD group neither sAβPPβ (standardized coefficient B = 0.350, $p = 0.199$) nor $A\beta_{42}$ (standardized coefficient B = 0.379, $p = 0.175$) were related to tau levels. No associations were detected between age (standardized coefficient B = 0.147, $p = 0.689$) and gender (standardized coefficient B = 0.142, $p = 0.532$) and tau concentrations in CSF. In all models, tolerance values were not less than 0.57. Tolerance values less than 0.2 are usually considered to indicate collinearity [40].

According to the regression analysis, CSF sAβPPβ levels correlated significantly with SORL1 concentrations (standardized coefficient B = 0.379; $p = 0.003$) in patients with MCI (Fig. 3) and were not influenced by age (standardized coefficient B = −0.158; $p = 0.202$) or gender (standardized coefficient B = 0.211; $p = 0.09$). In the AD group, significant associations between sAβPPβ and SORL1 (standardized coefficient B = 0.551; $p < 0.001$) (Fig. 3) and gender (standardized coefficient B = 0.398; $p = 0.003$) were observed, while age was not associated with SORL1 levels (standardized coefficient B = −0.045; $p = 0.734$). In patients with FTD, only SORL1 was found to be related to sAβPPβ levels in CSF (standardized coefficient B = 0.708; $p = 0.011$) (Fig. 3), whereas age (standardized coefficient B = −0.211; $p = 0.402$) and gender (standardized coefficient B = 0.347; $p = 0.141$) were not. Regarding relations between $A\beta_{42}$ and SORL1, the regression analysis did not reveal any associations between $A\beta_{42}$ and SORL1, age, or gender either in the MCI group (standardized coefficient B = 0.074, −0.201, 0.037, $p = 0.582$, 0.140, 0.785, respectively), or in the AD group (standardized coefficient B = −0.059, 0.096, 0.033, $p = 0.726$, 0.570, 0.838) respectively). The analysis did not show any

Table 2
Characteristics of patients with degenerative mild cognitive impairment (MCI) and with non degenerative MCI. Data presented as mean (SD)

| | Degenerative MCI | Non-degenerative MCI |
|---|---|---|
| *n* | 47 | 29 |
| Age (in years) | 67.3 (9.0) | 62.7 (9.4)* |
| Gender (men/women) | 23/24 | 15/14 |
| MMSE (standard deviation) [range] | 26.73 (2.22) [22–30] | 27.14 (1.84) [23–30]* |
| Tau (ng/ml) | 549.64 (247.08) | 171.07 (61.65)* |
| Soluble AβPPβ (ng/ml) | 1156.62 (491.57) | 903.26 (422.17)* |
| SORL1 (ng/ml) | (*n* = 32), 12.01 (5.16) | (*n* = 25), 11.80 (2.88) |
| Amyloid-$\beta_{1-42}$ (ng/ml) | 659.55 (314.33) | 863.72 (329.63)* |

MMSE: Mini-mental state examination, SORL1: Sortilin-related receptor with A-type repeats; *Statistically significant differences, $p < 0.05$.

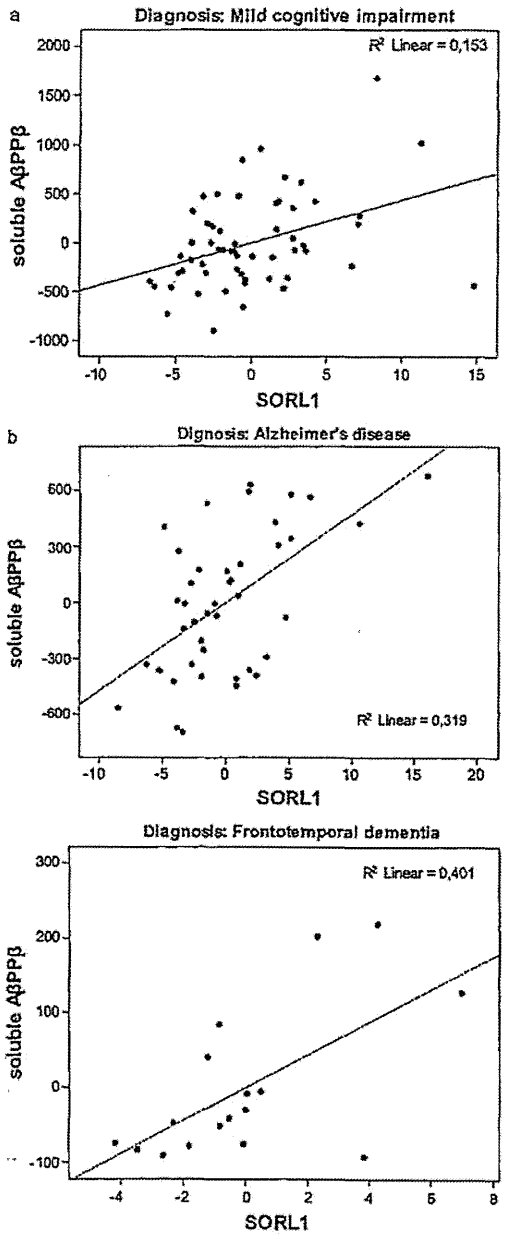


Fig. 3. Partial regression diagrams of soluble AβPPβ and SORL1 concentrations in cerebrospinal fluid in (a) patients with mild cognitive impairment, in (b) patients with Alzheimer's disease, and in (c) patients with frontotemporal dementia. Values are standardized and at zero centered.

statistically significant relations between $A\beta_{42}$ and SORL1 (standardized coefficient B=0.514, $p=0.077$), age (B=−0–030, $p=0.915$), or gender (standardized coefficient B=0.382, $p=0.146$) in patients with FTD too. Tolerance values were not less than 0.73.

## DISCUSSION

The main findings of the present study are the statistically significant positive correlations between sAβPPβ and tau in patients with AD and MCI, but not in the group of FTD patients, and the significant associations between sAβPPβ and soluble SORL1 in all groups of participants.

The positive correlation between sAβPPβ and tau in CSF of patients with AD and MCI, especially with MCI developing on a neurodegenerative basis, and the absence of such an association in the non-degenerative MCI subgroup are observations, which further support the concept of an interrelation between amyloid and tau pathology in AD, even though they do not establish any straightforward facilitatory causal effect of sAβPPβ on the increase of tau concentrations in CSF. These findings are in line with the reported positive correlation between tau levels and total soluble AβPP in CSF [41, 42], as well as with the association between β-secretase activity and tau levels [43]. One plausible explanation for this result is that the link between the two facets of AD pathology is possibly mediated by the binding of Aβ oligomers to neuronal target receptors, which aberrantly activates trophic signaling and activates an incomplete set of downstream events (e.g., increased Akt activation, hyperphosphorylation of critical Akt substrates, excessive activation of the PI3K/Akt pathway, leading to tau hyperphosphorylation, and neuronal degeneration [5]. Alternatively, the positive correlation between sAβPPβ and tau in CSF in AD and MCI could be attributable to an unspecific protein release from dying neurons and axons [43]. In line with the hypothesis that Aβ oligomers induce tau hyperphosphorylation and subsequently neurodegeneration, we detected an association between tau levels and sAβPPβ, mirroring the generation of all Aβ peptides, and not only of $A\beta_{42}$. However, it should be underscored that there is no experimental evidence for a relation between sAβPPβ and Aβ oligomers.

