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Soluble SorLA in CSF, a novel biomarker to explore disrupted trafficking of SorLA protein in Alzheimer disease

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Abstract

Background The SorLA protein, encoded by the *Sortilin-related receptor 1* (*SORL1*) gene, is a major player in Alzheimer's disease (AD) pathophysiology. Functional studies demonstrated that SorLA deficiency results in increased production of Aβ peptide, and thus a higher risk of AD. SorLA can be subject to proteolytic shedding at the cell surface, leading to the release of the soluble ectodomain of the protein (sSorLA) in the extracellular space. Recently, we demonstrated that a large proportion (~25%) of rare *SORL1* missense variants found in AD patients alter SorLA trafficking along the constitutive secretory pathway, resulting in reduced delivery of SorLA to the plasma membrane and thus a loss of function. Here, we aimed to determine if CSF levels of sSORLA in AD patients carrying *SORL1* rare variants that impact or not the trafficking of the protein can be used as a novel biomarker to explore disrupted trafficking of SorLA protein in AD.

Methods A total of 151 participants were categorized into 5 study groups: controls without any neurodegenerative disease (n=30), patients suffering from Fronto-Temporal Lobar Degeneration (FTLD, n=34), AD patients not carrying a *SORL1* rare variant (AD $^{SORL1\ WT}$, n=40), AD patients carrying *SORL1* trafficking-defective variants or a protein-truncating variant (PTV) (AD$^{SORL1\ TD}$, n=16), and AD patients carrying a *SORL1* variant with no evidence of trafficking defect (AD $^{SORL1\ nTD,}$ n=31). Thirty-three unique rare variants of *SORL1* were included for this study: 3 PTVs, 13 missense variants that impact SorLA protein trafficking in in vitro cellular assays, and 17 variants with no detectable effect on SorLA protein trafficking. We measured amounts of cleaved sSorLA by western blot in CSF samples.

Results We found significantly decreased levels of sSorLA in AD$^{SORL1\ TD}$, compared to all other groups. According to ROC curve analysis, levels of sSorLA showed good performances to distinguish AD$^{SORL1\ TD}$ patients from other AD patients (AUC=0.89 [95%CI: 0.81-0.97]).

Conclusions Our results suggest that differential levels of sSorLA in CSF could be used as a novel marker to explore disrupted trafficking of SorLA protein in Alzheimer disease. This could help solve some proportion of variants of uncertain significance in *SORL1*.

Keywords Alzheimer's disease, Biomarker, sSorLA, CSF

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Background

The SorLA protein, encoded by the *Sortilin-related receptor 1* (*SORL1*) gene, is a major player in Alzheimer's disease (AD) pathophysiology [1–4]. SorLA is a large transmembrane protein (~250-kDa), that is part of the mammalian VPS10p sorting receptor family. These receptors interact with various cargo proteins and are known to play diverse roles in intracellular sorting and trafficking processes.

Newly synthesized SorLA molecules follow the constitutive secretory pathway from the endoplasmic reticulum (ER) to the cell surface, through the Golgi. Intact SorLA molecules undergo clathrin-dependent endocytosis. Internalized molecules move to the early endosomes, with most sorting to the trans-Golgi network (TGN) for continuous shuttling between TGN and endosomes [5]. Alternatively, SorLA may sort from endosomes to the cell surface [6] or to lysosomes [7].

Functional studies demonstrated that SorLA deficiency results in increased production of Aβ peptides [8–12], and thus a higher risk of AD. The Aβ peptide is the major constituent of senile plaques in the brain of patients, one of the two pathological hallmarks of AD. To perform its protective function against the secretion of the Aβ peptide, the SorLA protein acts at several levels [5, 13, 14]: (1) SorLA limits the access of APP to late endosomes, where it undergoes cleavage into Aβ by the β- and γ-secretases, ultimately leading to reduced Aβ production, and (2) it targets nascent Aβ molecules to the lysosomal compartment, thereby contributing to the catabolism of the Aβ peptide.

