Sorting Out the Role of the Sortilin-Related Receptor 1 in Alzheimer’s Disease

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Abstract. Sortilin-related receptor 1 (SORL1) encodes a large, multi-domain containing, membrane-bound receptor involved in endosomal sorting of proteins between the trans-Golgi network, endosomes and the plasma membrane. It is genetically associated with Alzheimer’s disease (AD), the most common form of dementia. SORL1 is a unique gene in AD, as it appears to show strong associations with the common, late-onset, sporadic form of AD and the rare, early-onset familial form of AD. Here, we review the genetics of SORL1 in AD and discuss potential roles it could play in AD pathogenesis.

Keywords: Alzheimer’s disease, amyloid, amyloid-beta protein precursor, endocytosis, endosomes, protein transport

INTRODUCTION

Sortilin-related receptor 1 (SORL1, also known as LR11 or SORLA) encodes a hybrid receptor with multiple domains involved in intracellular sorting and trafficking of proteins into their respective subcellular compartments. The sorting of proteins is essential for normal cell function and defects in these pathways are thought to be important factors in the pathogenesis of Alzheimer’s disease (AD).

AD, a progressive neurodegenerative disorder, is the most common form of dementia. It is defined by progressive cognitive decline, widespread neurodegeneration and two proteinopathies: senile neuritic plaques [consisting of the product of the proteolytic processing of the amyloid-β protein precursor (AβPP), amyloid-β (Aβ)], and neurofibrillary tangles (consisting of hyperphosphorylated tau protein) (reviewed in [1]). AD can be classified into two broad subtypes defined by the age of disease onset: early-onset AD (<65 years of age, EOAD) and late onset AD (>65 years of age, LOAD). There can be both sporadic and familial varieties of both EOAD and LOAD. The majority of AD cases (~95%) belong to the LOAD subgroup, and these mainly arise sporadically. Conversely, ~5% of cases belong to the EOAD subgroup [2]. A portion of EOAD cases are caused by autosomal dominant variants in AβPP [3–7], presenilin 1 (PSEN1) [8–13], and presenilin 2 (PSEN2) [14–17] (EOfAD). Recently, variants in SORL1 have been recognized as likely causing EOfAD [18, 19]. Intriguingly, SORL1 is also recognized as a genetic risk factor for development of late-onset, sporadic AD (sAD) [20–22]. The exact role of SORL1 in the pathogenesis of AD is unclear since there are multiple pathways which link it to AD pathological mechanisms.

This review will discuss the normal expression of SORL1 and the genetic evidence which supports a role in LOAD and EOAD. We then discuss the possible roles which SORL1 may play in different pathologies observed in AD. SORL1 appears to be strongly associated with both EOAD and LOAD and...
provides a unique opportunity to understand the similarities and differences between these two subtypes of AD.

**SORL1 HAS MULTIPLE TRANSCRIPT SPlicing PRODUCTS**

**SORL1** is located on chromosome 11q23.2-24.2 in humans and is widely expressed in the brain. It is particularly highly expressed in neurons of the hippocampus and some nuclei of the brainstem and Purkinje cells, and has slightly weaker expression in neurons of the thalamus and the hypothalamus [23]. It is also expressed in other tissue types such as the testes, ovaries, thyroid, and lymph nodes [24].

Alternative splicing occurs for **SORL1**, with at least 5 protein-coding transcripts arising from the gene in humans according to the ENSEMBL database [25] (Fig. 1). The shortest alternative splice product, **SORL1-206**, has a transcription initiation site in intron 30 and is expressed highly in the hippocampus and temporal lobe, moderately in the entorhinal cortex and frontal cortex and is undetectable in the testes or kidney [26]. Expression of this particular splice product is unchanged in the cerebellum of AD patients. However, total **SORL1** mRNA levels are relatively constant in the cerebellum of AD brains [27], so this is not surprising. Grear et al. [28] reported two **SORL1** mRNA isoforms expressed in the temporal lobe in both AD and non-AD samples: **SORL1-Δ2 and SORL1-Δ9**, which lack exon 2 and exon 19 coding sequences respectively. **SORL1-Δ2** comprises of up to 5% of total **SORL1** expression and **SORL1-Δ19** comprises of less than 1% (and was not investigated further). The **SORL1-Δ2** isoform lacks sequences coding for amino acid residues V96 to D134, the N-terminal proximal region of the VPS10 domain of SORL1 protein. It has increased abundance in white matter compared to grey matter, which suggests varying expression across cell types (as white and grey matter contain different ratios of cell types). However, expression levels of the **SORL1-Δ2** isoform did not correlate with Braak staging of AD progression (while the expression of the longest **SORL1** transcript splice form did show correlation). Finally, a non-coding RNA (ncRNA) termed 51A maps in antisense configuration to intron 1 of the **SORL1** gene and drives the alternative splicing of **SORL1** towards two splice products: 1) splice product B (**SORL1-B**), with a coding sequence beginning in exon 23 and ending with the stop codon used for translation of the full length protein and 2) splice product F (**SORL1-F**), with a coding sequence beginning with the start codon used in translation of the full length protein within exon 1 and ending in exon 14. Overexpression of the 51A ncRNA in a neuroblastoma cell line resulted in increased levels of Aβ peptides. Interestingly, 51A is upregulated in AD brains (although the authors observed substantial variation among individuals) [29].