$A\beta_{42}$ was found to be associated with tau in the MCI group and marginally in the AD. Previous studies found a correlation between $A\beta_{42}$ and tau in CSF

in healthy elderly individuals and in patients with non-neurodegenerative MCI, but not in patients suffering from AD pathology [41, 44]. Our MCI group was not restricted to patients with MCI due to neurodegeneration, since patients with MCI were recruited according to clinical criteria and not values of markers of degeneration. Nonetheless, the dichotomization of the MCI group with regard to values of the neurodegeneration marker tau revealed a significant negative correlation between tau and $A\beta_{42}$ in the degenerative MCI subsample, whereas in the non-degenerative MCI subsample tau was found to correlate positively with CSF $A\beta_{42}$. These findings in conjunction with the presence only of a tendency to a correlation between $A\beta_{42}$ and tau in CSF in patients with AD possibly indicate that the progression of AD pathology is likely to result in the attenuation of the association between $A\beta_{42}$ and tau possibly via deficient clearance mechanisms of $A\beta_{42}$ or high rates of $A\beta_{42}$ aggregation in amyloid plaques [1]. The observed discrepancies, concerning the relationship between tau and $A\beta_{42}$ in CSF in the patients with AD and MCI, obviously warrant further investigation, especially in the light of the limited size of the non-degenerative MCI subgroup in our study.

The regression analysis model revealed an impact of gender on CSF sAβPPβ levels in patients with AD. This observation implies a sexual dimorphism. Interestingly, recent reports from AD transgenic animal models have reported higher β-secretase activity and a more aggressive Aβ pathology in female than male mice [45]. Such findings are compatible with previous observations, which indicate an upregulation of both α- and β- pathways in women compared with men with AD [46]. Moreover, it is noteworthy that epidemiological studies have shown that women have higher risk of AD even after adjustment for age [47, 48]. Nonetheless, the influence of gender on CSF sAβPPβ concentrations needs to be replicated in studies including larger samples.

To our knowledge this is the first study to elucidate a correlation between SORL1 concentrations and sAβPPβ in CSF of patients with AD, MCI, and FTD. SORL1 was previously found to be reduced in the Golgi and early endosomal compartments in AD [49–51], allowing or fostering AβPP to be processed by β- and α-secretase, resulting in the generation of sAβPPβ [12, 52, 53]. The positive correlation in our study seems to be a contradiction in this regard. However, the employed ELISA determines the soluble form of SORL1, which is the product of SORL1 processing by proteases. It consists of the extracellular domain of the membrane-spanning SORL1 protein [37], which was found to be elevated in patients with AD [54] and is assumed to be less efficient than full-length SORL1 with regard to mediating AβPP transport through the Golgi-apparatus [53]. However, a hypothesis claiming that in AD the intracellular decline in full-length SORL1 levels is caused by an elevation in the endoproteolytical cleavage of SORL1, resulting in an elevation of the concentrations of the less efficient soluble SORL1, which can be detected in CSF, is quite unlikely especially in the light of the absence of statistically significant differences in CSF SORL1 concentrations amid the three study groups. A further possible explanation for the detected positive correlation is the direct interaction of soluble SORL1 with sAβPPβ in CSF in association with apolipoprotein E, since SORL1 levels in CSF are particularly increased in patients with AD carrying the *APOE* ε4 allele [54], and SORL1 is a membrane receptor for APOE-containing lipoproteins in CSF [55]. Though in participants in whom CSF SORL1 was determined ($n=116$), no differences were elucidated in SORL1 levels between *APOE* ε4 allele carriers and non carriers either in the AD or MCI and FTD groups (data not shown); in the regression analysis the interaction term *APOE* ε4 × SORL1 levels showed a significant effect on sAβPPβ concentrations (independent variable) in the MCI and AD group (standardized coefficient $B=0.46$, 0.391, and $p<0.001$, $p=0.01$ respectively), while in patients with FTD, the association did not attain statistical significance (standardized coefficient $B=0.085$, $p=0.747$). Interestingly, the positive correlation between SORL1 and sAβPPβ was also found in FTD. This observation indicates that the association between the two molecules is not restricted to patients suffering from amyloid pathology. Therefore, future studies investigating the associations between the two molecules in further clinical entities, that are associated with alterations in processing of AβPP (e.g., multiple sclerosis, lyme neuroborreliosis) [56, 57], as well as in healthy subjects are required, since it is possible that the detected relation can be observed not only in patients with neurodegeneration.

Despite the detected significant association between CSF SORL1 and sAβPPβ levels in CSF in all study groups, the analysis did not reveal such an association between SORL1 and $A\beta_{42}$, possibly owing to the aggregation of $A\beta_{42}$ in amyloid plaques and/or impaired $A\beta_{42}$ clearance mechanisms, resulting in the undermining of a potential association between the two peptides in the CSF.

The present study should be viewed in the light of a number of limitations. The size of the study sample was relatively small and no control group was included. As a consequence, we were not in the position to explore possible associations between sAβPPβ, tau and SORL1 in physiological aging. However, a group of patients with FTD, which is pathologically not characterized by amyloid pathology, was included in the study. Only a few proteins related to amyloid metabolism were determined. Thus our analysis and the detected associations do not provide experimental evidence for causal effects. Unfortunately, *APOE* genotype data were not available for all study participants. As a result this genetic factor could not be included in the regression analysis as residual. Our investigation encompassed a sample which was restricted to participants recruited at university centers. Hence, the generalization of the results warrants further investigation. No pathological verification of diagnoses was available, but current diagnostic criteria for AD have been shown to be very accurate for populations recruited at specialized centers [58].

AD is a clinical entity which is assumed to reach the dimension of a health scourge in the near future. As a result it is worth trying to unravel the pathomechanisms underlying the disease in order to facilitate the development of new effective disease-modifying therapies. The elucidated interrelations between the amyloid cascade and axonal degeneration as well as between soluble SORL1 and sAβPPβ contribute to our understanding of the genesis of AD and probably to the developing of novel therapeutic strategies.