SORL1 is now considered as a major AD risk factor gene. Rare (frequency<1%) truncating (introducing a premature stop codon; PTV) or missense predicted damaging variants of the *SORL1* gene are identified in ~2.75% of early-onset Alzheimer's disease (EOAD) patients [15]. PTVs show the strongest effect on AD risk, with odds ratio up to 28 regarding EOAD cases [2]. When carried along another moderate-to-strong risk factor like APOE ε4, loss-of-function variants of *SORL1* associate with a high penetrance of AD [16], this might thus be of clinical interest. The interpretation of the biological effect of PTVs appears to be straightforward, i.e. leading to reduced SorLA levels through nonsense mediated decay (NMD) and protein truncation and hence increased Aβ production. Any *SORL1* PTV can then be interpreted as a probable strong risk factor for AD in the context of medical genetics [17]. At the gene level, rare predicted deleterious missense variants of *SORL1* are also associated with increased AD risk, as a whole. However, the functional effect of every single missense variant remains complicated to determine, with a large diversity of variants showing diverse degrees of decreased SorLA function, from a neutral effect to a complete loss of function. Thus, many patients remain without a clear genetic status regarding *SORL1*, following the identification of a variant of uncertain significance, most of the time being a missense rare variant [17]. In addition, implementing in vitro testing of every novel missense variant does not appear to be easily feasible in a medical setting. Recently, we identified a major pathophysiological mechanism wherein a large proportion of rare *SORL1* missense variants found in AD patients alter the maturation and trafficking of the SorLA protein along the constitutive secretory pathway (19/90 variants tested), resulting in reduced delivery of SorLA to the plasma membrane and in a loss of SorLA function regarding Aβ production [18]. Maturation and shedding defects were subsequently demonstrated for two additional variants [19, 20]. Beside this mechanism, one variant has been shown to decrease SorLA binding to Aβ, leading to decreased lysosomal targeting and degradation of Aβ [7, 21].

As a type-1 transmembrane protein, SorLA can undergo regulated intramembrane proteolysis at the cell surface, leading to the release of the soluble ectodomain of the protein (sSorLA) in the extracellular space [22, 23]. The ectodomain of SorLA is processed by the tumour necrosis factor-α converting enzyme (TACE/ADAM17) [22, 24], but the physiological relevance of this fragment is unclear. The value of the cerebrospinal fluid (CSF) sSorLA as a potential AD diagnostic biomarker has been explored in several studies but the results are somewhat inconsistent. Some studies reported increased levels of sSorLA in AD [25, 26], other found decreased levels of sSorLA in AD [27], while some observed no difference [28–30]. Importantly, the genetic status of *SORL1* was not known in the above-mentioned studies [25–30]. One can assume that only carriers of certain *SORL1* rare variants may show decreased levels of sSorLA in the CSF, thus explaining that CSF sSorLA would not be a biomarker of AD as a disease, but maybe in a subset of patients. Depending on the underlying pathophysiological mechanisms, one can anticipate a strong reduction of CSF sSorLA levels in carriers of PTVs resulting in loss of protein expression, in carriers of maturation/trafficking variants reducing the amount of protein at the plasma membrane, while no effect is expected on sSorLA levels for variants affecting binding to Aβ for which the transport along the secretory pathway remains identical to the wild-type protein as well as, obviously, for neutral variants. Consistently, a significant decrease in the shedding of two maturation/trafficking defectives variants SorLA D1105H and SorLA R953C was observed in transfected HEK cells [19, 20].

In this study, we aimed to determine CSF levels of sSORLA in AD patients carrying *SORL1* rare variants

that impact or not the trafficking of the protein in cellular assays, in comparison to AD patients without *SORL1* variants and two control groups, i.e. patients with a Fronto-Temporal Lobar Degeneration (FTLD) and controls without any neurodegenerative disease. We found significantly decreased levels of sSorLA in AD patients with *SORL1* trafficking variants or PTVs, compared to all other groups, suggesting that differential levels of sSorLA in CSF could be used as a novel marker to explore disrupted trafficking of SorLA protein in Alzheimer disease.