Together, these studies show the complexity of alternative splicing of **SORL1** transcripts. However, the scientific literature lacks functional studies investigating the roles of these alternative splice products.

**SORL1 ENCODES A MEMBRANE-BOUND RECEPTOR WITH MULTIPLE FUNCTIONAL DOMAINS**

The full-length transcript of **SORL1** encodes a ∼250 kDa, membrane-bound protein which primarily localizes in the endosomal and Golgi compartments [30]. The protein is comprised of multiple functional domains. These include a vacuolar protein sorting 10 (VPS10) domain, five low-density lipoprotein receptor (LDLR) class B repeats, an epidermal growth factor-like (EGF-like) domain, eleven LDLR class A repeats, six fibronectin-type (FN-type) repeats, a transmembrane domain and a cytosolic domain containing recognition motifs for cytosolic adaptors [31–34] (Fig. 1B).

Nascent SORL1 proteins are generated in endoplasmic reticulum (ER) and are then transported through the trans-Golgi network (TGN) and eventually to the cell surface. They are initially inactive due to the pro-peptide at the NH2-terminal which is removed by furin-mediated cleavage in the TGN due to the RRKR furin recognition sequence [35]. This allows the receptor to be directed to the cell surface. Once at the cell surface, it appears that SORL1 can be utilized either in a signaling pathway or a trafficking pathway. In the signaling pathway, SORL1 is subject to ectodomain shedding by the α-secretase tumor necrosis factor-A converting enzyme (TACE/ADAM-17) [36–38]. The exact site at which TACE cleaves SORL1 is unclear. SORL1 is then processed further by γ-secretase at the plasma membrane, releasing a fragment of SORL1 into the extracellular space and a SORL1 intracellular domain (SORL1-ICD) into the cytosol [39, 40]. The SORL1-ICD contains a nuclear localization motif KHRR. It was demonstrated in a reporter assay that the SORL1-
Fig. 1. *SORL1* encodes a multi-domain containing protein and its transcripts are subject to alternative splicing. (A) depicts gene models for alternative *SORL1* splice products from the ENSEMBL database (ENSG00000137642) and published literature. Exons are numbered and color-coded to indicate which protein domains they encode. Protein-coding domains are as given for the human *SORL1* protein (Uniprot ID: Q92673) with SMART database annotations. VPS10, vacuolar protein sorting 10; LDLR, low density lipoprotein receptor; EGF, epidermal growth factor. (B) depicts a schematic of the full length *SORL1* protein consisting of a pro-peptide sequence, a VPS10 domain, five LDLR class B repeats, an EGF-like domain, eleven LDLR class A repeats, six fibronectin-type (FN) repeats, a transmembrane domain (TMD) and a cytosolic intracellular domain (ICD) containing recognition motifs for cytosolic adaptors. Binding sites of amyloid-β protein precursor (AβPP), amyloid-β (Aβ), sorting nexin 27 (SNX27), Golgi-localizing, γ-adaptin ear homology domain ARF-interaction (GGA), clathrin adaptor protein 1/2 (AP1/2), and phosphofurin acidic cluster sorting protein 1 (PACS1) and the nuclear localization signal (NLS) are indicated.

ICD can enter the nucleus to regulate transcription [39]. Whether the ICD has this function in reality and what genes it might regulate are yet to be determined. As an alternative to processing at the cell surface for signaling, *SORL1* can also enter a trafficking pathway if it is internalized by clathrin-mediated
endocytosis employing the chaperone clathrin adaptor protein 2 (AP2) [34]. Subsequently, internalized SORL1 receptors generally shuttle between the TGN and endosomes, guided by cytosolic adaptor proteins which will be discussed later in this review in the context of AβPP trafficking (Fig. 2) (reviewed in [41, 42]).

