Acta Neuropathologica (2019) 138:173–186
https://doi.org/10.1007/s00401-019-01991-4

REVIEW

SORL1 genetic variants and Alzheimer disease risk: a literature review and meta-analysis of sequencing data

Dominique Campion[1,2] · **Camille Charbonnier**[1] · **Gaël Nicolas**[1]

Received: 31 January 2019 / Revised: 11 March 2019 / Accepted: 11 March 2019 / Published online: 25 March 2019

Abstract

Massive parallel sequencing recently allowed the identification of three genes carrying a higher burden of rare, protein-truncating and missense predicted damaging variants in Alzheimer disease (AD) cases as compared to controls: *TREM2*, *SORL1*, and *ABCA7*. *SORL1* encodes SorLA, a key protein involved in the processing of the amyloid-beta (Aβ) precursor protein (APP) and the secretion of the Aβ peptide, the aggregation of which triggers AD pathophysiology. Common *SORL1* single nucleotide polymorphisms had originally been associated with AD with modest odds ratios (ORs). The association of AD with rare *SORL1* coding variants has been demonstrated at the gene level by aggregating protein-truncating (PTV) and rare predicted damaging missense variants. In addition to the loss of SorLA function induced by PTVs, a few missense variants were studied in vitro, showing diverse degrees of decreased SorLA function and leading to increased Aβ secretion. However, the exact functional consequences of most of the missense variants remain to be determined as well as corresponding levels of AD risk. Hereby we review the evidence of the association of *SORL1* common and rare variants with AD risk and conduct a meta-analysis of published data on *SORL1* rare variants in five large sequencing studies. We observe a significant enrichment in PTVs with ORs of 12.29 (95% confidence interval = [4.22–35.78]) among all AD cases and 27.50 [7.38–102.42] among early-onset cases. Rare [minor allele frequency (MAF) < 1%] and ultra-rare (MAF < 10^{-4}) missense variants that are predicted damaging by 3/3 bioinformatics tools also show significant associations with corresponding ORs of 1.87 [1.54–2.28] and 3.14 [2.30–4.28], respectively. Per-domain analyses show significant association with the APP-binding CR cluster class A repeats and the Aβ-binding VPS10P domains, as well as the fibronectin type III domain, the function of which remains to be specified. These results further support a critical role for *SORL1* rare coding variants in AD, although functional and segregation analyses are required to allow an accurate use in a clinical setting.

Keywords SORL1 · Alzheimer · Amyloid · Rare variants · Meta-analysis

Introduction

The genetics of Alzheimer disease (AD) is heterogeneous. Some families harbor extremely rare mutations in *APP*, *PSEN1* or *PSEN2*. Most of these pathogenic variants cause early-onset AD (EOAD, onset before 66 years) with an autosomal dominant transmission. However, in the majority of AD cases, the determinism is more complex, whatever the age of onset. In these forms, a wide diversity of genetic risk factors has been identified, varying both in terms of frequencies and strength of effect on AD risk (for review, see [33]). Among common loci, the *APOE ε4* allele is one of the strongest AD genetic risk factors identified so far, especially when carried at the homozygous state. By the age of 85 years, the lifetime risk of AD without reference to the *APOE* genotype in Caucasians has been evaluated at 11% in

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00401-019-01991-4) contains supplementary material, which is available to authorized users.

✉ Dominique Campion
dominique.campion@univ-rouen.fr

✉ Gaël Nicolas
gaelnicolas@hotmail.com

[1] Department of Genetics and CNR-MAJ, Normandy Center for Genomic and Personalized Medicine, Normandie Univ, UNIROUEN, Inserm U1245 and Rouen University Hospital, 76000 Rouen, France

[2] Department of Research, Rouvray Psychiatric Hospital, Sotteville-Lès-Rouen, France

males and 14% in females. At the same age, this risk reaches 23% and 30%, respectively, for *APOE ε3ε4* male and female carriers. The risk rises up to 51% and 60%, respectively, for *APOE ε4ε4* male and female carriers [15]. Other common loci, most of them having been identified through Genome Wide Association Studies (GWAS), are associated with AD risk with modest odds ratios (OR ≤ 1.3) [22]. More recently, rare coding variants [minor allele frequency (MAF) < 1%] with a moderate-to-high effect have been described in three genes in gene-based analyses: *TREM2*, *SORL1* and *ABCA7* [12, 17, 20, 23, 34, 47]. In addition, single rare variants have also been associated with AD risk (in *TREM2* and *ABI3*) or with AD protection (in *APP* and *PLCG2*) [19, 20, 46]. Of note, two of these genes (*ABCA7* and *SORL1*) show both frequent and rare variants associated with AD risk. Common *ABCA7* and *SORL1* variants were associated with a modest effect size and rare coding variants with a moderate-to-high risk. Here, we will review the role of *SORL1* common and rare variants in the genetics of AD with a specific focus on rare variants. After a meta-analysis of case–control association studies at the gene and the protein domain levels, we will discuss the implications of these results on AD pathophysiology and putative implications for clinical practice.

SORL1 common variants and AD risk

In 2007, Rogaeva et al. [41] studied multiple common single nucleotide polymorphisms (SNPs) in seven genes belonging to endocytic pathways thought to be involved in Aβ generation, including a panel of 29 SNPs located at the *SORL1* locus, in six different datasets of multiple ethnicities. In *SORL1*, two clusters of frequent SNPs defined haplotypes that were independently associated with AD risk. First, SNPs 8, 9, 10 in near complete linkage disequilibrium within intron 6 of *SORL1* were referred to as the 5′ cluster. The "T-A-T" haplotype at SNP 8–10 (frequency of about 40% in Caucasian populations; N.B. should be referred to as the T-A-A haplotype when using the + strand as reference for all three SNPs) was associated with a decreased risk of AD. Among the second cluster, referred to as the 3′ cluster and including SNPs 19–25 spanning exon 25 to intron 39, risk and protective haplotypes were also identified. Further studies attempted to replicate these findings with mixed results (e.g., [6, 25, 27]). Finally, a meta-analysis performed on data of 30,393 individuals provided confirmatory evidence that these two frequent *SORL1* variant clusters in different linkage disequilibrium blocks were associated with AD risk with modest and similar odds ratios [39]. The minor allele of SNP8 (rs668387) from the 5′ block displayed a protective effect with an OR of 0.92 [0.88–0.97]. Likewise, SNP19 (rs2070045), from the 3′ block, was associated with an OR of 0.92 [0.88–0.99] [39]. This association was further probed using endophenotypes such as white matter hyperintensities and hippocampal atrophy on magnetic resonance imaging, cerebrospinal fluid measures of $A\beta_{42}$ peptides or *SORL1* expression in the human brain [1, 11, 29].

Different mechanisms have been proposed to establish a correlation between the risk haplotypes and the regulation of *SORL1* expression. Rogaeva et al. [41] showed that the expression of *SORL1* mRNA was decreased in lymphoblasts from carriers of the at-risk 3′ haplotype in the primary study. Consistent with the hypothesis that this haplotype may be associated with reduced *SORL1* expression, Caglayan et al. [8] showed that two SNPs within the 3′ haplotype were associated with decreased levels of SorlA protein in AD brains. Using human induced pluripotent stem cells, Young et al. [52] demonstrated that human induced neurons carrying the 5′ risk haplotype showed a reduced response to *SORL1* mRNA expression induced by BDNF.