Methods

Participants selection

This study was made possible by the use of CSF samples taken in routine practice for diagnostic purposes by lumbar puncture. Patients with AD were recruited through the « Centre National de Référence Malades Alzheimer Jeunes » (CNRMAJ), which is responsible for genetic analysis of EOAD throughout France. All AD patients had compatible AD biomarkers in CSF and gave written consent to genetic analysis by exome sequencing (for exome sequencing procedures and variant interpretation, see ref. [17]). We selected patients after exome interpretation, among those without any pathogenic variants in *PSEN1*, *PSEN2* or *APP* and presenting or not a rare (frequency <1%) *SORL1* truncating (PTV, i.e. nonsense, canonical splice site or frameshift variants predicted to trigger nonsense-mediated decay) or missense variant. Among the CSF samples centralized retrospectively at Rouen University Hospital, those of selected patients were analyzed as described below. Patients with FTLD were all recruited from the Rouen Memory Centre of the Rouen University Hospital and diagnosis were made according to current diagnostic criteria [31–34], including CSF biomarkers excluding a diagnosis of AD. The second control group consisted of patients who consulted for memory complaint and for whom the final diagnosis based on clinical, neuropsychological, brain imaging and lumbar puncture biomarker data ruled out a neurodegenerative disease. DNA of controls and FTLD patient was not available. This study was approved by the Institutional Review Board of Rouen University Hospital (CERDE # E2024-81) and was conducted in accordance with the Declaration of Helsinki.

CSF sampling and preservation

CSF was obtained by lumbar puncture (LP). All centers used a common 10-mL polypropylene tube to collect CSF (catalog number 62.610.201; Sarstedt, Nümbrecht, Germany). All samples were aliquoted after centrifugation (1,700 x g) for 10 min at +4 °C in polypropylene Eppendorf tubes. Tubes were then rapidly frozen and stored at – 80 °C. All preanalytical steps were harmonized following the ePLM network as previously published [35] to reduce impact of these factors on sSorLA quantification. All samples were processed blindly of the diagnosis, *SORL1* status, and in random order.

cDNA constructs

The SorLA^{2131} cDNA construct has been previously described in [18]. *SORL1* missense variants were introduced by site-directed mutagenesis over the pCMV6-XL5-WT-SorLA ^{2131} plasmids using the QuikChange II XL Site-Directed Mutagenesis Kit from Agilent (Santa Clara, CA, USA) or the Q5® Site-Directed Mutagenesis Kit (New England Biolabs, Ipswich, MA) according to the manufacturer's instructions. The sequences of mutagenized oligonucleotides are indicated in Table S1. All constructs were sequence-verified.

HEK293 cell culture and transfection

HEK293 cells were grown and maintained in DMEM/F12 medium (Gibco/Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FCS (Eurobio, Les Ulis, France). Cells were plated in 12-well plates 48 h before transfection, grown to approximately 90% confluence, and transfected with the plasmid constructs using the lipofectamine 3000 reagent (Invitrogen™,Thermo Fischer Scientific) according to the manufacturer's protocol. Supernatants were collected and cells were harvested 48 hours after transfection, and processed for western blotting.

Protein extraction

HEK293 cells were homogenized in RIPA buffer (Tris-HCl pH8 0.05 M, NaCl 0.15 M, NP-40 1%, Sodium deoxycholate 0.5%, Glycerol 10%, DTT 2 mM), plus protease inhibitors (Sigma-Aldrich, St-Louis, MI, USA) and phosphatase inhibitors (Halt phosphatase, Thermo Fisher Scientific). After 10 min on ice, lysates were centrifuged (12,000 × g, 10 min, 4 °C) and the supernatant containing soluble proteins was collected. Protein concentrations were measured with the DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA).

Western blotting

Proteins were resolved by Tris-acetate NOVEX NuPAGE 3–8% (Invitrogen™) gels and then transferred onto nitrocellulose membrane using the Trans-Blot Turbo system (Bio-Rad Laboratories). Membranes were then blocked in 5% non-fat milk and immunoblotted with the appropriate monoclonal anti-SorLA primary antibody: 48/LR11 mAb# 611860, BD Bioscience (Franklin Lakes, NJ, USA), 1:5,000 or c-terminal ab226339, Abcam (Cambridge, UK), 1:1,000; D8D4G mAb#79322. Membranes were then incubated with secondary peroxidase-labeled

anti-mouse or anti-rabbit antibody (1:20,000) from Jackson Immunoresearch Laboratories (WestGrove, PA, USA), and signals were detected with chemiluminescence reagents (ECL prime, Cytiva, France). Signals were acquired with a GBOX (Syngene, Cambridge, UK), monitored by the Gene Snap software (Syngene). When appropriate, the signal intensity in each lane was quantified using the Genetools software (Syngene).