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ORIGINAL PAPER

# SORL1 genetic variants and cerebrospinal fluid biomarkers of Alzheimer's disease

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**Abstract** The neuronal sortilin-related receptor with A-type repeats (SORL1, also called LR11 or sorLA) is involved in amyloidogenesis, and the *SORL1* gene is a major risk factor for Alzheimer's disease (AD). We investigated AD-related CSF biomarkers for associations with *SORL1* genetic variants in 105 German patients with mild cognitive impairment (MCI) and AD. The homozygous CC-allele of single nucleotide polymorphism (SNP) 4 was associated with increased Tau concentrations in AD, and the minor alleles of SNP8, SNP9, and SNP10 and the haplotype CGT of these SNPs were associated with increased SORL1 concentrations in MCI. SNP22 and SNP23, and the haplotypes TCT of SNP19-21-23, and TTC of SNP22-23-24 were correlated with decreased A$\beta$42 levels in AD. These results strengthen the functional role of *SORL1* in AD.

**Keywords** Amyloid cascade · Biomarker · Mild cognitive impairment · Dementia · Genetic risk

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## Introduction

The neuronal sortilin-related receptor with A-type repeats (SORL1, also called LR11 or sorLA) has been linked to protective effects against amyloidogenesis in Alzheimer's disease (AD) [1]. SORL1 seems to be capable of regulating the intracellular trafficking and processing of amyloid precursor protein (APP) by impairing the cleavage of APP through $\alpha$-secretase, $\beta$-secretase ($\beta$-site APP-cleaving-enzyme-1, BACE1), and $\gamma$-secretase in a way that leads to reduced levels of soluble APP (sAPP) and amyloid beta protein (A$\beta$), the major component of amyloid plaques [2]. In line with this theory, reduced SORL1 expression has been demonstrated in human brains with amyloid pathology [3]. *SORL1* gene variants can reduce SORL1 expression or function and thereby increase A$\beta$ production as well as AD risk [4]. Recently, multiple single nucleotide polymorphisms (SNP) within the *SORL1* gene have emerged as risk factors for sporadic AD in a variety of populations. Although replications are inconsistent, implicating influences of multi-ethnicity and allelic heterogeneity [4, 5], several independent studies have observed that significant associations were located in 2 distinct regions: the 5′ end and the 3′ end of the *SORL1* gene [4]. So far, only few studies have reported associations of *SORL1* variants with cerebrospinal fluid (CSF) endophenotypes in

AD [6–8]. In the present study, we have investigated eleven AD risk SNPs in a German sample to evaluate the effect of *SORL1* variants on the CSF levels of A$\beta$42, total TAU, sAPP$\alpha$, sAPP$\beta$, and SORL1 protein as well as on the CSF activity of BACE1.

## Methods

The study population consisted of 44 Caucasian patients with probable AD according to NINCDS-ADRDA criteria and 61 patients with mild cognitive impairment (MCI) according to the revised International Working Group on MCI consensus criteria recruited from a university-based memory clinic in compliance with standardized guidelines [9, 10]. Written informed consent was obtained according to the 1975 Helsinki Declaration and the study protocol was approved by the ethics committee of the medical faculty at Technische Universität München.

The CSF concentrations of A$\beta$42, Tau (Innogenetics, Zwijndrecht, Belgium) as well as sAPP$\alpha$ and sAPP$\beta$ (Immuno-Biological Laboratories Co. Ltd., Gunma, Japan) were measured by enzyme-linked immunosorbent assay (ELISA) as described previously [11]. BACE1 activity in CSF was determined as the fluorescence signal of europium, which is proportional to the activity of BACE1, by a commercial BACE1 assay kit (Perkin Elmer Inc., Turku, Finland) according to a standard protocol [12, 13]. SORL1 concentration in CSF was quantified by ELISA in the laboratories of Sekisui Medical Co Ltd. (Ryugasaki, Japan) according to published procedures [14]. Genomic DNA was extracted from whole blood, and the apolipoprotein E *(APOE)* genotype was determined by a polymerase chain reaction and restriction enzyme digestion, simultaneously utilizing two distinct restriction enzymes, according to standard procedures.

Five marker SNPs at the 5′ end of the *SORL1* gene, rs661057 (SNP4), rs11600875, rs668387 (SNP8), rs689021 (SNP9), and rs641120 (SNP10), as well as 6 markers at the 3′ end, rs2070045 (SNP19), 21rs18ex26 (SNP21), rs1699102 (SNP22), rs3824968 (SNP23), rs2282649 (SNP24), and rs1010159 (SNP25), were selected from the published data based on their significant association with AD risk in Caucasian populations [4, 5, 7]. The genotypes were determined using TaqMan assays (SNP assays-on-demand) on a StepOne analyzer with StepOne software v2.1 (all assays, machine, and software from Applied Biosystems, Carlsbad, CA, USA).

Deviations from the Hardy–Weinberg equilibrium to exclude population stratification were tested for all 11 *SORL1* SNPs (http://www.oege.org/software/hwe-mr-calc.shtml) [15]. The sample size required to detect a significant difference between carriers and non-carriers with 90% power and a type I error rate of 0.05 was estimated in G-Power v3.1.3 [16] at $N = 14$ per group according to previous results [7] (mean A$\beta$42 concentration difference between carriers and non-carriers of the *SORL1* SNP23 T-allele of 56.60 ng/L with a shared standard deviation of 41.59 ng/L).

Patient characteristics were compared between the AD and the MCI groups using parametric tests for normally distributed data in the Predictive Analytics Software package (PASW) v18 (The SPSS Inc., Chicago, IL, USA). Analysis of covariance (ANCOVA) in PASW was used to test for the genotypic or allelic effect of all 11 SNPs of interest on CSF biomarker concentrations, adjusting for age, gender, and *APOE*, which was coded as a dichotomous variable for carriers and non-carriers of the $\varepsilon$4 allele. In addition, three-marker haplotypes of SNP8/SNP9/SNP10, SNP19-21-23, SNP22-23-24, and SNP23-24-25, again selected from the literature according to their linkage disequilibrium (LD) and the significant association with AD risk, were reconstructed and assessed with the Haplo.stats package in R software v2.1 (http://www.r-project.org/). The associations between *SORL1* haplotypic variants and CSF biomarker concentrations were examined in multivariate linear models after adjustment for age, gender, and *APOE* $\varepsilon$4 carrier status. Only genetic frequency higher than 5% was considered. Significance was set at $p < 0.05$. The study was driven by a priori hypotheses; therefore, no correction for multiple comparisons was applied [17] in accordance with similar previous studies [18].