**GENETIC EVIDENCE FOR A ROLE OF SORL1 IN AD**

The first characterization of genetic variation of SORL1 in AD came from a candidate gene approach in 2007. Rogaeva et al. [21] investigated 29 single nucleotide polymorphisms (SNPs) associated with AD throughout the SORL1 locus in 6 cohorts of familial and sporadic forms of LOAD from different ethnicities. Associations of these individual SNPs with AD were modest, with odds ratios (ORs) ranging from 1.4–2.6 (compared to 14.9 for homozygosity for the ε4 allele of APOE [43]). Haplotype analysis using a sliding window covering 3 SNPs confirmed these associations by demonstrating that two clusters of SNPs in SORL1 were independently associated with AD: the 5’ cluster and the 3’ cluster. The 5’ cluster consists of SNPs 8, 9 and 10 which are located within intron 6 of SORL1. Possession of the T – A – T haplotype was associated with decreased risk of AD in Caribbean-Hispanic families (p = 0.0086) and in Israeli-Arab (p = 0.0037) and North European (p = 0.068) case-control cohorts. Conversely, the C – G – C haplotype for these SNPs was associated with increased risk for developing AD. The 3’ cluster consists of SNPs 22 – 25 and two overlapping haplotypes were associated with increased risk of AD: the C – T – T alleles at SNPs 22–24 and the T – T – C alleles at SNPs 23–25 in North European families and North European case-control cohorts. This region was also associated with AD in an African-American cohort. However, it was the A – C – T alleles for

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**Fig. 2. SORL1 trafficking pathways.** Nascent SORL1 peptides are generated in the endoplasmic reticulum (ER) and follow the constituent secretory pathway to the trans-Golgi network (TGN) where the pro-peptide is removed by furin-mediated cleavage. This allows the receptor to move to the plasma membrane where it can follow a signaling pathway (left) or a trafficking pathway (right). In the signaling pathway, SORL1 is cleaved by tumor necrosis factor-A converting enzyme (TACE) and then by γ-secretase, releasing luminal fragments of SORL1 and a cytosolic SORL1 intracellular domain (SORL1-ICD). SORL1-ICD can move to the nucleus (N) and regulate transcription of as yet unknown genes. In the trafficking pathway, SORL1 can be internalized via clathrin-mediated endocytosis utilizing the chaperone clathrin adaptor protein 2 (AP2). Internalized SORL1 receptors then shuttle between the TGN and the endosomes, guided by cytosolic adaptor proteins such as adaptor protein 1 (AP1), phosphofurin acidic cluster sorting protein (PACS1), Golgi-localizing, γ-adaptin ear homology domain ARF-interaction (GGA) and the retromer complex.
SNPs 23 – 24 \((p = 0.0025)\) which showed the latter association.

Further evidence for the association of the \textit{SORL1} locus with LOAD comes from a large-scale meta-analysis of four published genome-wide association studies (GWAS) with a combined sample size of 74,046 individuals \((25,580\ LOAD\ cases\ and\ 48,466\ controls)\). The \textit{SORL1} locus was one of the 14 genomic regions to be associated with LOAD \((p < 5 \times 10^{-8})\). The strongest signal in the \textit{SORL1} region was rs11218343, an intronic variant which was observed to be protective with an odds ratio of 0.77 \((95\%\ CI = 0.72 – 0.82)\) \[44\]. This study was extended to include 35,274 cases and 59,163 controls and \textit{SORL1} remained as one of the 25 genes reaching the threshold for genome-wide significance \((p < 5 \times 10^{-8})\) \[45\].

Due to the nature of GWAS, only common variants can be identified. With the advancement of next generation sequencing, whole exome sequencing (WES) experiments have identified rare variants in \textit{SORL1} associated with AD. Intriguingly, an early WES study performed on families showing autosomal dominant inheritance of EOAD but without pathological variants in \textit{AβPP}, \textit{PSEN1}, or \textit{PSEN2} found that 7/29 families carried putatively pathological variants in \textit{SORL1}. This supported the idea that \textit{SORL1} could be a fourth autosomal dominant EOaD gene alongside the presenilins and \textit{AβPP}. These variants were found throughout the coding sequence of the gene and were either missense (Gly511Arg, Asn924Ser, Asn1358Ser, Gly1681Asp, and Tyr141Cys) or nonsense (Cys1478X and Trp1821X) \[18\] (Fig. 3). However, Campion et al. \[46\] noted that some of the affected individuals in these families possessed variants in \textit{SORL1} and other AD risk loci \((e.g., APOE e4, TREM2, and ABCA7)\). It is also worthy of note that the ages of disease onset of some of these reported patients were close to the conventional threshold of 65 years of age. Therefore, it is still somewhat uncertain whether \textit{SORL1} variants can cause EOaD.