Determination of sSorLA levels

The levels of sSorLA in CSF were determined by western blot. Briefly, for each CSF sample, 10 µL were analyzed at least twice on separate gels. On each gel, two deposits from a single pool of 10 CSF samples were systematically included to serve as an internal normalization control. The signal intensities of the sSorLA protein were quantified and normalized to the mean of the two deposits of the pooled samples arbitrarily set to 100. When appropriate, a further level of normalization, in relation to the total amount of protein detected in the CSF, was applied.

Statistical analysis

To identify trafficking-defective variant, we compared the variants to the wild-type (WT) form of SorLA by performing a regression model where the outcome was the mean of normalized signal intensities measured in western blot. For each variant, the p-value refers to the nullity test of its associated coefficient in the model.

We compared the levels of CSF sSorLA between groups using an ANOVA followed by Tukey post-hoc tests and Bonferroni correction. ANOVA was performed both before and after normalization of CSF sSorLA levels on total protein levels. To test the robustness of our results, we also performed ANOVA adjusted for age at PL or gender; and in the subset of AD patients, we performed ANOVA adjusted for age at onset or the number of *APOE*-E4 alleles.

Then, based on the subset of AD patients, we computed receiver operating characteristic (ROC) curve associated with the ability of the levels of CSF sSorLA to distinguish AD patient carrying a trafficking-defective *SORL1* variant from the other AD patients. All statistical analyses were performed using the R software. All tests were two-tailed at the significance level of 5% (ns: not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Results

Demographic and clinical characteristics

A total of 151 participants, including 47 AD patients with a *SORL1* PTV (n=3) or missense rare variant (n=44), 40 AD patients not harboring any PTV or missense rare variant of *SORL1*, 34 patients suffering from FTLD, and 30 controls without any neurodegenerative disease, were retrospectively recruited for this study. We observed 30 distinct *SORL1* missense rare variants as some are shared by several participants (Figure S1A, Table S2). For 7 of them, the impact on protein transport along the secretory pathway had not been previously investigated. To assess this, we first examined the impact of these 7 variants on SorLA trafficking using a secreted SorLA^{2131} protein model. Following transient transfection in HEK293 cells, both intracellular and secreted SorLA proteins were analyzed by western blotting. As expected, the WT-SorLA^{2131} proteins were detected both in the intracellular lysate and the conditioned medium (secreted) (Figure S1B). All assessed SorLA variants were present in the cellular lysate, confirming proper expression in HEK293 cells. We observed that the R953C, A1812V, V1459I and P1654T variants resulted in a statistically significant decrease in secretion levels compared to the wild-type construct, indicating a functional impact of these variants on SorLA trafficking to the cell surface. In contrast, the R480C and D1345N variants exhibited wild-type trafficking patterns. Additionally, the W804 C showed a statistically significant increase in secretion levels compared to the wild-type construct, although the signification of this increase in SorLA levels remains unclear. Overall, among the 30 distinct *SORL1* variants selected for this study, 13 were found to impact the trafficking of the SorLA protein, while 17 showed no detectable impact in our cellular assays. These results allowed us to categorize the participants into 5 study groups: controls without any neurodegenerative disease (Control group, n=30), patients suffering from FLTD (FTLD, n=34), AD non-*SORL1* variant carriers (AD$^{\text{SORL1 WT}}$, n=40), AD with *SORL1* trafficking-defective variants or PTV (AD$^{\text{SORL1 TD}}$, n=16), and AD carrying *SORL1* variants that do not alter trafficking (AD$^{\text{SORL1 nTD,}}$ n=31). Group characteristics are shown in Table 1.

CSF sSorLA levels are lower in patients carrying a *SORL1* maturation/trafficking-defective or truncating variant

We then determined CSF sSorLA levels by western blot in our five study groups. First, to confirm the specificity of our signal, we used two different antibodies targeting distinct domains of the protein (Figure S2). The 48/LR11 antibody targeted the CR-cluster domain of the ectodomain, while the ab226339 antibody was directed against the cytoplasmic part of the protein, a domain absent in the sSorLA fragment. As expected, the antibody directed against the ectodomain of SorLA were able to detect a signal, which was absent when using the antibody directed against the cytoplasmic domain. Western