More recently, genome-wide association studies (GWAS) performed on Japanese and Caucasian individuals identified a third association signal mapping between the above mentioned 5′ and 3′ blocks [30]. The alternate alleles of the most associated SNPs, rs11218343 and rs3781834 were protective in both populations with similar odds ratios (0.74–0.83) despite different frequencies (4 and 2%, and 34% and 23%, respectively, for rs11218343 and rs3781834 in the Caucasian and in the Japanese samples). These SNPs appeared to be independent from the previously reported 5′ cluster, but not necessarily from the 3′ cluster. Meanwhile, a meta-analysis performed on 74,538 individuals further confirmed these results [22]. *SORL1* was one of the 20 loci associated with AD risk with genome-wide significance ($p < 5 \times 10^{-8}$). The intronic rs11218343 SNP (MAF = 0.04) provided the second strongest signal with a protective effect of the C allele leading to an OR of 0.77 (95% CI = [0.72–0.82], $p = 9.7 \times 10^{-15}$) [22]. A recent update of the GWAS analysis, with 94,437 individuals now included, confirmed this result (OR = 0.80, 95% CI = [0.75–0.85], $p = 2.9 \times 10^{-12}$) [21].

In summary, from targeted SNP analyses and GWAS, there is now clear evidence of an association of several *SORL1* common variants with AD risk. Although the protective effects seem to be modest as measured by ORs, they remain in the ranges of most of the ORs associated with common variants in GWAS and the signal is even among the highest ones, far after the notable exception of *APOE ε4*. The relationship between these different *SORL1* signals, if any, remains elusive.

SORL1 rare variants and AD risk

With the advent of massive parallel sequencing, the attention was progressively drawn towards rare coding variants. In 2012, using the strategy described by Ng et al. [31] in a seminal whole exome sequencing (WES) report, we performed WES in 14 EOAD unrelated probands from

multiplex families in which the mode of inheritance was suggestive of an autosomal dominant transmission, despite a negative screen of the *APP*, *PSEN1* and *PSEN2* genes [37]. *SORL1* was the gene that was the most frequently hit (5/14 cases) by protein-truncating variants (PTVs, i.e., variants that introduce a premature stop codon due to nonsense single nucleotide substitutions, frameshifts insertions or deletions or alteration of canonical splice sites) or missense variants not listed in databases [37]. This suggested a strong link between familial EOAD and *SORL1* rare coding variants. However, due to the lack of DNA in affected relatives, it was not possible to assess the segregation of these variants with disease in these pedigrees. We next conducted a WES analysis among 484 unrelated French EOAD patients and 498 ethnically matched controls. After collapsing *SORL1* rare variants (MAF ≤ 1%) in a burden test, we detected an enrichment of the combination of PTVs and predicted damaging Mis3 missense variants (i.e., variants predicted damaging by 3/3 prediction software: Polyphen-2, SIFT and Mutation Taster) in cases (OR = 5.03, 95% CI = [2.02–14.99], $p = 7.49 \times 10^{-5}$) [34]. This enrichment increased when restricting the analysis to the 205 cases with a positive family history (OR = 8.86, 95% CI = [3.35–27.31], $p = 3.82 \times 10^{-7}$), even reaching the so-called exome-wide significance (p value threshold: 2.5×10^{-6}) after correction for multiple testing for 20,000 genes [34]. Finally, expanding our dataset to 927 late-onset Alzheimer disease (LOAD) cases, 852 EOAD cases and 1273 controls, the enrichment in PTVs and Mis3 rare variants in EOAD patients reached exome-wide significance whatever the family history (OR = 3.41, 95% CI = [2.09–5.55], $p = 1.61 \times 10^{-7}$) [5]. However, we detected a significant and positive trend from LOAD to sporadic EOAD and EOAD with positive family history, with ORs for PTVs and Mis3 rare variants increasing from 1.48 [0.87–2.53] in LOAD patients to 2.11 [1.04–4.09] in sporadic EOAD and finally to 4.83 [2.80–8.46] in EOAD patients with a positive family history.

Meanwhile, using WES, whole genome sequencing (WGS) or targeted resequencing, other groups gathered data on *SORL1* rare variants in a case–control setting [7, 16, 18, 38, 43, 48, 50, 51]. Two studies resorted to an unbalanced design, involving actual sequencing of cases but selective genotyping of controls, thereby preventing the discovery of control-specific variants. In a study performed on data from individuals of Caribbean Hispanic ancestry, Vardarajan et al. [50] used a complex design, including the sequencing of *SORL1* in probands of 151 families and the genotyping of a limited set of variants in controls and in affected and unaffected relatives, which precluded any classical gene-based association study based on the collapsing of rare variants. Nevertheless, they concluded that a set of 17 variants, gathering mostly rare variants but also two low-frequency variants, was enriched in cases with a modest p value. Likewise, Gomez-Tortoza et al. [16] sequenced *SORL1* in 124 cases with a positive family history, but genotyped selected variants in controls. The following studies sequenced both cases and controls. Verheijen et al. [51] sequenced *SORL1* in 1255 EOAD cases and 1938 age and origin-matched controls from multiple European countries and observed a significant enrichment of rare PTVs and missense variants in patients (SKAT-O p value of 10^{-4}, OR is not relevant when using SKAT-O hence not provided). Holstege et al. [18] added 640 AD cases (including 320 EOAD cases) and 1268 controls from the Netherlands to this sample. In the combined dataset, *SORL1* variants with a Combined Annotation Dependent Depletion (CADD) score > 30 and a MAF < 10^{-4} (based on ExAC frequencies [26]) were associated with a strong increase of AD risk (OR = 10.9; 95% CI = [4.6–25.67], $p = 1.8 \times 10^{-11}$, Fisher exact test). The effect was even stronger for extremely rare variants (MAF < 10^{-5} with a 12-fold increase in AD risk) [18]. More recently, WES data from the American Alzheimer's Disease Sequencing Project (ADSP) including 5740 AD cases with an age of onset > 60 years and 5096 cognitively normal controls of European or Caribbean Hispanic ancestry have been reported [7]. Using SKAT-O gene-based test aggregating all PTV and missense *SORL1* variants without further prioritization based on in silico predictions, an enrichment was observed in cases ($p = 8.68 \times 10^{-5}$). Raghavan et al. [38] analyzed the same dataset in combination with 1371 LOAD cases and 2331 controls from the Washington Heights-Inwood Community Aging Project (WHICAP) as well as 6395 additional controls. They found an enrichment of ultra-rare *SORL1* PTVs (MAF < 10^{-4}) in cases with an exome-wide significant level (OR = 36 [95% CI: 5.8–1493.0], $p = 2.17 \times 10^{-8}$). Overall, only one study of limited sample size (332 WES of cases—mainly LOAD—and WES or WGS of 676 controls,) failed in identifying an enrichment in *SORL1* rare variants among cases [43].

Thus, despite differences in the effect size between studies that are primarily related to different inclusion criteria for patients and different criteria for variant prioritization, all but one published studies showed a clear increase in the frequency of rare variants in cases compared to controls, which in three independent studies reached exome-wide significance. Of note, the more damaging the variants were predicted by in silico tools and the rarer they were (both conditions being non-independent), the stronger the association appeared to be. The highest ORs were detected when focusing on PTVs, most of which were present as singletons in these datasets.