Despite the observations of Campion et al. \[46\], numerous other studies have found that variants in \textit{SORL1} are associated with EOaD. An independent study found 3 additional variants in \textit{SORL1} segregating with EOaD in families \((\text{Arg}416\text{X}, \text{Gly}1017\text{-Glu}1074\text{del},\ \text{and}\ \text{Arg}1303\text{Cys};\ \text{Fig. 3})\), although some of the affected individuals had \textit{APOE} genotype \textit{e3/e4} and had a later age of disease onset \[19\]. Nicolas et al. \[47\] found an enrichment of rare variants of \textit{SORL1} in AD cases compared to controls in a WES study in a French cohort \((OR = 5.03, 95\%\ CI = 2.02 – 14.99, p = 7.49 \times 10^{-5})\). When the authors restricted the analysis to cases showing a family history of AD, the OR increased to 8.86 \((95\%\ CI = 3.35 – 27.31, p = 3.82 \times 10^{-7})\). Other studies have shown variants in \textit{SORL1} to be associated with EOaD in different cohorts \[48, 49\]. However, these studies do not describe family histories of the EOaD variants, so no conclusions can be made about Mendelian inheritance.

\textit{SORL1} expression levels are also reduced in the sporadic form of AD. Gene expression profiling of lymphoblasts from LOAD patients and age-matched controls found that \textit{SORL1} was downregulated approximately 2-fold at the mRNA level, and 2.5-fold at the protein level \[50\]. Sager et al. \[51\] quantified \textit{SORL1} protein levels in frontal cortex of AD patients and found that approximately 30% of AD cases had reduced \textit{SORL1} protein levels. However,
they did not find any significant differences between AD and control brains overall. Another independent study found that SORL1 protein levels were decreased in post mortem, sporadic AD brains but not in EOfAD brains [52]. These results suggest that SORL1 plays different roles in EOfAD and the sporadic forms of AD. Thus, the relationship between SORL1 and the different subtypes of AD is still unclear.

Taken together, the above genetic studies support that SORL1 is a unique gene in AD as, currently, no other genes are strongly implicated in both the early and late onset forms of the disease. Understanding the molecular and cellular changes occurring due to variation in SORL1 could help us illuminate the differences between EOfAD and LOAD.

FUNCTIONAL STUDIES OF SORL1 VARIANTS

Functional studies characterizing the effects of genetic variation in SORL1 have largely been based on variants’ effects on AβPP processing (AβPP processing and trafficking are discussed in detail later in this review). Cuccaro et al. [53] showed that the EOfAD variants T588I and T2134M of SORL1 reduce the binding affinity of SORL1 protein for AβPP, resulting in altered AβPP trafficking in HEK293 cell lines. Similarly, Vardarajan et al. [54] showed that the E270K, A528K, and T947M variants found in LOAD patients also have reductive binding affinities for AβPP and alter AβPP trafficking in HEK293 cell lines.

Young et al. [55] used human induced pluripotent stem cell (hiPSC)-derived neurons from LOAD patients to investigate the cellular effects of the risk (R) or protective (P) haplotype at the 5’ SNP cluster in SORL1. They found expression of SORL1 was variable among their hiPSC-derived neurons, and there were no significant differences between levels of SORL1 mRNAs due to SORL1 haplotype or disease state. This supported that the effect of this haplotype was likely not due to altered basal SORL1 expression levels. However, they did find that patients carrying the R/R haplotype generally do not increase SORL1 expression levels in response to brain-derived neurotrophic factor (BDNF, a known inducer of SORL1 expression [56, 57]) that is also implicated in AD [58, 59]). This led to increased Aβ levels in R/R haplotype-containing hiPSC-derived neurons relative to hiPSC-derived neurons carrying the R/P or P/P haplotypes and, therefore, can partially explain the increased risk associated with the R/R haplotype. Having one copy of the protective haplotype appears to be sufficient to increase SORL1 expression in response to BDNF. Therefore, increasing SORL1 expression in response to BDNF is likely a protective mechanism.