## Results

The demographic and clinical characteristics are summarized in Table 1; genotype and allele frequencies are provided in the Supplementary Tables 1 and 2. None of the 11 SNPs showed significant deviation from the Hardy–Weinberg equilibrium in the AD group; in the MCI group, deviation was only observed for SNP21 (Supplementary Table 1). The APOE $\varepsilon$4 allele was associated with lower A$\beta$42 levels in the MCI group ($p < 0.001$, $N = 61$). The single-marker analysis revealed significant associations between A$\beta$42 concentrations and the synonymous coding SNP22 and SNP23 at the 5′ end of the gene in the AD group. Carriers of the SNP22 C-allele ($p = 0.04$, $N = 22$) and SNP23 A-allele ($p = 0.04$, $N = 23$) had lower levels of A$\beta$42 than non-carriers (Supplementary Table 3). In the haplotype analyses, we observed associations of haplotype TCT (frequency 36.9%) of SNP19-21-23 ($p = 0.04$, $N = 38$) and TTC (frequency 24.7%) of SNP22-23-24 ($p = 0.04$, $N = 38$) with decreased CSF A$\beta$42 levels in the AD group (Fig. 1a). At the 3′ end of the gene, a significant association between the homozygous minor allele CC of

**Table 1** Characteristics of the study sample

| | AD ($N$ = 44) | MCI ($N$ = 61) | $p$ value |
|---|---|---|---|
| Age at lumbar puncture* | 66 (9.6) | 65 (8.7) | 0.44 |
| Age at onset of symptoms* | 6 (8.8) | 63 (8.8) | 0.62 |
| Men:women | 23:21 | 35:26 | 0.81 |
| Schooling, years* | 13 (2.9) | 1 (2.7) | 0.91 |
| MMSE score* | 23 (3.1) | 27 (1.9) | <0.001** |
| ApoE4 carrier, $n$ (%) | 26 (59.1%) | 27 (44.3%) | 0.55 |
| Aβ42 (ng/L)* | 551.8 (233.52) | 771.1 (350.84) | <0.001** |
| TAU (ng/L)* | 627.8 (384.24) | 383.9 (255.87) | <0.001** |
| sAPPα (ng/mL)* | 287.1 (159.21) | 332.2 (166.75) | 0.17 |
| sAPPβ (ng/mL)* | 897.0 (402.65) | 1047.2 (493.75) | 0.10 |
| BACE1 (FU/μL)* | 8333.06 (2585.76) | 9381.67 (3239.94) | 0.08 |
| SORL1 (μg/L)* | 11.9 (4.69) | 11.9 (4.28) | 0.95 |

*SNP* single nucleotide polymorphism, *CSF* cerebrospinal fluid, *Aβ42* amyloid beta 42, *sAPPα, sAPPβ* alpha- and beta-soluble amyloid precursor protein, *BACE1* β-site APP-cleaving-enzyme-1, *SORL1* sortilin-related receptor with A-type repeats, *AD* Alzheimer's disease, *MCI* mild cognitive impairment, *FU* fluorescence units

* Mean (SD), ** significant at $p < 0.05$

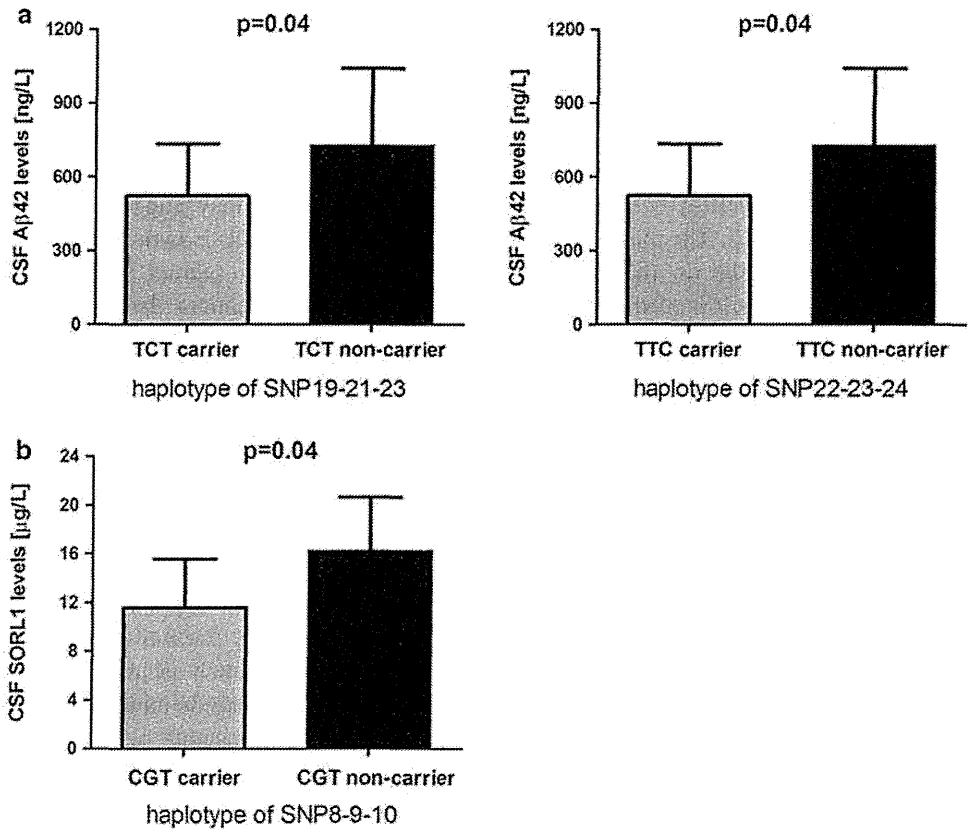


**Fig. 1** **a** Effects of SORL1 haplotypes on CSF Aβ42 levels in the AD group; and **b** effects of SORL1 haplotypes on CSF SORL1 levels in the MCI group

SNP4 and increased Tau levels was observed ($p$ = 0.03, $N$ = 7) in the AD group. No association was found in heterozygous carriers, which points to a strong gene dosage effect (Supplementary Table 4). In the MCI group, at the 3′ end of the gene, SNP8, SNP9, and SNP10 showed significant associations with CSF SORL1 levels in a way that minor allele carriers had increased SORL1 concentrations (SNP8 TT: $p$ = 0.04; SNP9 AA: $p$ = 0.04; SNP10 CC: $p$ = 0.04) (Supplementary Table 5). Again, these associations were driven by the homozygous carriers of the minor alleles of each of the three SNPs. In the haplotype analyses, a significant association between reduced CSF SORL1

levels was found with haplotype CGT (frequency 22.5%) of SNP8-9-10 in the MCI group ($p = 0.04$, $N = 55$) (Fig. 1b). There were no associations between sAPP levels and BACE1 activity with any of the SNPs or haplotypes.

## Discussion

SORL1 regulates the intracellular sorting of APP and hinders APP cleavage and thereby A$\beta$ production [1, 2]. The *SORL1* gene has been identified as a major risk factor for sporadic AD [4]. In the present study, associations between *SORL1* genetic variants and CSF levels of A$\beta$42, Tau, and SORL1 were observed at two distinct gene regions in patients with MCI and probable AD. Associations between *SORL1* genetic variants and CSF sAPP$\alpha$ and sAPP$\beta$ concentrations as well as BACE1 activity were not observed.