A meta-analysis of publicly available data

As previously seen, gene-based case–control association studies on rare variants applied diverse strategies in terms of

variant filtration. To allow a global view, we reanalyzed (see Supplementary information) the five studies with publicly available non overlapping data, which included a total of 9204 cases and 9646 controls of European ancestry (Table 1) [5, 7, 18, 43, 51]. In the combined data set, the mean age at onset of AD for PTVs + Mis3 rare *SORL1* missense variant carriers was 67.9 years (range: [35.0–90.9]). It should be remembered, however, that this distribution does not reflect the average age at onset prevailing in the general population because patients included in WES/resequencing studies were not randomly ascertained, with an over-representation of EOAD cases.

In this dataset, we performed a meta-analysis of gene-based burden association tests using fixed-effect Mantel–Haenszel estimates of the ORs for different levels of filtration. We first applied a sequence of allele frequency filters, all previously adopted in several studies: rare variants (MAF < 0.01 in gnomAD v2.1, non-neuro dataset [5, 34, 51]), ultra-rare variants (MAF < 10^{-4} in gnomAD v2.1, non-neuro dataset [18, 38]), as well as MAF < 10^{-5} and MAF < 5×10^{-6} in gnomAD v2.1, non-neuro dataset, the latter corresponding to singleton variants in gnomAD v2.1 [18]. Secondly, we classified missense variants either according to our previous classification (Mis0, Mis1, Mis2, Mis3 for variants predicted damaging by 0 to 3 tools among SIFT, Mutation Taster and Polyphen2 HumDiv) [5, 34] or using CADD thresholds (≤ 20, 20–30, > 30) [18]. Of note, PTVs were not included in these categories, whatever their CADD scores. Thirdly, we performed the same analyses after restriction to EOAD cases, among studies with available data on EOAD status. A summary of all variants included in the meta-analysis ($n = 383$) can be found in Supplementary Table 1.

An overview of all gene-based meta-analyses is provided in Fig. 1 while the forest-plots of most significant results among rare variants (MAF < 0.01) are displayed in Fig. 2. The estimated risk was maximal for PTVs. Only two PTV carriers were present among the control subjects. The age at last evaluation of the ADES-FR control carrier was 75 years. Age information was not available for the p.(Arg1207*) control carrier [43]. Of note, three individuals from gnomAD v2.1 non-neuro carry this variant (age range is available for 2/3: 40–45 and 60–65 years, respectively). By contrast, 39 PTV carriers were found in patients. All but three PTVs were private. One nonsense variant, p.(Arg744*) was found in two cases from the ADSP study and one Dutch case (MAF in gnomAD, non-neuro dataset: 4.81×10^{-6}) while the remaining two were each found in two cases. Overall, the burden of PTVs was strongly associated with AD risk, displaying ORs of 12.29 (95% CI = [4.22–35.78], $p = 4.2 \times 10^{-6}$) in all AD cases and 27.50 [7.38–102.42] ($p = 7.8 \times 10^{-7}$) after restriction to EOAD cases (Fig. 2). Of note, in gnomAD v2.1 gene constraint metrics, only 42 *SORL1* protein-truncating single-nucleotide variants (nonsense or canonical splice site) are listed, compared to 124.5 expected from a mutational model accounting for tri-nucleotide context, coverage and methylation pattern, suggesting that *SORL1* is moderately intolerant to loss of function (o/e = 0.34 [0.26–0.44]) [26]. Part of the AD patients from ADSP could be included in this count, possibly overestimating slightly the burden of PTVs in the general population.

Regarding missense variants, both the Mis3 and CADD > 30 categories of variants were associated with AD risk at an exome-wide significant level (or of the order) whatever the allele frequency threshold (Figs. 1 and 2, Supplementary material). CADD > 30 rare variants displayed a higher OR but case carriers were twice as less numerous as Mis3 rare variant case carriers. There was no association of the burden of any other category of rare variants although the Mis2 variants and variants with a CADD score between 20 and 30 showed a suggestive association (Supplementary material). When using decreasing thresholds of allele frequencies, the OR increased both for Mis3 and CADD > 30 variants.

Finally, in all analyses, the association was stronger when restricting the sample to EOAD cases as compared to all AD cases (Figs. 1 and 2).

The case of *SORL1* low-frequency coding variants and rare recurrent coding variants

The effects of common and rare variants have been studied in multiple large case–control studies. However, the case of coding variants in between in terms of frequency remains less clear.

In the gnomAD database, only two *SORL1* non-synonymous variants with a frequency above 1% are reported within the canonical transcript (ENST00000260197) (see supplementary material): p.Ala528Thr (Mis2, CADD score = 26.5, MAF = 7.3% in gnomAD v2.1, non-neuro dataset, MAF ranging from 3.2 to 12.2% in diverse populations from gnomAD) and p.Glu270Lys variant (Mis3, CADD score = 24, MAF = 1.5%). In vitro, both p.Glu270Lys and p.Ala528Thr variants were associated with increased secretion of Aβ when transfected in HEK293 cell lines stably expressing the Swedish *APP* mutant, thus suggesting a potentially deleterious effect regarding *SORL1* function [50].

Of note, the p.Ala528Thr variant, also known as rs2298813, corresponded to SNP13 in large common variant association studies [39, 41]. No significant association was claimed in these studies. Intriguingly, in the American ADSP sequencing study, it was associated with AD risk with a p value of 8.7×10^{-5} [7] despite lower sample sizes. Likewise, among the five sequencing studies included in our meta-analysis, this variant was associated with AD risk with a p value of 1.3×10^{-3} (OR = 1.19 [1.07–1.32]).

Table 1 Summary of case–control association studies based on sequencing data included in the meta-analysis of burden tests

Study	*n* cases	*n* controls	Ethnicity	Type of variants	MAF filter	Prioritization of missense variants	Most significant result	*n* cases and controls included in the meta-analysis
Bellenguez and Charbonnier et al. [5]	852 EOAD 927 LOAD	1273	European (French)	PTV, missense	1%	Mis3, i.e., predicted damaging by Polyphen2 HumDiv, SIFT and Mutation Taster	Burden or rare PTV and Mis3 in EOAD cases: OR = 3.41, 95% CI = [2.09–5.55], $p = 1.61 \times 10^{-7}$	1779 cases 1273 controls
Verheijen et al. [51]	1255 EOAD	1938	European (diverse)	PTV, missense	1%	No further prioritization	SKAT-O *p* value of the meta-analysis of 1085 patients and 1752 controls from five countries: 10^{-4}	1255 EOAD cases 1938 controls
Holstege et al. [18]	640 cases including 320 EOAD cases	1268	European (Dutch)	PTV, missense	1% 0.1% 0.01% 0.05%	CADD score: 0–20, 20–30, > 30	Burden of PTV and missense singleton variants ($MAF < 5 \times 10^{-4}$) OR = 11.3 [4.0–32.1], $p = 4.9 \times 10^{-6}$ Meta-analysis of data with Verheijen et al. burden of PTV and singleton missense variants ($MAF < 5 \times 10^{-4}$) OR = 12.0 (4.2–34.3), $p = 5.0 \times 10^{-9}$	640 cases 1268 controls
Bis et al. [7]	5740, age at inclusion ≥ 60 (ADSP)	5096	European American and Hispanic	PTV, missense	1%	No further prioritization	SKAT-O *p* value: 8.68×10^{-5}	5198 cases 4491 controls
Sassi et al. [43]	332 (mostly LOAD)	676	European (British and North American)	All non-synonymous, synonymous, splice site, and UTR	5%	No further prioritization	No association ($p = 1$)	332 cases 676 controls