Table 1 Description of the dataset

					AD (SORL1)		
		Control	FTLD	AD (SORL1 WT)	AD (SORL1 nTD)	AD (SORL1 TD)	AD (SORL1 nTD + TD)
N		**30**	**34**	**40**	**31**	**16**	**47**
Age at LP	years, mean (min-max)	63 (40–75)	65 (45–77)	63 (51–76)	65 (52–76)	61 (46–69)	64 (46–76)
AAO	years, mean (min-max)			60 (48–72)	60 (49–73)	57 (44–65)	59 (44–73)
Time AA0-LP	years, mean (min-max)			3.9 (0.6–13.9)	4.7 (0.3–10.1)	3.7 (1.3–9.8)	4.4 (0.3–10.1)
Gender	Male N (%)	15 (50.00%)	18 (52.94%)	16 (40.00%)	9 (29.03%)	6 (37.50)	15 (31.91 %)
	Female N (%)	15 (50.00%)	16 (47.06%)	24 (60.00%)	22 (70.97%)	10 (62.50)	32 (68.09%)
APOE	ε2/ε2 N (%)			0	0	0	0
	ε2/ε3 N (%)			1 (2.50%)	1 (3.23%)	0	1 (2.13%)
	ε2/ε4 N (%)			0	2 (6.45%)	0	2 (4.25%)
	ε3/ε3 N (%)			16 (40.00%)	9 (29.03%)	8 (50.00%)	17 (36.17%)
	ε3/ε4 N (%)			16 (40.00%)	15 (48.39%)	7 (43.75%)	22 (46.81%)
	ε4/ε4 N (%)			7 (17.50%)	4 (12.90%)	1 (6.25%)	5 (10.64%)

Mean (min-max) means that the variable is described by the mean (minimum-maximum)

N (%) means that the variable is described by the number (and percentage) of individuals in each category

AAO and *APOE* status are available for AD patients only

LP Lumbar Puncture, *AAO* Age At Onset, *FTLD* Frontotemporal Lobar Degeneration, *AD* Alzheimer Disease, *TD* Trafficking Defective, *nTD* not Trafficking Defective

blot analysis of CSF samples using the 48/LR11 antibodies revealed that the levels of CSF sSorLA were significantly decreased in $AD^{SORL1\ TD}$ group compared to the 4 others groups (ANOVA $F_{4,146}$=8.27, p=4.88e-06) (Fig. 1; Figure S3, Table S3). Importantly, normalization of the sSorLA signal by the CSF total protein concentration did not change this result (ANOVA $F_{4,146}$=6.37, p=9.48e-05) (Figure S4, Table S3). Adjustment on age at LP or gender did not affect the conclusions. Similarly, in the subset of AD patients, adjustment for age at onset or *APOE* status also did not affect the conclusions.

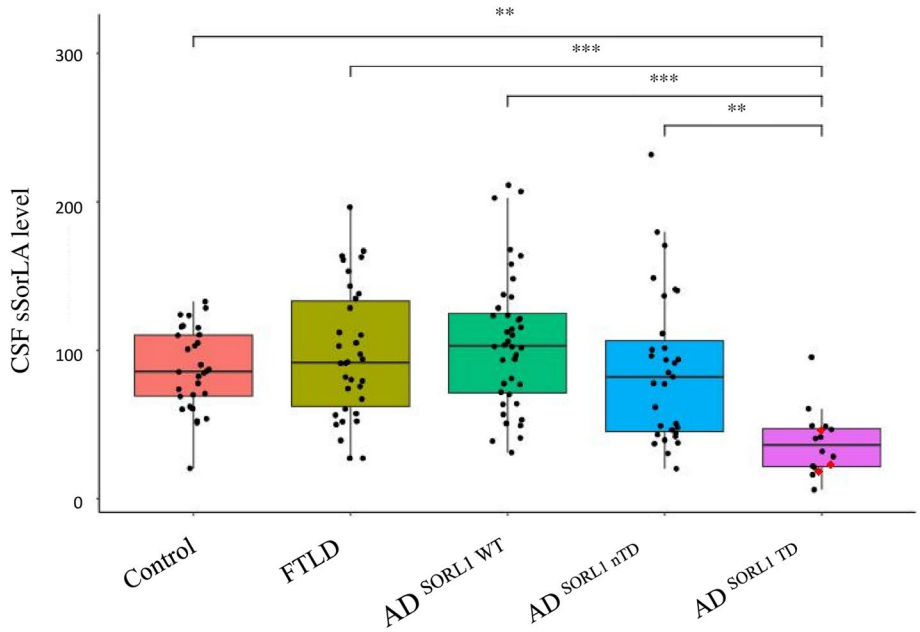

Fig. 1 Distribution of levels of CSF sSorlA in each group. Significance levels of post-hoc tests were displayed after Bonferroni correction for significant differences (*p < 0.05, **p < 0.01, ***p < 0.001). PTV variants are highlighted as red dots