In the AD group, lower CSF A$\beta$42 levels were found in carriers of the exonic SNP22 (C-allele) and SNP23 (A-allele), and haplotypes TCT of SNP19-21-23 and TTC of SNP22-23-24 at the 3′ gene end. It has been demonstrated that SNP19 is in strong linkage disequilibrium with SNP22 and SNP23 in various Caucasian cohorts [19]. SNP21, on the other hand, has been reported as AD-related *SORL1* polymorphism in a German cohort [3] and the haplotype TGA of SNP19-21-22 correlated with lower CSF A$\beta$42 in AD before [7]. This finding was not replicated in our work, probably due to the low frequency of these markers in our sample (Supplemental Tables 1 and 2). In the initial genetic association study [4], the SNP22 C-allele, SNP23 T-allele, and haplotype CTT of SNP22-23-24 were associated with an increased risk for AD. In contrast, in our study, reduced A$\beta$42 levels were correlated with genotypes and haplotypes consisting of the alternative alleles. This inconsistency suggests that *SORL1* allelic heterogeneity and ethnic variants may also play a role [20]. Since exonic SNPs of the *SORL1* gene are present in the mature mRNA, they could directly alter translation and thus protein levels [21]. Therefore, the 3′ end SNPs, in particular the synonymous coding SNPs, might directly influence the function of the SORL1 protein and thereby alter the CSF levels of A$\beta$42.

We also found that Tau levels were associated with CC homozygotes of SNP4 in the AD group. The C-allele of SNP4 has been associated with AD among Caucasian populations in multiple independent cohorts and genome-wide association studies before [4, 5, 20, 22–25]. Although the present work is a case-only study that precludes a statement on the association of *SORL1* SNPs with AD risk per se, our data still confirm that the SNP4 C-allele is significantly associated with upregulated CSF Tau levels, which in turn are correlated to neurodegenerative pathology.

SORL1 protein is considered an important regulator of amyloidogenesis since reduced SORL1 levels may lead to dysfunctional retromer trafficking and upregulated cerebral A$\beta$ production [1]. It remains inconclusive how reduced SORL1 protein expression in AD brain is related to alterations of SORL1 in CSF. It has been reported that the expression of SORL1 protein is reduced in brain tissue from patients with sporadic AD [3]. The two published CSF studies are inconsistent in this regard, reporting both decreased [26] and increased [27] SORL1 levels in AD compared with healthy controls. We identified associations between CSF SORL1 concentrations and three AD risk marker SNPs in the MCI group; the homozygous minor allele carriers of the intronic SNP8 (T-allele), SNP9 (A-allele), and SNP10 (A-allele) had increased SORL1 concentrations in CSF. Moreover, the haplotype analysis confirmed that a three-marker haplotype CGT (a combination of the major alleles) of SNP8/SNP9/SNP10 was associated with reduced CSF SORL1 levels in the MCI group. These three SNPs have been confirmed as the most significant AD risk markers within the *SORL1* gene in Caucasian samples in a recent meta-analysis including 11,592 cases and 17,048 controls [28]. The association of three 5′ end SNPs in our study with CSF SORL1 concentrations is consistent with the allelic disease association in this meta-analysis. Since MCI often represents pre-dementia AD, our data may suggest that the influence of *SORL1* genetic variants is particularly relevant in early clinical AD stages.

Our current study extends the existing literature on associations between SORL1 genetic variants and AD biomarkers, thereby supporting the role of SORL1 as an important influence factor on AD pathogenesis. Limitations include the rather small study sample and the lack of longitudinal data as well as neuropathological verification of the diagnoses. Therefore, replication studies with independent larger samples are warranted. We did not aim to replicate the results from previous genetic association studies; neither did we aim to identify new risk SNPs, and no control group was included because of this study design choice. Lack of consistent replication of genetic findings is a common occurrence in the study of complex phenotypes and may be indicative of inadequate power resulting from small sample size and genetic or environmental heterogeneity. The use of CSF biomarkers for genetic studies of AD may provide increased statistical power and important insight into the biological mechanisms by which these variants modulate disease risk. In any study attempting to associate genetic information with pathology, the exact effect of genetic variants on phenotypic variation often remains unclear. On the one hand, the genetic variants may have a direct effect on markers of pathology; on the other hand, neighboring SNPs in LD with the variant tested or other downstream factors may also have an influence.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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# β-Site amyloid precursor protein–cleaving enzyme 1 activity is related to cerebrospinal fluid concentrations of sortilin-related receptor with A-type repeats, soluble amyloid precursor protein, and tau

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**Abstract**

**Background:** β-Site amyloid precursor protein (APP)–cleaving enzyme 1 (BACE1) activity determines the rate of APP cleavage and is therefore the main driver of amyloid β production, which is a pathological hallmark of Alzheimer's disease (AD).
**Methods:** The present study explored the correlation between BACE1 activity and cerebrospinal fluid (CSF) markers of APP metabolism and axonal degeneration in 63 patients with mild AD and 12 healthy control subjects.
**Results:** In the AD group, positive correlations between BACE1 activity and soluble APP β, the APP sorting receptor sortilin-related receptor with A-type repeats (also known as SorLA or LR11), and tau were detected. BACE1 activity was not associated with amyloid $\beta_{1-42}$ or soluble APP α concentrations in the AD group, and no associations between BACE1 activity and any of the protein concentrations were found in the control group.
**Conclusion:** Our results confirm the relevance of BACE1 and sortilin-related receptor with A-type repeats within the amyloid cascade and also provide a further piece of evidence for the link between amyloid and tau pathology in AD.


*Keywords:* Alzheimer's disease; Dementia; Biomarker; Amyloid cascade; β-secretase

## 1. Background

The cerebral pathologic hallmarks of Alzheimer's disease (AD) include the extracellular accumulation of amyloid β (Aβ) plaques, synaptic and neuronal degeneration, and the presence of tau protein tangles [1]. Aβ plaques mainly consist of the 4-kDa Aβ peptide, which is generated by the enzymatic cleavage of the transmembrane amyloid precursor protein (APP). The first, and rate-limiting, APP cleavage step by the β-site APP-cleaving enzyme 1 (BACE1) [2] results in the production of the N-terminal soluble APP (sAPP) β and a C-99 fragment, which is subsequently cleaved by the γ-secretase complex, resulting in Aβ. The alternative processing of APP by the α-secretases precludes the generation of Aβ because the cleavage site lies within the Aβ sequence; sAPPα is a product of this processing pathway [3]. The relevance of BACE1 in AD is supported by its increased expression and activity in the brain tissues [4,5] and cerebrospinal fluid (CSF) of patients with AD [6,7].