In the meta-analysis, only cases and controls from European ancestry and for which data were publicly available were included. For example, data from the American Alzheimer Disease Sequencing Project (ADSP) study, analyzed both in Bis et al. [7] and Raghavan et al. [38] were included, but the data provided exclusively by Raghavan et al. were not. Studies genotyping selected variants could not be included in the meta-analysis [16, 50]. We did not include studies with less than 100 individuals (e.g., [48])

CADD Combined Annotation Dependent Depletion, *EOAD* Early-Onset Alzheimer Disease, *LOAD* Late-Onset Alzheimer Disease, *MAF* Minor Allele Frequency, *PTV* Protein Truncating Variant, *UTR* untranslated region

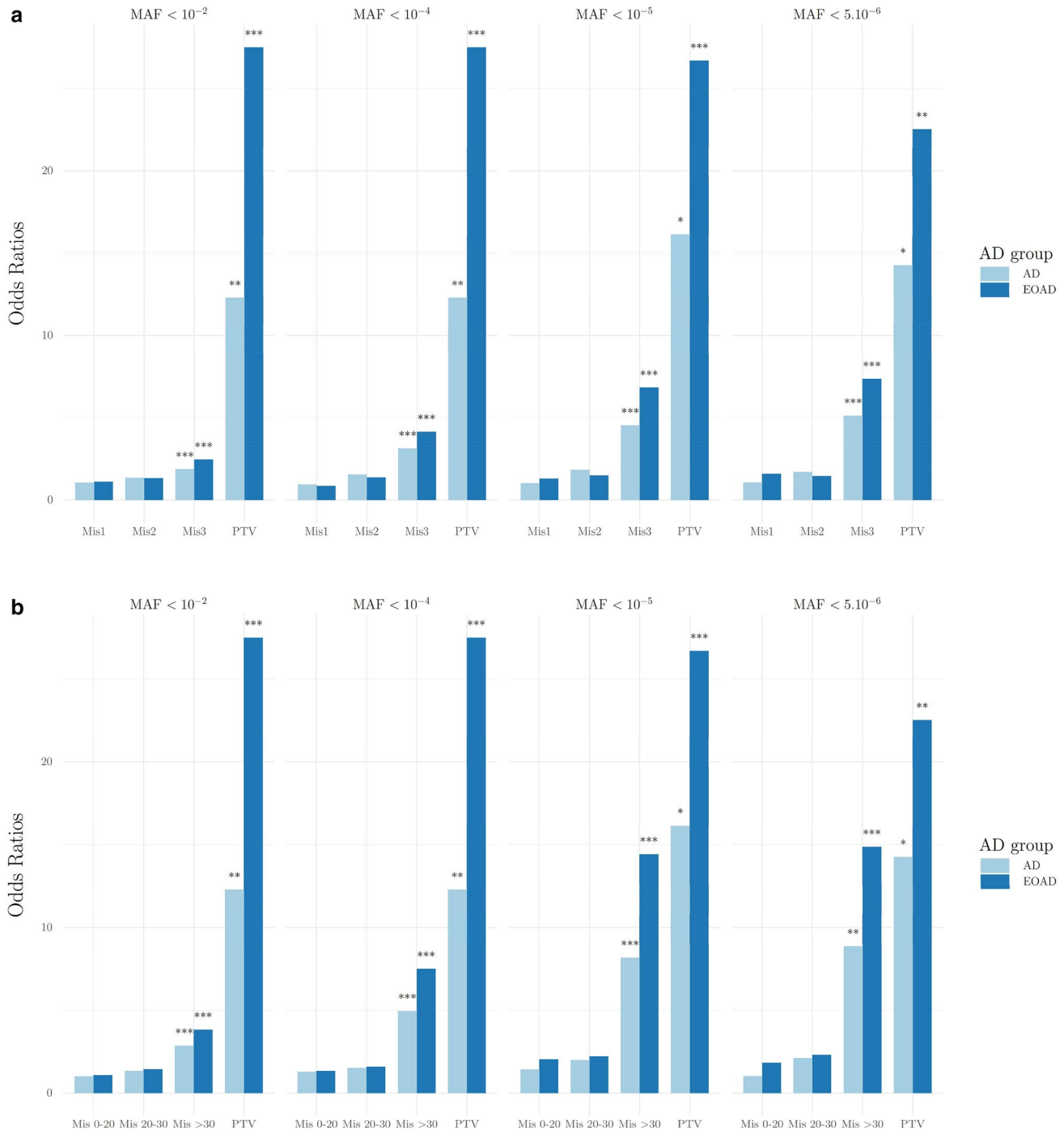

Fig. 1 Overview of burden test meta-analyses at the gene level for varying levels of allele frequency filters and stringency of pathogenicity predictions according to Mis0-3 and CADD classifications. The gene-based meta-analysis was performed for all protein-truncating (nonsense, frameshift indels, and canonical splice site) variants (PTVs) and all missense variants. Variants were filtered by decreasing MAF frequency thresholds in gnomAD v2.1 non-neuro (MAF < 1%, MAF < 10^{-4}, MAF < 10^{-5}, MAF < 5×10^{-6}). **a** Missense variants were classified according to the number of bioinformatics software predicting the variant as damaging, i.e., Mis0, Mis1, Mis2 and Mis3 were predicted damaging by 0, 1, 2, or 3 tools (Mutation Taster, Polyphen2 HumDiv and SIFT), respectively. **b** Missense variants were classified according to CADD scores using the following thresholds 0–20, 20–30 or > 30. ***Stands for exome-wide significant p values ($p < 10^{-6}$), **indicates p values of the order of exome-wide significance ($p < 10^{-5}$), *indicates p values $p < 10^{-4}$