CSF sSorLA is an argument for classification of rare missense *SORL1* variants as a loss of function, AD-associated risk factor variant

We assessed the diagnostic accuracy of CSF sSorLA to discriminate between *SORL1* variants impacting or not the trafficking of SorLA protein as determined in cellular assays. To this regard, a ROC curve analysis was undertaken (Fig. 2, Figure S5). The area under the curve (AUC) was 0.89 (95% CI 0.81–0.97). Confidence intervals denote that the AUC of CSF sSorLA is significantly different from the area under the diagonal, which corresponds to a random performance of a test.

Discussion

In the present study, we detected significantly decreased CSF levels of sSORLA in AD patients carrying *SORL1* rare variants that impact the trafficking of the protein in in vitro tests or introduce a premature stop codon (PTV), compared to AD patients carrying *SORL1* rare variants that do not impact the trafficking of the protein, AD non-*SORL1* variant carriers, patients with FTLD and controls without any neurodegenerative disease. Furthermore, AUC analysis showed that levels of sSorLA show good performances to distinguish AD patients carrying *SORL1* rare variants that impact the trafficking of the protein in in vitro tests from other AD patients, supporting a role of sSorLA CSF levels as a novel marker to explore disrupted trafficking of SorLA protein in AD.

In the field of medical genetics, variants are classified using the American College of Medical Genetics and Genomics – American for Molecular Pathology (ACMG-AMP) recommendations into five categories, based on variant and patient-specific arguments [36], namely benign (class 1), likely benign, uncertain significance, likely pathogenic and pathogenic (class 5). Missense variants remain a major source of variants of uncertain significance. Such variants do not allow any use as a diagnostic confirmation or for genetic counseling. In vitro functional assessment can help reclassify some of the missense variants of uncertain significance, but they remain difficult to set up in a clinical setting and there is a lack of availability of such tests, when existing. *SORL1* rare