SORL1 AND AβPP

The most studied role of SORL1 in AD is its role in AβPP trafficking. AβPP is a single transmembrane-spanning protein which can be subjected to an amyloidogenic, or non-amyloidogenic proteolytic processing pathway. In the non-amyloidogenic pathway, AβPP is cleaved by α-secretase, giving two fragments: a secreted form of AβPP, sAβPPα, and C-terminal fragment C83. The ADAM (A disintegrin and metalloprotease domain) family of proteolytic enzymes are responsible for α-secretase cleavage of AβPP. ADAM-9 [60], ADAM-10 [60, 61], ADAM-17 [60, 62], and ADAM-19 [63] have been shown to contain α-secretase activity. However, ADAM-10 appears to be the main α-secretase [64]. Further processing of sAβPPα by γ-secretase (of which the PSENs are catalytic subunits (reviewed in [65])) gives a p3 fragment and an AβPP intracellular domain (AICD). This pathway is known as non-amyloidogenic as α-secretase cleaves within, and prevents the formation of, the Aβ peptide. In the amyloidogenic pathway, AβPP is cleaved by β-secretase (β-site AβPP cleaving enzyme 1, BACE1), giving sAβPPβ and C99 fragments. Then C99 is further processed by γ-secretase to give Aβ peptides of mainly 40-42 amino acids in length (Fig. 4) (reviewed in [66]).

AβPP undergoes a series of trafficking steps within cells (Fig. 5). Nascent AβPP holoprotein is generated in the ER, before further processing in the Golgi. Some AβPP molecules can be transported through secretory vesicles to the plasma membrane, where the majority are cleaved by α-secretase and so are non-amyloidogenically processed [67]. Some surface AβPP may be internalized within endosomes by clathrin-mediated endocytosis. Internalization occurs due to interaction of the NPXY motif in the cytoplasmic tail of AβPP and the clathrin adaptor AP2 [68, 69]. Amyloidogenic processing of AβPP has been shown to proceed in many membranous organelles in the cell including the early and late-endosomal compartments [70, 71], the plasma membrane [71, 72],
Fig. 4. Proteolytic processing of AβPP. Membrane bound amyloid-β protein precursor (AβPP) can be subjected to non-amyloidogenic or amyloidogenic processing. In the non-amyloidogenic pathway, AβPP is first cleaved by α-secretase within the amyloid-β (Aβ) sequence, producing a soluble sAβPP fragment and a membrane-bound C83 fragment. C83 can be processed further by the γ-secretase complex to give a p3 fragment and an AβPP intracellular domain (AICD). In the amyloidogenic pathway, AβPP can be cleaved by β-secretase, giving the C99 and sAβPPβ fragments. Then C99 is cleaved by γ-secretase to give Aβ peptides and an AICD. β-secretase and γ-secretase may function together as a supramolecular complex. Yellow depicts a lipid bi-layer and pink depicts a cytosolic region.

The TGN [73] (non-amyloidogenic processing is also thought to occur in the TGN [74]), the lysosomes [75, 76], and the ER [77, 78]. In regards to amyloidogenic processing at the ER, Area-Gomez et al. [79] showed that PSENs (i.e., γ-secretase) are particularly enriched in the mitochondrial associated membranes (MAMs) of the ER, which are lipid raft-like regions of the ER that form close associations with mitochondria and regulate their activity (reviewed in [80, 81]). BACE1 also is enriched in lipid rafts, and has been specifically detected in MAMs [82]. Therefore, the proteolytic cleavage of AβPP to form Aβ, which appears to occur in the ER, likely occurs in MAMs. Intriguingly, MAMs have been implicated in AD (see [83, 84] for excellent reviews on this topic).

An interesting observation is that SORL1 forms a complex with BACE1 in perinuclear regions in neurons [85]. BACE1 and γ-secretase have also been shown to form a complex, which sequentially cleaves AβPP holoprotein to form Aβ and this also occurs in perinuclear regions of the cell [86]. MAMs also have a perinuclear subcellular distribution [79, 87] and, as mentioned above, are enriched in PSENs and contain the other components of γ-secretase [79]. We have also detected SORL1 protein in the MAM [88]. Taken together, these results support the idea that SORL1, BACE1, and γ-secretase may form a complex in MAMs which proteolytically cleaves AβPP holoprotein to form Aβ. However, direct evidence for this SORL1/AβPP/BACE1/γ-secretase complex has not been observed. As mentioned previously, SORL1 is also cleaved by γ-secretase [39, 40] and this could also occur in this complex. This idea is intriguing, as it links, functionally, all four EOFAAD loci (if SORL1 indeed is an EOFAAD locus). Further characterization of the interactions between these AD-related proteins is required.