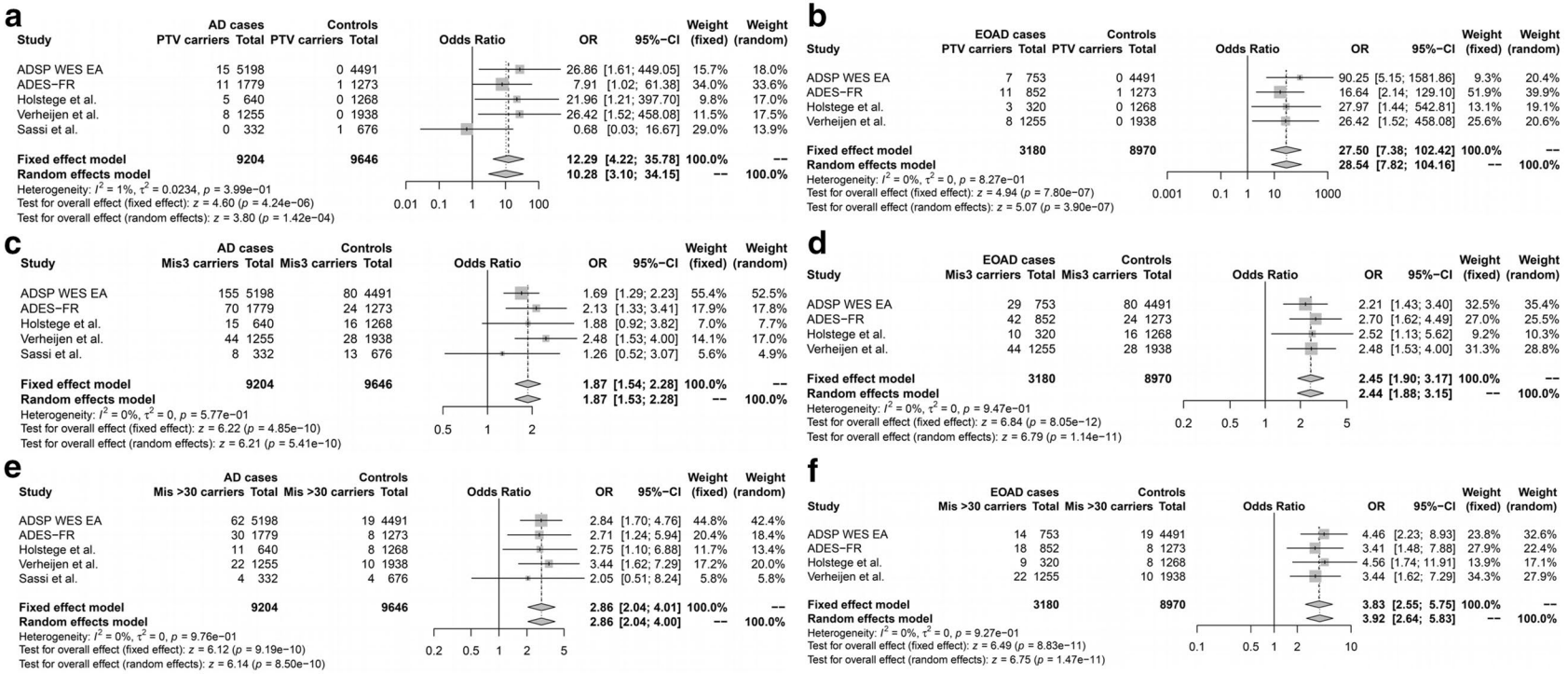

Fig. 2 Meta-analysis forest-plots of burden tests at the gene level among rare variants. Results of the gene-based meta-analysis performed on protein-truncating (nonsense, frameshift indels, and canonical splice site) variants (PTVs, **a**, **b**) and all rare (minor allele frequency < 0.01 in gnomAD v2.1, non-neuro dataset) missense variants are displayed (**c**–**f**). Regarding missense variants, Mis3 results are depicted in **c** and **d** while CADD > 30 results are depicted in **e** and **f**. Results obtained on all patients are presented in **a** (PTVs), **c** (Mis3) and **e** (CADD > 30). Results obtained on early-onset AD (EOAD) patients are presented in **b** (PTVs), **d** (Mis3) and **f** (CADD > 30). For the remaining categories of variants and for additional MAF threshold filtering categories, the results are presented as Supplementary material

The p.Glu270Lys variant (rs117260922) has been claimed to be associated with AD in one study of limited sample size (OR = 3.4, $p = 0.04$) [14]. In our meta-analysis, we did not confirm this result, the OR being 1.04 [0.89–1.21] ($p = 0.621$). As the frequency of these two variants, p.Ala528Thr and p.Glu270Lys, allows variant imputation and as they are present in some SNP arrays, a specific case–control meta-analysis of all available data (including the powerful IGAP study [21] gathering 94,437 individuals) and taking into account linkage disequilibrium with the other SNPs already associated with AD in previous GWAS would be the most efficient approach to answer the question of their association with AD.

Besides these two variants, some recurrent but rarer variants also deserve single variant association analysis. The meta-analysis results (fixed effect) of single variant analysis among all AD cases with available data for all non-synonymous variants displaying more than ten occurrences in the combined data set are presented in Supplementary Material. Although no significant result emerged, a lack of power is a concern and, likewise, imputation in large datasets is warranted to allow conclusions on the presence or absence of effect of each variant and to tackle the heterogeneity observed within studies.

SORL1: the fourth autosomal dominant AD gene or a more complex determinism?

The initial identification of rare coding *SORL1* variants in EOAD probands with a positive family history of EOAD [37] raised the question of an autosomal dominant pattern of inheritance in some families. Up to now, the formal statistical evidence of an association of rare *SORL1* variants with AD risk came from case–control studies, not allowing any conclusion on Mendelian inheritance [18, 34].

However, supporting a claim for a putative autosomal dominant transmission, several pedigrees where rare *SORL1* variants co-segregated with AD have been reported. For example, five affected family members (including four LOAD patients) carried a p.Asn674Ser variant (Mis2) in a Dutch family. Of note, all five affected individuals were *APOE4* homozygous [28]. In another report [49], three families were described which showed co-segregation of rare missense variants with AD. The p.Arg1303Cys variant (Mis3) was found in four affected patients (all but one had an early onset) and was absent in two non-demented subjects from a two-generation family; the p.Gly1732Ala variant (Mis3) was detected in two affected siblings from the second family while, in the third family, the c.3050-2A>G splice variant segregated with the disease in two affected EOAD patients, one patient described as having possible mixed AD and vascular dementia, and was absent in one unaffected member. Of note, in this study, of the nine affected individuals carrying one of these rare *SORL1* variants, six carried an *APOE3-4* genotype, and three were *APOE4* homozygous [49].

Contrasting with these results, some other familial studies assessing other rare missense variants reported unaffected elderly carriers and affected non-carriers, raising interrogations on the inheritance pattern. For example, in a recent report [10], the p.Thr588Ile variant (Mis3) was identified in four patients (including one EOAD and 3 LOAD patients) and two unaffected subjects (aged 81 and 84 years, respectively) in a first family while the p.Thr2134Met variant (Mis3) was found in only three of four cases in a second family. Likewise, Gomez-Tortoza et al. [16] reported non-segregation of rare *SORL1* variants with AD in three families: the p.Asn1809Ser variant (Mis2) was absent in 1 out of 2 affected subjects from the first family, the p.Asp2065Val (Mis2) variant was absent in 1 out of 3 affected members of the second one and the Gly1871Val (Mis3) was absent in 2 out of 3 affected individuals from the third family. The conclusion of the authors was that a lack of segregation in these pedigrees ruled out the implication of these variants as a direct cause of AD.

Of note, although only heterozygous carriers were reported in these studies, we recently described a compound heterozygous *SORL1* PTV carrier [24]. The patient exhibited dementia starting around the age of 55 and the family history was positive for dementia in both paternal and maternal lineages (onset before 78 in the mother, before 70 and 65 in the father and the paternal grandfather, respectively), complicating a bit more the case of *SORL1* rare variants inheritance.

In addition, we assessed a putative role of somatic mutations (i.e., post-zygotic de novo mutations) in the etiology of sporadic EOAD [32]. We identified five somatic SORL1 mutations with low allele fraction (0.36–7.91%). Of them, one was a Mis3 variant, detected in 3.61% of the reads in a blood sample and another one, detected in 0.36% of the reads in a blood sample of another patient, was predicted to strongly enhance a cryptic 5′ splicing site. However, the functional impact of each variant could not be assessed and the low allele fractions in the blood make these results difficult to interpret regarding the etiology of AD.