In addition to the secretases, the sortilin-related receptor with A-type repeats (SORL1, also termed LR11 or sorLA), a member of the apolipoprotein E and low-density lipoprotein receptor family [8,9], has emerged as another relevant regulator of APP processing; SORL1 is probably involved in the intracellular sorting of APP and its interactions with the secretases, including BACE1 [10]. According to recent

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evidence, SORL1 promotes the retention of APP in subcellular compartments that are less favorable for secretase processing, thereby reducing the extent of its proteolytic breakdown into both amyloidogenic and nonamyloidogenic products [11]. In line with this finding, the neuronal expression of SORL1 is dramatically decreased in the brains of patients with sporadic AD [12–14]. The large extracellular part of the receptor is released after endoproteolytic cleavage [15] and can therefore be measured in CSF; however, no general consensus has yet been reached regarding the effects of AD on SORL1 concentrations in CSF [16,17].

The aforementioned evidence and theoretical considerations suggest that BACE1 activity should be positively correlated with $A\beta_{1-42}$ and sAPPβ (but not sAPPα), and possibly also with tau as well as SORL1, concentrations in CSF. Some of these assumptions, such as the positive association between BACE1 activity and sAPPβ and tau concentrations, are backed by previous research, whereas others are not [18,19], which warrants replication. Furthermore, the correlation between the concentrations of SORL1, the encoding gene of which is among the strongest known genetic risk factors for sporadic AD [20], and BACE1 activity in CSF has not been studied thus far. The main aim of the present study was to provide evidence in relation to these issues.

## 2. Methods

### 2.1. Participant selection

Sixty-three patients with probable AD and available lumbar CSF samples were identified in the electronic database of the Department of Psychiatry and Psychotherapy at the Technische Universität München (Munich, Germany). Informed written consent was available for all patients; the study protocol was approved by the ethics committee of the faculty of medicine at the Technische Universität München. The clinical diagnoses had been established by consensus of two experienced clinicians according to National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria for probable AD in conjunction with International Classification of Diseases (10th revision) criteria for mild AD dementia. The diagnostic workup included patient and proxy interviews, physical examination, psychometric testing, routine blood sampling, and structural imaging of the brain (magnetic resonance imaging or computed tomography). None of the patients showed signs of relevant cerebrovascular disease or any plausible cause for cognitive impairment other than AD. The psychometric assessment was based on the Consortium to Establish a Registry for Alzheimer's disease neuropsychological assessment battery, which incorporates the Mini-Mental State Examination. An additional group of 12 healthy control subjects, recruited at the Department of Neurology of the University of Bari in Italy, was included to explore the associations between the CSF protein levels in the absence of any relevant neurodegenerative pathology. The control subjects had no subjective memory complaints and no history of cognitive impairment. They were independent in their activities of daily living and did not show any signs of a relevant psychiatric or neurological illness.

### 2.2. CSF sampling and analyses

CSF was collected in sterile polypropylene tubes using atraumatic cannulas placed in the L3/L4 or L4/L5 intervertebral space. The CSF was centrifuged (1800 $\times g$ at 4°C for 10 minutes) immediately after collection to remove cells. Aliquots of the remaining CSF supernatants were stored in polypropylene tubes at −80°C for further processing.

### 2.3. Determination of $A\beta_{1-42}$, tau, sAPPα, and sAPPβ levels

$A\beta_{1-42}$, total tau (Innogenetics, Ghent, Belgium), and sAPPα/sAPPβ (IBL, Gunma, Japan) levels in CSF were measured in duplicate using commercially available enzyme-linked immunosorbent assays (ELISAs) according to the manufacturers' instructions as described previously in greater detail [21-23].

### 2.4. BACE1 activity assay

BACE1 activity was measured using a time-resolved fluorescence activity assay based on SignalClimb technology (TruePoint Perkin Elmer, Turku, Finland) according to optimized manufacturer's instructions [7]. The synthetic TruePoint BACE1 substrate is a 10-amino acid–long peptide with a fluorescent europium chelate coupled to one end and a quencher of europium fluorescence (QSY7) coupled through lysine to the other end. The hydrolysis of the substrate's protein sequence CEVNLDAEFK by BACE1 results in a fluorescence signal proportional to the activity of BACE1. The fluorescence signal was measured at 37°C in a microplate reader using time-resolved fluorescence (FLUOstar Omega, BMG Labtech, Offenburg, Germany; excitation wavelength: 320 nm, emission wavelength: 615 nm) in black 96-well plates (Perkin Elmer, Turku, Finland) at a final volume of 27 μL, including 10 μL of CSF, 2 μL of dimethyl sulfoxide, and 15 μL of BACE1 substrate (0.80 nM/mL). The continuous measurement of BACE1 activity was started immediately after adding the CSF sample; BACE1 activity was defined as the maximal activity within the first 30 minutes. Each sample was measured at least four times to verify reproducibility. Proteinase inhibitors were added to block all non-BACE1 aspartyl protease activity.

### 2.5. SORL1 concentrations

SORL1 concentrations in CSF were quantified using ELISA in the laboratories of Sekisui Medical Co Ltd. (Ryugasaki, Japan) as described previously [24]. Briefly, 10 μL of CSF was diluted with 100 μL of sample buffer and added to

the plate coated with mouse monoclonal antibody M3 [25]. After incubating with the biotinylated rat monoclonal antibody R14, the SORL–antibody complex was reacted with horseradish peroxidase–conjugated streptavidin. A standard curve was constructed using purified SORL1 protein. The final absorbance of each sample was determined at 450 nm [24]. SORL1 concentrations were only determined in a subsample of 40 patients with probable AD and in the entire control group. The first published study using this assay [24] showed that purified SORL1 in CSF was immunologically identical to SORL1 from cell culture, strongly arguing that the ELISA measures the soluble form of the membrane-bound receptor.

### 2.6. Statistical analysis

Data were analyzed using the Predictive Analytics Software package version 18 (SPSS Inc., Chicago, IL) using two-sided tests. Normal distribution was checked using the Kolmogorov–Smirnov test. Correlations between BACE1 activity and CSF protein concentrations were investigated using the Pearson correlation coefficient. $P$ values were regarded significant at a level of 5%; the false discovery rate (http://sdmproject.com/utilities/?show=FDR), which controls the expected proportion of incorrectly rejected null hypotheses (type I errors), was used to account for the error in multiple comparisons (i.e., results at $P_{corr} < .05$ were regarded significant).