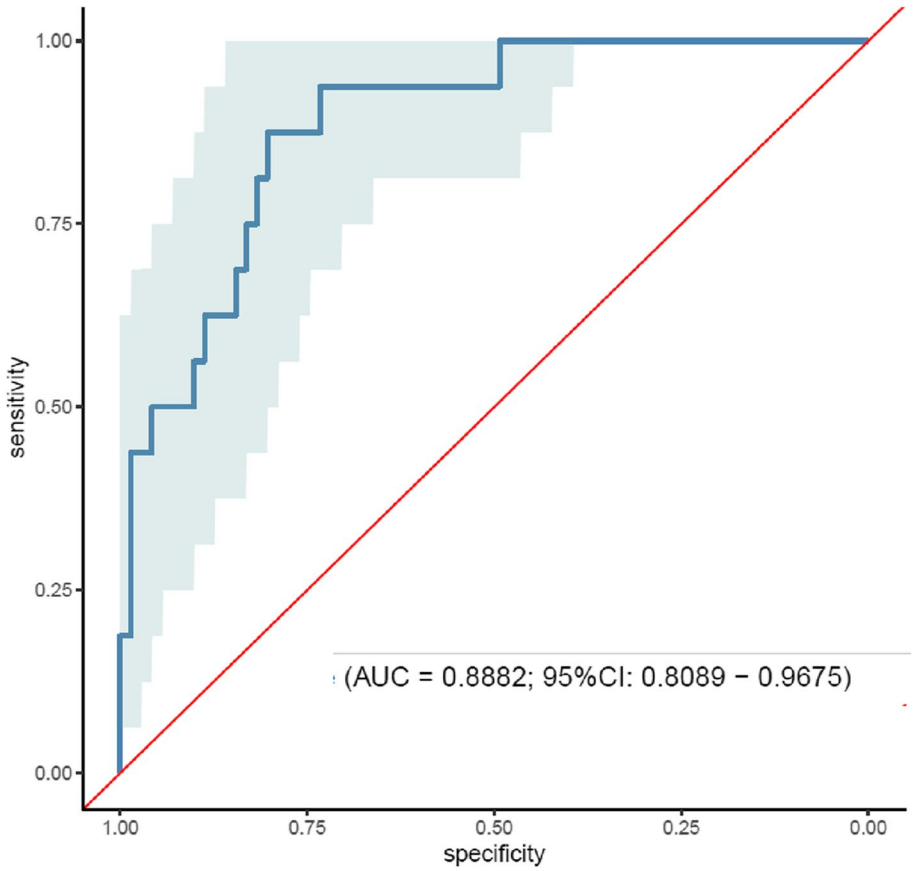

Fig. 2 ROC curve associated with ability of CSF sSorlA levels to predict if a patient is carrier of a trafficking defective *SORL1* variant. The ROC curve was obtained by computing sensitivity and specificity associated with different thresholds of CSF sSorlA levels to distinguish between patients in AD $^{SORL1\ TD}$ group and patients in AD $^{SORL1\ nTD}$ + AD $^{SORL1\ WT}$ group

variants are considered as a strong AD risk factor, as none of them has been shown to be fully penetrant *per se*, up to now [2, 16]. However, loss-of-function *SORL1* variants in the context of other risk factors, such as the *APOE4* allele, show a full or nearly full penetrance, suggesting that they might be used in genetic counseling, provided that (i) *SORL1* rare variants can be accurately classified and (ii) knowledge on risk factors other than the *APOE4* allele is sufficient, not to miss any strong risk or protective variant that might change the individual level of risk. In addition, they might also be used in prevention trials [37]. Recently, we proposed a framework to classify modest, moderate and strong risk factors in a clinical setting [17]. Loss-of-function *SORL1* variants are considered as a strong risk factor, including truncating variants and those showing a strong level of loss of function in vitro. However, as for many other genes, it remains challenging to propose a patient-based in vitro assessment of every novel missense variant identified in a patient. In addition, there are some discrepancies between some in vitro test results depending on the specific assays that are applied. Here, we propose that sSorLA CSF levels may reflect the degree of loss of function that a *SORL1* variant might be responsible of, through decreased expression, protein truncation, or maturation/trafficking defects. Variants associated with a significant decrease in sSorLA levels in the CSF might thus be reclassified as definite strong AD risk factors in the clinical risk factor classification [17], thus not necessarily requiring to perform an in vitro assessment. However, because of inter-individual variability and some overlap between sSorLA CSF levels between different groups, intermediate levels might not be sufficient to conclude. Conversely, normal levels of sSorLA in the CSF do not preclude a loss of function effect of a given *SORL1* variant, as some variants may keep normal level of protein membrane surface expression, but still show some degree of loss of function, for example in their Aβ binding abilities [7, 21]. The result of such an assay may thus be used only for a positive classification as a definite risk factor, and not to reclassify a given variant as likely benign.

Of note, sSorLA CSF levels have previously been assessed as a putative AD biomarker, as previously mentioned [25–30] before rare variants of *SORL1* were discovered [3, 4]. Our results, together with the results of genetic studies on rare variants, do not support a role of sSorLA CSF levels as an AD diagnosis biomarker, but rather as a strong argument facilitating the reclassification of certain rare *SORL1* variants. Alternatively, in case sSorLA would be assessed prior to any genetic study, the identification of low levels of sSorLA in the CSF should lead to propose the sequencing of the *SORL1* gene, as it might be a consequence of a truncating or a maturation/trafficking deficient variant.

The primary limitation of this study is the use of western blotting as a quantification method. Western blotting involves a series of interdependent steps that can introduce small variations, potentially influencing the accuracy of data analysis. Additionally, western blot only allows for relative protein abundance measurement, and not absolute concentration. Hence, it would be helpful to confirm and to complete those findings using ELISA (enzyme-linked immunosorbent assay). Furthermore, the classification of *SORL1* missense variants as "trafficking-defective" was primarily assessed using a model based on the overexpression of secreted truncated SorLA^{2131} protein in HEK cells that evaluates the impact of *SORL1* variants on protein transport along the secretory pathway. The use of the HEK cell line, with a truncated form of SorLA, presents some limitations: first, we cannot rule out the possibility that certain variants might affect protein trafficking solely in the context of the full-length protein and/or in neuronal cell types. Second, we cannot directly assess the cleavage of the protein by TACE at the plasma membrane, an event that, if affected, could also lead to a reduction in the quantity of protein in the extracellular space. Thus, it is possible that some variants labeled as "non-trafficking variants" in this study may still affect protein trafficking and extracellular level in vivo. Finally, although the effect of preanalytical factors such as tube composition, time before freezing the sample or blood contamination on sSorLA quantification is unknown, the compliance with harmonized procedures and the exclusion of any blood-contaminated CSF sample by blood cell count (as recommended for Alzheimer's disease biomarker assays) are likely to have reduced this risk.