There is no doubt that most cases carrying a *SORL1* rare risk variant have a positive family history of AD—but not necessarily EOAD—and some pedigrees exhibit a pattern consistent with an autosomal dominant inheritance. However, we can assume that there is a tendency towards the publication of positive co-segregation of variants with AD, leading to a putative publication bias.

Compared with *APP*, *PSEN1* or even *PSEN2* mutation carriers, the mean age at onset of *SORL1* rare variants carriers is clearly shifted towards older ages. Consequently, segregation analyses may face the coincidental occurrence in the same pedigree of cases carrying rare, potentially highly

penetrant, *SORL1* variants, with late-onset cases related to another determinism (phenocopies). Therefore, we have to remain cautious when interpreting segregation data. Ideally, the age-dependent penetrance curve of each variant should be established before allowing any use in a clinical setting for the counseling of unaffected relatives. Although it appears impossible to perform such an analysis for each variant given their respective extreme rarity, assessing the penetrance of all *SORL1* PTVs as a single averaged signal, with or without the inclusion of functionally assessed missense variants (see below), seems to be a realistic study design in the near future. Notably, the observation of co-occurrences of *SORL1* rare variants with an *APOE ε4* homozygous genotype and even *ABCA7* or *TREM2* rare risk variants in some patients [5] further complicates the matter, suggesting that the etiology of AD in some families should be viewed as oligogenic. Indeed, as shown in Fig. 3, in some of our families, some affected relatives are non-carriers while oligogenic inheritance rather than strict autosomal dominant inheritance is encountered in other families.

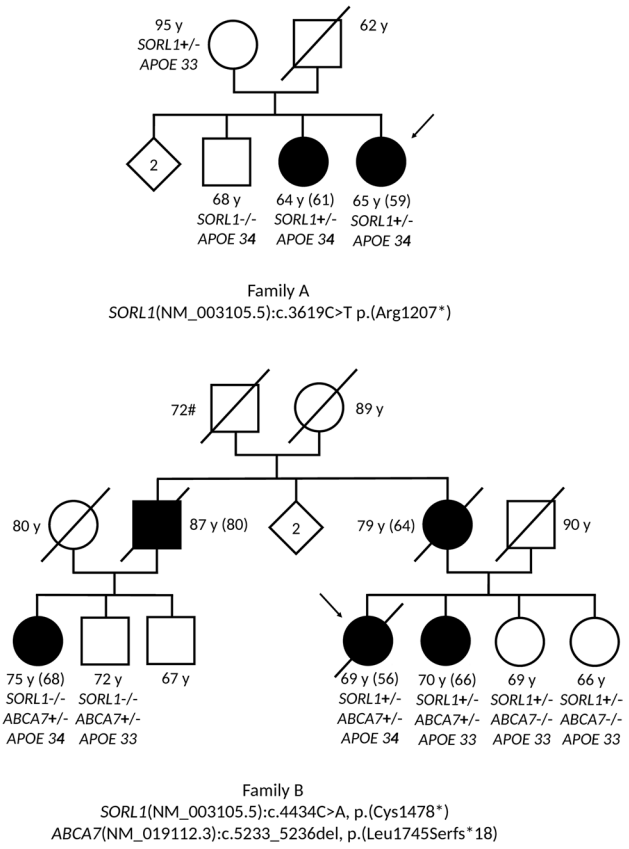

Fig. 3 Reduced pedigrees of two families with *SORL1* protein-truncating variants suggesting oligogenic inheritance. In Family A (upper panel), the mother transmitted a nonsense *SORL1* variant while being unaffected by AD at the age of 95 years. In Family B (lower panel, unpublished), both proband and affected sister carried protein-truncating variants in *ABCA7* and in *SORL1*, while the affected cousin carried only the *ABCA7* variant. In addition to different *APOE* genotypes in affected individuals, this family suggests an oligogenic determinism of AD. Squares: males, circles: females, diamonds: undetermined. Filled symbols: deceased individuals. Ages (y: years) appear next or under the individual symbols and indicate the age at last examination or the age at death. Ages in parentheses: ages of onset. When DNA is available, the results of targeted sequencing appear with a + for the presence of the variant and a − for the absence of the variant (+/−: heterozygous). Arrows point to probands, # history of psychiatric or behavioral troubles a few years before death

SorLA protective role against AD

The *SORL1* gene encodes a 250-kDa type-I transmembrane protein termed sortilin-related receptor (short name: SorLA; UniProt entry: Q92673) that belongs to the vacuolar protein sorting 10 (VPS10)-related sortilin family. SorLA is highly expressed in the brain where it shows predominant localization in neurons of the cerebral cortex, hippocampus, and cerebellum, but it is expressed in multiple other cell types as well. At the subcellular level, SorLA is mainly localized in the Golgi compartments and endosomes, with a minor fraction on the cellular surface. SorLA is synthesized as a pro-receptor containing a 53 amino acid propeptide that folds back on the VPS10P domain to block the binding of ligands. Cleavage of the propeptide in the trans-golgi network (TGN) produces the mature receptor, which is then able to interact with its target proteins. From the TGN, the mature receptor is directed to the plasma membrane through constitutive secretory vesicles. Most of the SorLA molecules at the cell surface undergo clathrin-dependent endocytosis. Internalized receptors move to the early endosomes from where they are sorted to the TGN to continuously shuttle between TGN and endosomes afterwards (for review see [3] and [45]).

Several studies have highlighted a protective function of wild-type SorLA against AD. SorLA interacts both with the amyloid protein precursor (APP) and with its toxic derivative, the Aβ peptide itself. As shown in Fig. 4, during its maturation, APP follows the constitutive secretory pathway and moves from the endoplasmic reticulum through the Golgi complex and the TGN to the plasma membrane. At the cell surface, most precursor molecules are cleaved by the α-secretase within the Aβ sequence, which results in the release of non-amyloidogenic fragments. APP molecules not cleaved by the α-secretase are internalized from the cell surface through clathrin-mediated endocytosis. From the early endosomes, they move to the late endosomal–lysosomal compartments or retrogradely to the TGN. In late endosomes, APP molecules are processed by β- and γ-secretases. This amyloidogenic pathway produces the toxic Aβ peptide, which is either released from cell by exocytosis or degraded by lysosomes [9].

Mouse models support an important role for SorLA in the control of the amyloidogenic processes. Indeed, loss of *Sorl1* expression in several mouse models of AD increases