## 3. Results

The characteristics of the study sample are shown in Table 1. The CSF concentrations of $A\beta_{1-42}$ and tau were in the expected range for this kind of sample [26]. The Mini-Mental State Examination score range confirmed the mild degree of dementia in the AD group and the lack of any objective cognitive impairment in the control group. In the AD group, there was a significant positive correlation after false discovery rate correction for multiple comparisons between BACE1 activity on the one hand and tau ($r = 0.30, P_{corr} = .04, N = 63$) as well as SORL1 concentrations ($r = 0.37, P_{corr} = .04, N = 40$) on the other hand (Figure 1). BACE1 activity was not correlated with sAPPα ($P_{corr} = .17$) or $A\beta_{1-42}$ ($P_{corr} = .91$) levels in the AD group. At a more liberal threshold of $P < .05$ uncorrected for multiple comparisons, BACE1 activity was also positively correlated with sAPPβ levels in patients with AD ($r = 0.26, P_{uncorr} = .05, N = 63$). BACE1 activity was not correlated with the levels of any of the CSF proteins in the control group (tau: $P_{corr} = .96$; $A\beta_{1-42}$: $P_{corr} = .75$; sAPPα: $P_{corr} = .55$; sAPPβ: $P_{corr} = .55$; SORL1: $P_{corr} = .90$).

Table 1
Characteristics of the study groups

| Variable | Control group | AD group |
|---|---|---|
| N | 12 | 63 |
| Age, years* | 47.50 (13.70) | 66.87 (9.39) |
| Age at onset, years* | na | 62.83 (9.09) |
| Men/women | 6:6 | 34:29 |
| MMSE score* | 30 (0.00) | 22.54 (3.27) |
| BACE1 activity, FU/μL* | 7468.43 (1966.75) | 8757.01 (2636.36) |
| $A\beta_{1-42}$, ng/L* | 708.33 (378.17) | 540.48 (232.99) |
| Tau, ng/L* | 125.58 (57.29) | 605.21 (361.99) |
| sAPPα, ng/mL* | 265.19 (218.35) | 263.83 (145.58) |
| sAPPβ, ng/mL* | 746.35 (519.92) | 864.95 (405.522) |
| SORL1 ng/mL* | 10.36 (2.61) | 11.83 (4.74)† |

Abbreviations: na, not applicable; nd, not done; AD, Alzheimer's disease; MMSE, mini-mental state examination; FU, fluorescence units; Aβ, amyloid β; sAPP, soluble amyloid precursor protein; BACE1, β-site amyloid precursor protein–cleaving enzyme 1; SORL1, sortilin-related receptor with A-type repeats.
*Mean (SD).
†N = 40.

## 4. Discussion

The present study explored the association between BACE1 activity, which is regarded as the rate-limiting factor of Aβ production, and the concentrations of several AD markers in CSF. Our findings partly confirm the results of previous research, but they also add new evidence to the existing literature, for example, by demonstrating a positive correlation between BACE1 activity and SORL1 concentration in CSF.

### 4.1. BACE1 and tau

In the present study, BACE1 activity correlated positively with tau concentrations in the AD group. This is a challenging observation at first glance, which is, nevertheless, in line with three previous individual reports [19,18,28] and a meta-analysis [18]. Tau is a marker of axonal degeneration, which may enhance BACE1 shedding, resulting in higher CSF levels and activity of BACE1 in AD [27]. Alternatively, as both tau and BACE1 are primarily located in neurons, their correlation may indicate that increased BACE1 activity in CSF is associated with protein release from decaying neurons.

### 4.2. BACE1 and markers of APP metabolism

We also found a positive correlation between BACE1 activity and sAPPβ levels. This association was expected because sAPPβ is a direct product of APP cleavage by BACE1 [29]. BACE1 activity did not correlate with the CSF levels of sAPPα in our study; this was also an expected result because there is no direct association between BACE1 and sAPPα, which is a product of α-cleavage rather than of β-cleavage [3]. In contrast to our findings, an association between sAPPα levels and BACE1 activity in CSF was observed in a previous study [19]. The authors argued that this surprising finding might be explained by the strong correlation between sAPPα and sAPPβ in CSF, suggesting tightly linked regulating processes, or alternatively by the fact that sAPPα may reflect overall APP levels. The expected correlation between BACE1 activity and $A\beta_{1-42}$ levels was probably obscured in our study by other factors