SORL1 can act to modulate the distribution of AβPP within cells. It has been shown that overexpression of SORL1 results in an accumulation of AβPP in the Golgi and prevents it from being sorted to the late endosomal membranes where some Aβ is produced [27, 30, 89]. Conversely, Sorl1 deficiency results in increased Aβ levels compared to controls [30, 90]. SORL1 interacts with the carbohydrate-linked domain of AβPP through its LDLR class A domain (repeats 5-8 appear to be crucial region for binding) [30, 91, 92], as well as with the cytoplasmic domain of AβPP through its intracellular domain [93] (Fig. 1B). There is evidence supporting that this interaction between SORL1 and AβPP prevents oligomerization of AβPP, which is required for the γ-secretase-cleavage of AβPP [94]. It has also been shown that SORL1 acts as an adaptor between AβPP and other intracellular sorting proteins (including the retromer complex, AP1, GGA, and PACS1) which guide AβPP through the endosomal/secretory pathways, keeping it away from subcellular compartments where Aβ is thought to form (such as in endosomes). These adaptors are discussed below and are summarized in Fig. 5. Binding sites of these adaptors in the SORL1 cytoplasmic tail are shown in Fig. 1B.
Fig. 5. SORL1 dependent trafficking of AβPP. AβPP is translated at the endoplasmic reticulum (ER) and is processed in the Golgi for direction to the cell surface. Some AβPP is cleaved in the non-amyloidogenic pathway by α-secretase and some AβPP is internalized by clathrin-mediated endocytosis via the chaperone clathrin adaptor protein 2 (AP2). SORL1 is present in early endosomes and can guide AβPP throughout different pathways in the cell by interacting with different adaptor proteins. SORL1 and AβPP can move directly back to the plasma membrane (orange) mediated by sorting nexin 27 (SNX27). They can also move retrogradely to the trans-Golgi network (TGN) mediated by the retromer complex, clathrin adaptor protein 1 (AP1), and/or phosphofurin acidic cluster sorting protein (PACS1). They can also move anterogradely from the TGN to the early endosomes mediated by Golgi-localizing, γ-adaptin ear homology domain ARF-interaction (GGA) proteins. Without SORL1, AβPP can move to late endosomal compartments where some β- and γ-secretase activities are thought to be located and can be proteolytically cleaved to form Aβ. SORL1 can also bind newly-formed Aβ and direct it to the lysosome for degradation. SORL1, AβPP, β- and γ-secretases are also present in the mitochondrial associated membranes (MAMs) of the ER.

The retromer complex is part of the endocytic machinery in cells and is responsible for retrograde transport of cargo from endosomes to the TGN as well as recycling of cargo from endosomes to the plasma membrane. It is composed of two subcomplexes of proteins encoded by genes from the vacuolar protein sorting (VPS) family. The subcomplex responsible for cargo selection consists of the proteins VPS35, VPS29 and VPS26 while the subcomplex responsible for vesicle formation consists of VPS5 and VPS17 (reviewed in [95, 96]). SORL1 interacts with the VPS26 subunit of the retromer complex through a FANSHY motif (Fig. 1B) in SORL1’s cytoplasmic domain and loss of this binding motif in SORL1 resulted in aberrant subcellular localization of SORL1 and an accumulation of AβPP in late endosomes [31]. Loss of retrograde transport of SORL1 back to the TGN would result in retention of SORL1 in endosomes. This would, in turn, increase retention of AβPP in endosomes where some Aβ is formed. This is supported by results from a cell culture study, where siRNA knockdown of the retromer complex caused impaired AβPP trafficking to the TGN [97]. Interestingly, gene expression profiling of the entorhinal cortex and the dentate gyrus of AD brains found that expression levels of genes encoding retromer components VPS35 and VPS26 were decreased in AD patients (a mix of EOAD and LOAD) compared to age-matched controls [98]. Also a GWAS showed that SNPs in retromer-associated genes were associated with sporadic AD in a Caucasian cohort [99]. In animal...
models, retromer activity knockdown in the mouse brain resulted in memory defects, synaptic dysfunction and increased Aβ levels, which is reminiscent of AD pathology [100, 101]. Taken together, these results indicate the possibility that loss of retromer function could also play a role in sporadic forms of AD.

Golgi-localizing, γ-adaptin ear homology domain ARF-interaction (GGA) proteins are responsible for anterograde transport from the TGN to endosomes. GGA binds to SORL1 via recognition of a DVPM motif (Fig. 1B) and an acidic cluster of amino acid residues in the cytoplasmic domain of SORL1 [32, 33]. Loss of the recognition motif for GGA proteins results in an inability of SORL1 to return to the TGN so that it accumulates in early endosomes and at the plasma membrane. This also results in increased sAβPPα products as AβPP also accumulates in the organelles in which α-secretase resides [33, 102, 103].