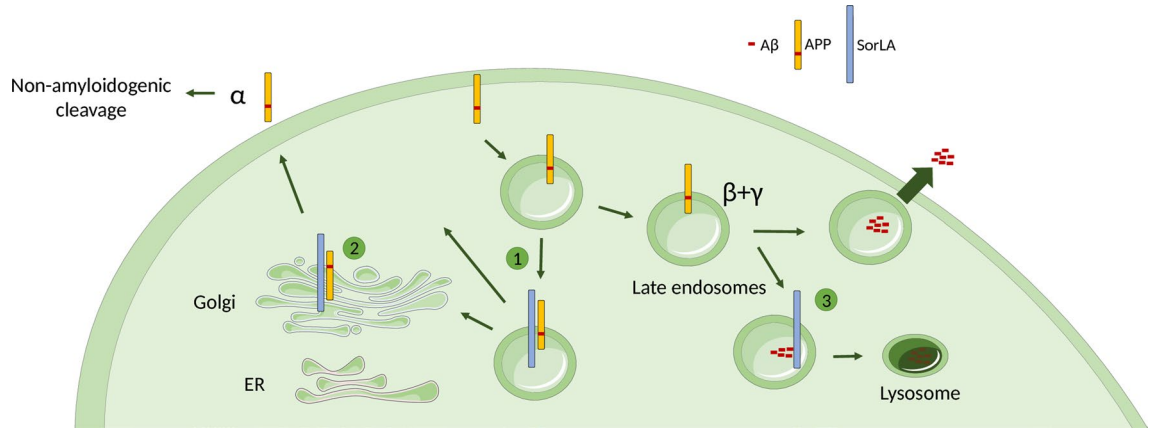

Fig. 4 SorLA: a protector against Aβ secretion. The amyloid β precursor protein (APP) follows the secretory pathway, from the endoplasmic reticulum (ER) to the membrane through the Golgi apparatus. At the membrane, it can be cleaved by the α-secretase within the Aβ coding sequence of APP, hence leading to the generation of non-amyloidogenic fragments. Alternatively, APP can undergo clathrin-dependant endocytosis and be directed to endosomes. In late endosomes, sequential cleavage by the β- and the γ-secretases results in the production of the Aβ peptide which can then be secreted in the extracellular compartment or be directed to the lysosome. SorLA can bind both APP and Aβ and acts as a repressor of Aβ secretion in at least three ways: (1) it can redirect APP to the Golgi apparatus or to the membrane; (2) it can slow down the exit of APP molecules from the Golgi, and (3) it can target nascent Aβ peptides to the lysosome. Reduced *SORL1* expression or loss of function in animal or cellular models and in patients carrying specific genetic variants can hence result in increased overall secretion of Aβ through diverse mechanisms

extracellular Aβ levels and senile plaque deposition [2, 13, 42]. On the other hand, overexpression of *SORL1* in neuronal and non-neuronal cell lines blocks the APP processing and decreases Aβ production [2, 36, 41, 44]. The mechanisms whereby SorLA acts to lower Aβ production have been reviewed recently in details [3, 45]. Briefly, SorLA acts as a neuronal sorting receptor for APP and Aβ. It can protect against amyloidogenic processes in three ways: (1) SorLA causes retrograde endosome to TGN retrieval of APP, preventing from both α- and β-cleavage; (2) SorLA slows down the exit of APP molecules from the Golgi complex and thereby reduces the number of APP molecules subjected to the amyloidogenic processing, and (3) SorLA targets produced Aβ molecules to lysosomal degradation (Fig. 4).

Consistent with these mechanisms, putative reduced *SORL1* expression or altered SorLA function induced by genetic variants is predicted to result in increased Aβ secretion, which is totally in line with AD pathophysiology.

A per-domain rare variants association analysis

The mature SorLA receptor contains several functional domains (Fig. 5) (for review see [45] and Uniprot and Pfam databases, https://www.uniprot.org/uniprot/Q92673, https://pfam.xfam.org/protein/Q92673). The VPS10P domain and the cluster of complement-type repeats (CR cluster, class A repeats) form major ligand-binding sites in the luminal receptor domain. The YWTD-β-propeller (LDL-R class B repeats) is involved in pH-dependent release of bound ligands. The fibronectin-type III cluster is engaged in protein–protein interactions but may also stabilize the conformation of the SorLA ectodomain. The short cytoplasmic tail encodes recognition motifs for cytosolic adaptors regulating SorLA trafficking. SorLA directly interacts with APP via the CR cluster, class A repeats domain, and via a six amino acid-stretching FANSHY motif located in the cytoplasmic tail, while the VPS10P domain binds monomeric Aβ in a pH-dependent manner.

Missense variants associated with AD risk are scattered throughout the different domains. To assess the contribution of each domain to the association of *SORL1* missense variants with AD, we have calculated the per-domain ORs, focusing on one of the strongest signals in the gene-based association, namely the one obtained after aggregation of ultra-rare (MAF $< 10^{-4}$) Mis3 variants (Fig. 5). We considered 16 different domains (including the linkers between each domain) and a p value threshold of 10^{-3} after Bonferroni correction for 16 domains tested both on all AD and EOAD patients. Three domains passed this threshold, both among all AD cases and among EOAD cases: the CR cluster, class A repeats domain (OR = 2.21 [1.38–3.52], $p = 8.92 \times 10^{-4}$) and 2.76 [1.55–4.91], $p = 5.5 \times 10^{-4}$, in all AD cases and in EOAD cases, respectively), the VPS10P domain (OR = 4.44 [1.83–10.75], $p = 9.77 \times 10^{-4}$ and 8.13 [2.82–23.50], $p = 1.1 \times 10^{-4}$) and the fibronectin type III domain (OR = 4.41 [2.02–9.62], $p = 1.89 \times 10^{-4}$ and 6.02 [2.40–15.09], $p = 1.31 \times 10^{-4}$). Both CR cluster, class A repeats, and VPS10P domains have already been identified as critical for the function of SorLA regarding AD, as APP binding and Aβ binding domains, respectively [4,

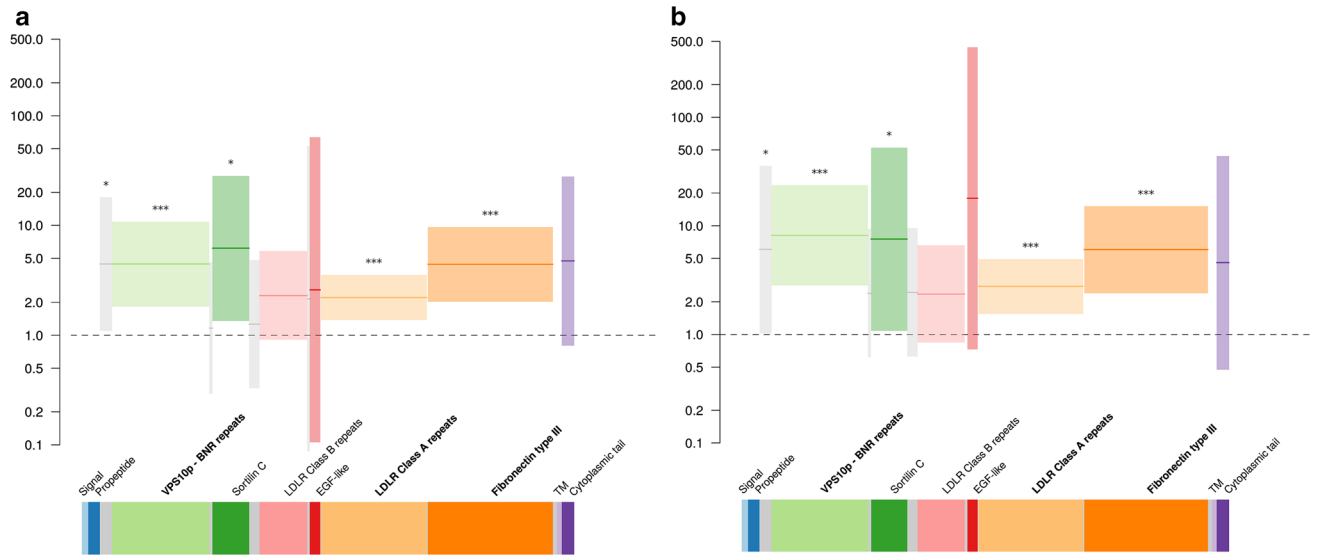

Fig. 5 Meta-analysis of burden tests at the protein domain level among ultra-rare variants. Protein domains were obtained from Uniprot and Pfam (regarding the Sortilin C domain). Each domain is represented with a different color in the lower part of each panel. Analyses were restricted to Mis3 ultra-rare (MAF $< 10^{-4}$) variants in all AD cases (**a**) and in EOAD cases (**b**). The corresponding odds ratios (OR) and 95% confidence intervals are represented above each domain. An absence of OR corresponds to the absence of ultra-rare Mis3 variants in the corresponding domain. In grey appear the linkers between each known domain. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. After Bonferroni correction for a double analysis of 16 domains or linkers, a p value threshold of 1.5×10^{-3} was considered significant

9]. The function of the fibronectin type III domain remains less clear. Interestingly, it has recently been shown that the fibronectin type III domain containing protein 5 interacts with APP [35]. In addition, its overexpression decreased Aβ secretion in a cellular model, although the specific domains of this protein involved in APP binding were not determined [35].