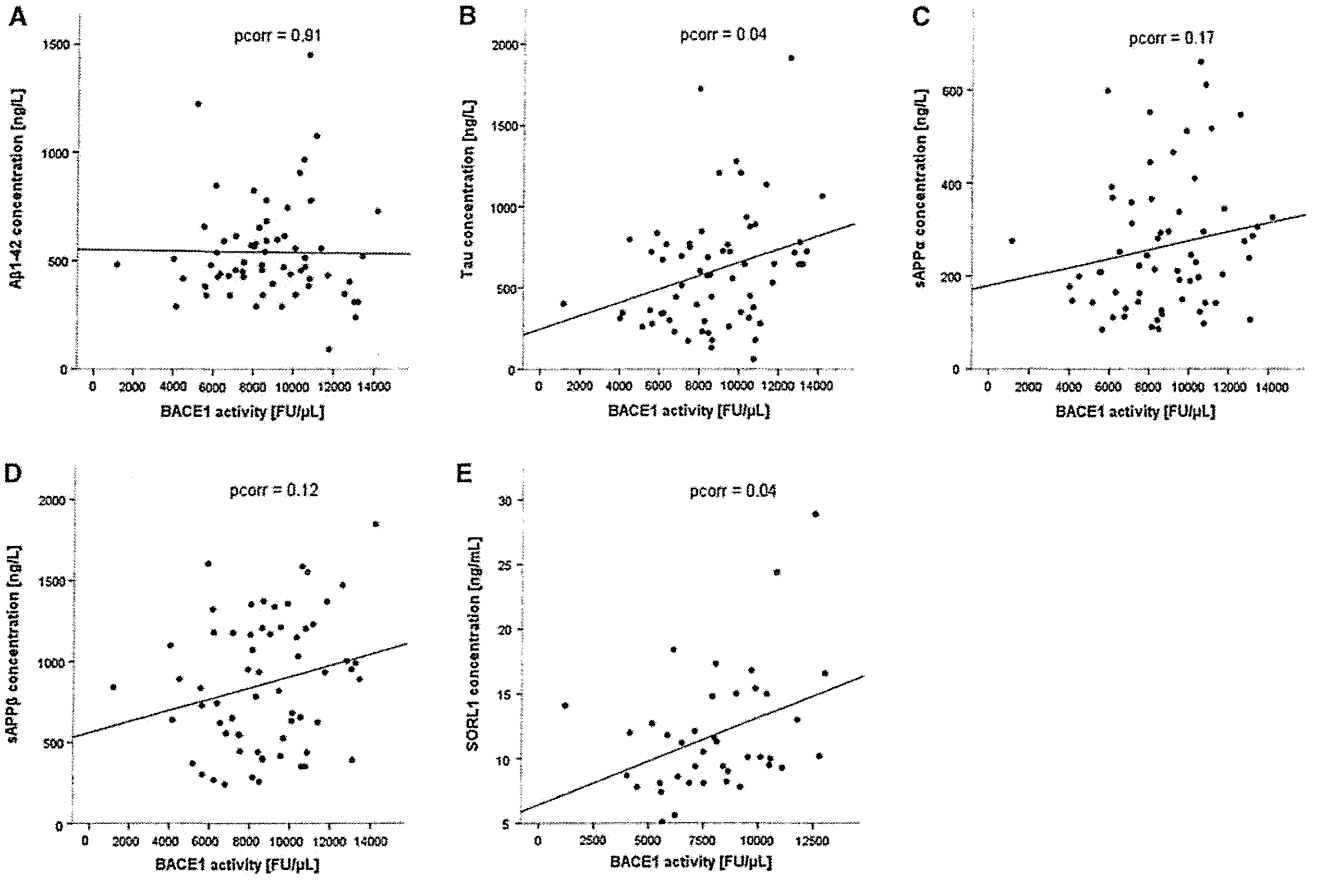


Fig. 1. Scatterplots showing the correlation between cerebrospinal fluid BACE1 activity and the concentrations of (A) $A\beta_{1-42}$, (B) tau, (C) sAPPα, (D) sAPPβ, and (E) SORL1 in the AD group.

influencing Aβ deposition in senile plaques, which is thought to be mirrored by decreased $A\beta_{1-42}$ concentrations in CSF.

### 4.3. BACE1 and SORL1

We also report a positive correlation between BACE1 activity and SORL1 concentrations in CSF. This finding is consistent with in vitro studies showing a direct interaction between BACE1 and SORL1 [30]. SORL1 levels were found to be reduced in the Golgi apparatus and early endosomal compartments in AD [31-33], allowing or promoting APP processing by BACE1 and α-secretase [30,34,35]. The ELISA used in our study determines the soluble form of SORL1, which consists of the extracellular domain of the membrane-spanning SORL1 protein [24]. This extracellular fragment seems to be less efficient than full-length SORL1 in mediating APP transport through the Golgi apparatus [30] because SORL1 fragments have altered binding capacities compared with the full-length SORL1 receptor [36,37]. Taking into account that AD pathology is associated with increased BACE1 activity, it can be hypothesized that the intracellular decline of full-length SORL1 levels in AD is caused by an elevation in the endoproteolytical cleavage of SORL1, resulting in increased concentrations of the less efficient soluble SORL1 that we measure in CSF. However, it has to be mentioned that no causalities can be derived from a study reporting associations between CSF protein levels and that the validity of our argumentation will have to be tested in future studies.

### 4.4. Limitations

The present study should be viewed in light of a number of limitations. The size of the control group was relatively small, and the control subjects were younger than the patients with AD. As a consequence, we were not in a position to explore the differences in BACE1 activity between physiological aging and AD. Furthermore, although not very likely, some patients with causes for cognitive impairment other than AD might have been included despite the rigorous diagnostic assessment. No pathological verification of diagnoses was available, but current diagnostic criteria for AD have been shown to be very accurate for populations recruited at specialized centers. On the one hand, further research on larger samples with age-matched control groups is needed to replicate our findings; on the other hand, genetic variants of SORL1 will also have to be considered in future analyses. The thus-far inconclusive findings on SORL1 in CSF [16,17]

might probably be partly explained by the genetic association of four other vacuolar protein sorting 10 protein-domain receptors with sporadic AD [35], which may dilute the effect of any individual marker including SORL1.

## 5. Conclusion

Our study provides a further piece of evidence pointing to the associations between BACE1 on the one hand and relevant CSF markers of AD on the other hand, including the soluble form of the APP sorting receptor SORL1, the first product of APP cleavage by BACE1 (sAPPβ), and a marker of axonal degeneration (tau). Although our investigation was not designed to establish any diagnostic validity of the studied CSF proteins, it still strongly supports the relevance of BACE1 and SORL1 in CSF for AD and their potential benefit as AD biomarkers and therapeutic targets.

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# Circulating soluble LR11/SorLA levels are highly increased and ameliorated by chemotherapy in acute leukemias

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ABSTRACT

*Background:* LR11/SorLA, a receptor interacting with CD87 on monocytes and macrophages, is highly expressed on human immature hematopoietic stem cells. However, it is unknown whether LR11 is expressed on premature leukemic cells, and whether the levels of circulating soluble LR11 (sLR11) shed from leukemic cells correlate with disease state.
*Methods:* The expression of LR11 on leucocytes and leukemic cells was examined by flow cytometry. Serum sLR11 levels were measured by ELISA in patients with various hematological diseases, including 43 acute myeloid leukemia (AML) and 23 acute lymphoblastic leukemia (ALL) patients. Data were subjected to statistical analysis for validation of sLR11 levels and patients' clinical data.
*Results:* LR11 is specifically expressed in monocytes, and surface levels on leukemic cells are highly induced in both AML and ALL. sLR11 levels of acute leukemia patients were significantly increased ($P<0.001$) (ALL, $73.5\pm93.5$ ng/ml; AML, $26.8\pm29.1$ ng/ml) in comparison to controls ($9.2\pm3.3$ ng/ml). Patients with AML and ALL in remission showed significantly decreased sLR11 levels to below 20 ng/ml.
*Conclusions:* LR11 and its released soluble form are strongly elevated in acute leukemias. Remarkably, this increase in circulating sLR11 levels is ameliorated at complete remission.


## 1. Introduction

Chemotherapy in combination with the use of allogeneic hematopoietic stem cell transplantation has improved the prognosis of acute leukemia patients [1–3]. However, primary induction failure and relapse are still major problems affecting survival, requiring efficient prognostic factors at diagnosis and for monitoring minimal residual disease (MRD). Migration through vascular endothelia and underlying extracellular matrices is essential for mobilization and homing processes of hematopoietic stem and progenitor cells (HSPC) between bone marrow and circulation [4]. Understanding the underlying regulatory features is pivotal for evaluating the (i) expansion of leukemic cells originating from post-chemotherapy bone marrow MRD and (ii) efficacy of HSPC mobilization induced by G-CSF and analogues for transplantation in patients with acute leukemias [5,6]. HSPC migration is strictly controlled by a close interplay between chemokines and adhesion molecules selectively expressed in these migrating cells, stromal cells, or endothelial cells [4–6]. Recent clinical studies suggest that the levels of circulating soluble forms of adhesion molecules and/or cell surface receptors are associated with G-CSF-induced mobilization of HSPC and with the prognosis of leukemia patients under chemotherapy [7,8]. However, our

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