Phosphofurin acidic cluster sorting protein 1 (PACS1) is another sorting protein which mediates both retrograde and anterograde transport between the Golgi and endosomes [104]. The furin binding region of PACS1 interacts with SORL1 by recognizing the acid cluster motif DDLGEDDED in the SORL1 cytoplasmic domain [33, 34] (Fig. 1B). Loss of PACS1 activity has a similar outcome to loss of GGA proteins, where AβPP accumulates in early endosomes, likely due to aberrant subcellular localization of SORL1 [105].

Clathrin adaptor protein 1 (AP1) binds to an overlapping region in the cytoplasmic domain of SORL1 (DDLGEDDE) (Fig. 1B) and knockdown of AP1 also results in aberrant subcellular localization of SORL1 [34].

Sorting nexin 27 (SNX27) is a sorting protein involved in retrograde transport from the endosomes to the plasma membrane. SNX27 mediates the transport of AβPP via binding to the membrane-proximal region of the SORL1 cytoplasmic tail. Knockdown of SNX27 resulted in a reduced amount of cell surface SORL1 and AβPP protein levels. Conversely, overexpression of SNX27 enhanced distribution of AβPP and SORL1 to the cell surface. An increase in AβPP protein levels at the cell surface also resulted in increased α-secretase cleavage of AβPP to give sAβPPα [106]. However, SNX27 has been shown to interact with PSEN1 protein and this appears to reduce γ-secretase activity (i.e., reduced cleavage of AβPP and Notch) [107] so this could also explain the increased levels of sAβPPα.

SORL1 also plays a role in trafficking of Aβ. It interacts with Aβ through its VPS10 domain as shown by fluorescence polarization assays. Overexpression of SORL1 in a neuronal cell line also expressing a human AβPP isoform resulted in faster turnover of intracellular Aβ and accumulation of Aβ in lysosomes. Furthermore, an EOAD variant in the VPS10 domain of SORL1 disrupts the ability for SORL1 to bind Aβ [108, 109]. This process also appears to be mediated by GGA proteins [103]. These results indicate that SORL1 directs Aβ to lysosomes for degradation.

In summary, SORL1 plays an important and central role in the complex trafficking pathways of AβPP within cells. Therefore, it is no surprise that the loss of SORL1 activity in sporadic and some familial forms of AD leads to AβPP-related pathologies.

OTHER POTENTIAL ROLES OF SORL1 IN AD

SORL1 and lipoprotein metabolism

When AD was first described by Alois Alzheimer over a century ago, he observed a third pathological hallmark of ‘adipose inclusions’ and ‘lipid granules’ in the glia of his deceased patient Auguste Deter [110]. Subsequently, these observations were largely overlooked, but it is now well established that aberrant lipid metabolism occurs in AD brains. Lipids in the brain include glycerophospholipids, sphingolipids and cholesterol. These lipids play important roles such as in the structure of the myelin sheath which insulates axons and in formation of the plasma membrane. Cholesterol is one of the most well studied lipids and its homeostasis is implicated in AD. Indeed, there is an enrichment of cholesterol in cell membranes in AD patients compared to controls and there appears to be a positive correlation between cholesterol level and disease severity [111]. It is thought that cholesterol in the CNS is synthesized by glial cells and then imported into neurons as APOE-containing lipoproteins. However, neurons have also been shown to express genes involved in cholesterol synthesis [112] so the reality of brain cholesterol metabolism may be more complex. SORL1 mediates cholesterol intake into neurons by acting as a receptor for APOE. SORL1 has preferential binding affinity for each of the isoforms of APOE which corresponds with the relative risk that each APOE allele presents for development of AD. SORL1’s highest affinity is with the ε4 isoform, followed by ε3 then ε2 [113]. Each isoform of APOE differs by cysteine (Cys) and
arginine (Arg) content at positions 112 and 158: e4 (Arg, Arg), e3 (Cys, Arg), and e2 (Cys, Cys). These amino acid residue changes result in conformational changes to the protein structure of APOE (reviewed in [114]) and could explain the differential affinities between each of the APOE isoforms and SORL1.

Overexpression of SORL1 increases cellular uptake of APOE e4 and e3 (but not e2, which is protective for AD (reviewed in [115])) as shown by a cell culture study [113]. Additionally, neural stem cells isolated from an AD patient with genotype e4/e4 had lower SORL1 expression after 5 weeks in culture than neural stem cells from AD and control subjects with the other possible APOE genotypes [116]. However, this study only analyzed one homozygous e4 patient. Collectively, these results indicate that SORL1 and APOE are functionally related.