Of note, statistical power may not be sufficient to exclude any role of the other domains so that a functional role of variants in other domains remains possible. For example, smaller domains with high OR may be of interest, as the linker region between the propeptide and the VPS10P domain, and the Sortilin C domain.

A functional assay to classify rare missense variants

Association studies with rare variants are based on collapsing subsets of these variants. A few PTVs have been subjected to RNA analysis in the blood, showing a clear decrease of the mutated allele in the cDNA, which suggests a strong effect of nonsense-mediated decay (NMD) [34, 37, 51]. Hence, it can reasonably be assumed that most of the PTVs constitute a homogeneous subset that results in haploinsufficiency. However, whether or not missense variants behave as actual loss-of-function variants remain to be determined for most of them. As previously shown, aggregating missense variants classified as Mis3 or with a CADD score > 30 according to in silico predictions [34] [18] with PTVs strengthened the association signal with AD as compared with PTVs alone. This indicates that a large subset of these variants are likely to functionally result in a loss of SorLA function. However, all Mis3 or CADD > 30 missense variants cannot be globally equated to PTVs unless functional experiments settle on whether the actual effect of a particular variant is equivalent to a PTV and induces a reduced functional activity, displays no effect at all on protein functionality, or anything in between those two extremes.

On the other hand, the fact that rare missense variants classified as Mis2 are not significantly associated with AD per se provides some validation to the in silico classification distinguishing Mis3 from Mis2 variants, but does not rule-out that a small subset of Mis2 variants behaves as genuine loss-of-function variants. Ultimately, only functional assays will provide a fine and accurate identification of all *SORL1* missense variants that behave as genuine loss-of-function variants.

To assess the functional consequence of *SORL1* missense mutations on a key parameter, namely the Aβ production, Rogaeva and collaborators designed a functional assay based on the expression of SorLA proteins in HEK293 cells stably overexpressing APP [41]. They showed that the expression of the wild-type form of SorLA resulted in a significant decrease of Aβ secretion. They reasoned that expression of hypomorphic variants of SorLA would be predicted to lead to an increase of Aβ levels compared with the wild-type

form of the protein. So far this assay has been used for three rare missense variants p.Thr588Ile (Mis3), p.Thr2134Met (Mis3), p.Thr947Met (Mis3), and 2 low-frequency variants p.Ala528Thr (Mis2), p.Glu270Lys (Mis3) [10, 50]. All these *SORL1* mutants increased Aβ production compared to the wild-type control, suggesting that these variants resulted in a partial loss of function of the protein. Consistent with a proposed role for SorLA in the retention of APP in the Golgi, these mutants showed an increase of the amount of APP at the cell surface. More APP molecules can therefore be delivered to the late endosomes where they would be cleaved to generate Aβ peptides. Interestingly, the underlying molecular mechanisms appear different between the variants. The p.Glu270Lys, p.Ala528Thr and p.Thr588Ile mutants showed normal levels of SorLA at the cell surface but a reduced binding affinity for APP. In contrast, the p.Thr947Met and p.Thr2134Met variants showed a decreased amount of SorLA proteins at the cell surface.

Another assay has been devised, which measures the turn-over of intracellular Aβ peptides: SH-SY5Y cells stably overexpressing APP with or without SorlA were treated with DAPT to block γ-secretase activity. At different time points, cells were lysed and the intracellular concentration of Aβ was determined by ELISA. This assay has been used to show that the p.Gly511Arg (Mis3) variant, which is located in the VPS10P domain, disrupts the ability of SorLA to promote the intracellular catabolism of Aβ. The mutation altered Aβ binding, and may affect the ability of SorLA to direct Aβ peptides to lysosomal degradation [9].

Concluding remarks

The association of *SORL1* rare coding variants with AD results from the collapsing of a large set of ultra-rare variants. This explains why imputation-based studies which were successful at identifying *TREM2* and *ABCA7* [20, 47] have failed in identifying *SORL1* as an AD risk factor. It also makes implausible that the association signal of rare variants was related to that obtained with common *SORL1* SNPs. Indeed, it is highly unlikely that the wealth of ultra-rare mutations constituting the rare variants signal have arisen on a same common haplotype. In this context, it is noteworthy that a strategy based on collapsing rare PTVs and predicted damaging missense variants in a rather limited sample of extreme cases successfully yielded an exome-wide *p* value. This result strongly implies that a large subset of *SORL1* missense variants behave as loss-of-function variants. However, development of functional tests is warranted to definitely classify missense variants. Although individually extremely rare, PTVs or rare Mis3 variants were identified in 1.8% of controls, 3.6% of all AD cases and 4.8% of EOAD cases, showing that these variants may concern a significant proportion of patients. Given the high effect size of PTVs, it appears reasonable to disclose the identification of such a variant in a patient as it may provide a critical piece of information on disease etiology and hence respond to patients' queries. However, to allow the use of such variants in asymptomatic relatives as a predictive result, segregation studies are warranted. Indeed, the penetrance of these variants cannot be directly estimated precisely from case–control analyses. Only a few segregation studies have been reported and we can expect that distinct variants may be associated with diverse levels of penetrance. However, as illustrated in published segregation studies, an incomplete penetrance can be encountered, even at advanced ages and for the variants associated with the highest risk, namely the PTVs. In addition, co-occurrence of other moderate-to-high genetic risk factors together with *SORL1* rare variants suggests an oligogenic inheritance in some families. Based on current knowledge, classical genetic counseling shall not be proposed to asymptomatic relatives nor should be applied the ACMG-AMP recommendations for variant pathogenicity as they apply to confirmed Mendelian genes [40]. Future functional and segregation studies will help concluding on (1) the context in which some *SORL1* rare variants should be used in a clinical setting, (2) which variants could be used and (3) with which level of associated risk (i.e., penetrance). These critical data will be required to finally allow accurate clinical information and putative medical use as well as inclusion of carriers in preventive clinical trials.

Acknowledgements We thank Magalie Lecourtois, Anne Rovelet–Lecrux and Olivier Quenez for their help. We are grateful to Henne Holstege for providing variant information on the EOAD subset from the Dutch study (Holstege et al. [18]) and to the NIAGADS for granting us access to the ADSP data. This work was supported by the Fondation pour la Recherche Médicale (Grant DEQ 20170336711).

Compliance with ethical standards

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

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