Conclusions

Here, we identified that soluble SorLA levels are associated with the carrier status of a *SORL1* rare variant affecting SorLA trafficking or introducing a premature stop codon. Such sSorLA levels might be used for the reclassification of *SORL1* variants of uncertain significance into the category of definite AD risk factors, in case of decreased sSorLA CSF levels, in a medical setting.

Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid-β
APP	Amyloid Precursor Protein
EOAD	Early-onset AD
HEK	Human embryonic kidney
LP	Lumbar Puncture
PTV	Protein-truncating variants
SORL1	Sortilin-related receptor 1gene
SorLA	Sortilin-related receptor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-025-01748-0.

Supplementary Material 1: Figure S1. Impact of SorLA genetic variants on SorLA trafficking in HEK293 cells. (A) Schematic representation of the location of SorLA variants examined in this study. Variants were classified according to their predicted deleteriousness assessed by three bioinformatic tools: PolyPhen-2, MutationTaster, and SIFT. Twenty two of them were predicted damaging by all three bioinformatics tools (Mis3 variants, red), 4 were predicted damaging by two of the three tools (Mis2, gold) and 7 were predicted damaging by one or none of the tools (Mis1-0, black). The 3 *SORL1* protein truncating variants (PTV) and the 13 missense variants presenting trafficking defects in in vitro cellular assays are represented on the bottom line of the schematic presentation of the SorLA protein. (B) Wild-type (WT) and mutant SorLA^{2131} proteins were expressed in HEK293 cells. The SorLA proteins secreted into the cellular medium and the corresponding cell lysate (Intrac.) were analyzed by immunoblotting using an anti-SorLA antibody. (C) Quantification of SorLA secretion efficiency from 3 independent experiments. WT-SorLA^{2131} were arbitrarily set at 1 arbitrary unit. Protein levels were compared by using a regression model where WT was the reference. (ns: not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). Figure S2. Western blot analysis of sSorLA in CSF with antibodies raised against different domains of the SorLA protein. (A) Schematic diagram of SorlA, with the locations of epitopes for mAbs. (B) Western blot analysis of sSorLA in CSF samples (L1233, L1237) and cellular lysate prepared from HEK293 cells overexpressing full-length SorLA protein (FL). Figure S3. Western blot analysis of sSorLA in CSF samples. Western blot analysis of sSorLA in all CSF samples collected in this study. Each CSF sample was analyzed in duplicate. On each gel, two deposits from a single pool of 10 CSF samples were systematically included to serve as an internal normalization control. The signal intensities of the sSorLA protein were quantified and normalized to the mean of the two deposits of the pooled samples, arbitrarily set to 100 (Fig. 1). When appropriate (Figure S4), a further level of normalization, in relation to the total amount of protein detected in the CSF, was applied. Figure S4. Distribution of sSorlA levels normalized by the CSF total protein concentration in each group. Significance levels of post-hoc tests was displayed after Bonferroni correction for significant differences ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$). Figure S5. ROC curve associated with ability of normalized CSF sSorlA levels to predict if a patient is carrier of a trafficking defective *SORL1* variant. The ROC curve was obtained by computing sensitivity and specificity associated with different thresholds of normalized CSF sSorlA levels to distinguish between patients in AD $^{SORL1\ TD}$ group and patients in AD $^{SORL1\ nTD}$ + AD $^{SORL1\ WT}$ group.

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Authors' contributions
Conceptualization: R. C., A. Z., D. W., C. S., A. R-L., G. N., M. L.; Resources : M.Q., A. Z., D. W., G. N., A. B., F. B., O. B., J. D., M. F., G. L., S. L., C. M., M. M., L. N., J. P., I. Q., A. R-S., S. S.; Acquisition, analyses: R. C., A. Z., D. W., C. S., A. R-L., G. N., M. L. Interpretation of data: R. C., A. Z., D. W., C. S., A. R-L., G. N., M. L.; Writing – original draft: R. C., A. Z., D. W., C. S., A. R-L., G. N., M. L.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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