**SORL1 and insulin signaling**

It has been proposed that AD may represent a ‘type 3 diabetes’ (reviewed in [117]) and that insulin resistance/deficiency underlies AD pathology. The insulin signaling pathway has been shown to be perturbed in AD brains [118, 119]. Since insulin signaling stimulates glucose metabolism, this could partly explain the aberrant glucose metabolism observed in AD brains in FDG-PET studies (reviewed in [120]).

**SORL1** has been shown to act as a sorting receptor for the insulin receptor in adipocytes. It directs internalized insulin receptor back to the cell surface, preventing lysosomal catabolism and, thereby amplifying insulin signals [121]. Loss of SORL1 activity could decrease the amount of insulin receptor at the cell surface and perturb the insulin signaling pathway. It is unknown whether this also occurs in the CNS and further investigation is required.

**Is SORL1 involved in iron homeostasis?**

There is strong evidence that perturbation of iron homeostasis may play a role in AD (reviewed in [122]). To our knowledge, SORL1 has not been linked directly to iron homeostasis. However, AβPP is thought to play a role in maintaining iron homeostasis and so SORL1 may have indirect effects.

Iron content is increased in both living [123] and post-mortem [124, 125] AD brains relative to controls. Iron dyshomeostasis may also be linked to many of the pathologies observed in AD such as mitochondrial dysfunction, vascular pathologies, changes in energy metabolism, and inflammation (reviewed in [122]).

A recent, elegant study by Yambire et al. [126] showed that acidification of the endo-lysosomal system was critical for ferric iron (Fe$^{3+}$) to be reduced to the more reactive ferrous (Fe$^{2+}$) form and released into the cytosol. Inhibition of endo-lysosomal acidification was shown to lead to a cellular ferrous iron deficiency and a pseudo-hypoxic response (as HIF-1α, a master regulator of cellular responses to hypoxia, is normally inhibited from acting under normoxia by a degradative mechanism requiring Fe$^{2+}$). Mitochondrial biogenesis and function were also disturbed and inflammation markers were increased. It has been shown that the C99 fragment of AβPP (and interestingly, also the PSEN1 holoprotein [127]) is required for acidification of the endo-lysosomal system [128]. Also, AβPP$^{-/-}$ mice are observed to have accumulation of intraneuronal iron [129, 130]. This demonstrates that interference with AβPP activity affects cellular iron homeostasis. If SORL1 is required for the proper subcellular localization of AβPP, then the loss of SORL1 activity observed in sporadic AD and some EO of AD cases may also indirectly affect iron homeostasis via AβPP. Further investigation of the role of SORL1 in iron homeostasis is required.

**SORL1 and estrogens**

One hypothesis to explain the female bias observed in AD incidence [141, 142], is the reduced estrogen levels in females due to menopause. This may increase the susceptibility of post-menopausal females to AD. Indeed, estrogen levels have been shown to be reduced in female AD brains relative to healthy, age-matched, female controls [143].

Estrogens have been shown to have neuroprotective effects in the brain. Estrogen administration reduces the severity of lesions in the brain after either permanent or transient ischemia [144]. This is relevant to AD as hypoxia/reduced blood flow is evident in AD brains [145, 146]. It has also been shown that estrogens are protective against Aβ toxicity *in vitro* [147, 148].

SORL1 expression appears to be stimulated by estrogens. Ratnakumar et al. [149] found that SORL1 was one of the 504 genes significantly differentially expressed in laser-dissected, serotonergic neurons from female, adult, ovariectomized Rhesus macaques treated with estrogen. Interestingly, AβPP and APOE
were also among these genes significantly differentially expressed \( (p < 0.05, \log \text{ fold change} > 2) \). \textit{SORL1} has also been shown to be differentially expressed in various cancer cell lines in response to estrogen treatments [150, 151]. Intriguingly, some studies have found an association between \textit{SORL1} variants and AD in a sex-specific manner in various cohorts [152, 153]. However, a detailed investigation of the relationship between estrogens and \textit{SORL1} in the context of AD is yet to be performed.

**CONCLUSION**

It is no surprise that variation at the \textit{SORL1} locus is associated with AD, as \textit{SORL1} plays roles in many of the cellular processes that have been linked to AD pathologies. \textit{SORL1} shows potential to illuminate mechanistic similarities and differences between rare EOaAD and the much more common sporadic forms of LOAD. However, more family-based studies analyzing the segregation of variants in \textit{SORL1} with EOaAD are required to confirm whether \textit{SORL1} can be regarded as a causative EOaAD locus alongside the \textit{PSEN} and \( \beta \text{PP} \).

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**CONFLICT OF INTEREST**

The authors have no conflicts of interest to report.

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