REVIEW

Associations of sorLA/*SORL1* with Alzheimer's disease

Ishita Guha Thakurta, Olav M. Andersen

The Lundbeck Foundation Research Center MIND, Danish Research Institute of Translational Neuroscience (DANDRITE) Nordic-EMBL Partnership, Department of Biomedicine, Ole Worms Allé 3, Aarhus University, DK-8000 Aarhus C, Denmark

Correspondence: Olav M. Andersen
E-mail: o.andersen@biomed.au.dk
Received: March 09, 2015
Published online: April 29, 2015

In recent years Alzheimer's disease (AD) has emerged as a research priority mainly due to an impressive increase in the average life expectancy in humans, which is associated with debilitating neurodegenerative disorders. The cardinal feature of the disease is accumulation of the amyloid-β peptide, which is derived from the sequential proteolytic cleavage of the amyloid precursor protein (APP). SorLA (sorting protein-related receptor with A-type repeats) is a member of the VPS10p-domain receptor gene family and identified as a significant sorting receptor that controls the processing and trafficking of APP. This review systematically discusses information on sorLA associations with AD including the mechanisms that regulate sorLA activity. We also describe how advances in understanding the mechanisms by which sorLA can reduce the amyloidogenic pathway may open for novel therapeutic strategies in tackling this devastating disorder.

Keywords: *SORL1*; sorLA; Amyloid Precursor Protein; Alzheimer's disease

To cite this article: Ishita Guha Thakurta, *et al*. Associations of sorLA/*SORL1* with Alzheimer's disease. Receptor Clin Invest 2015; 2: e700. doi: 10.14800/rci.700.

Introduction

Alzheimer's disease (AD) is the most common form of senile dementia. It is a progressive neurodegenerative disorder characterized by profound deterioration of memory and higher cognitive functions. The consistent features of the disease are amyloid plaques, neurofibrillary tangles and dystrophic neurites containing hyperphosphorylated tau [1]. Several studies have established the involvement of three key genes in early-onset (or familial) AD namely *amyloid precursor protein* (*APP*), *presenilin 1* (*PSEN1*), and *presenilin 2* (*PSEN2*). Similarly, *apolipoprotein E* (*APOE*) E4 allele has been identified as risk factor for late-onset (or sporadic) AD [2, 3]. Recently, genetic analysis revealed that also sorLA (*SORL1*) is a potential factor which can modulate the risk of early as well as late-onset AD (LOAD) [4, 5].

SorLA is a member of the vacuolar protein sorting 10 protein (VPS10p) domain receptor gene family but also shares structural similarity with the family of low density lipoprotein receptors (LDLRs). It was identified and characterized independently using biochemical purification of proteins from human brain binding to the receptor-associated protein, and subsequently confirmed by genetic screening of novel LDLR family members in other species [6, 7, 8]. This 250 kDa protein is encoded by the *SORL1* gene located on the human chromosome 11q23/24 [6]. The structure of sorLA comprises besides the N-terminal VPS10p domain and its associated 10 CC-domain, also an YWTD-repeat containing six-bladed β-propeller domain with an associated epidermal growth factor precursor like module, a cluster of 11 complement-type repeat (CR) domains, and a cassette of six structural elements found in neural cell-adhesion molecules (fibronectin type III repeats), a transmembrane region and a short tail in the cytoplasmic part (Figure 1.A) [9, 10]. SorLA is expressed widely in the mammalian central nervous system (CNS) notably the cerebral and entorhinal cortex, hippocampus, cerebellum and

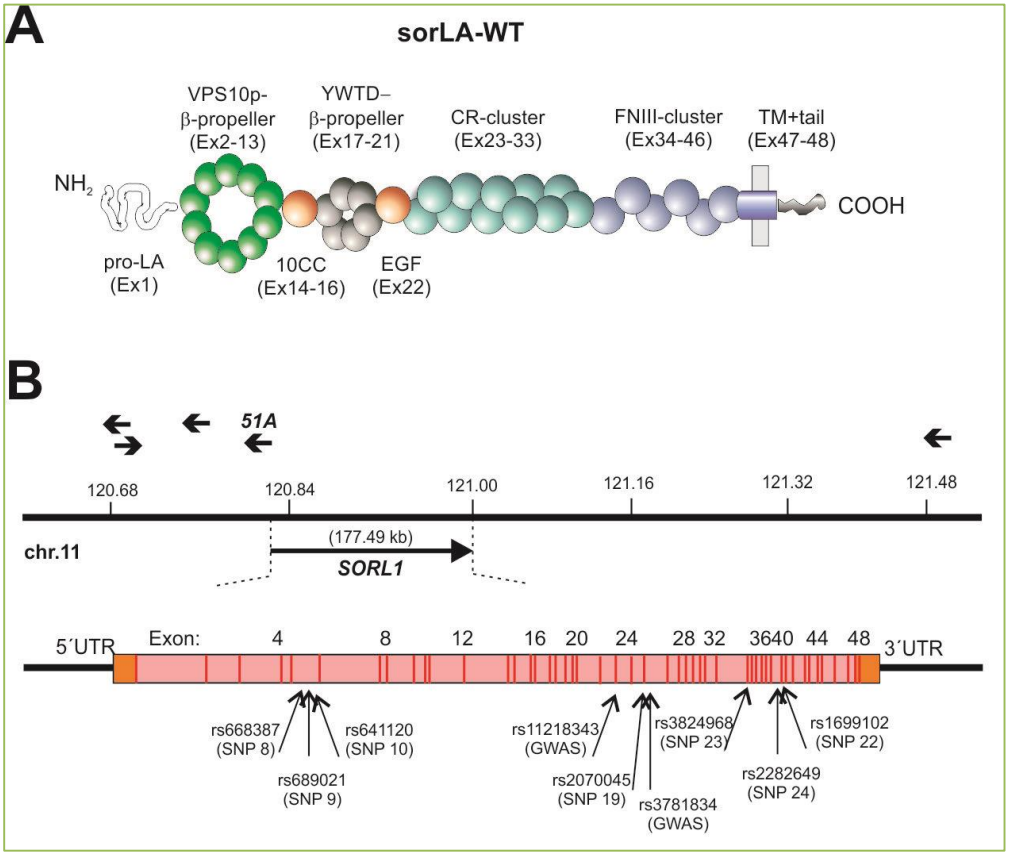

Figure 1. SorLA domain structure and genetic architecture of *SORL1* A. Schematic representation of the full-length sorLA-WT protein showing the mosaic domain structure encompassing the VPS10p β-propeller with its attached 10CC-domain, the YWTD β-propeller with its attached EGF domain, the cluster of 11 CR-domains, the cassette of 6 FnIII-domains, the transmembrane (TM) and the tail regions. The numbers below each protein region corresponds to its encoding exons **B.** Genomic map of human *SORL1* showing the location of some SNPs associated with Alzheimer's disease. Orange bars represent the 5' UTR and 3' UTR, pink bar represents intronic regions and red vertical bars represent each of the 48 exons.

brain stem as well as in non-neural tissues like ovary, testis, liver, adrenal gland, and lymph nodes [11, 12, 13].

This highly conserved putative receptor is located mainly in Golgi bodies, trans-Golgi network (TGN), early endosomes, and the plasma membrane of the cell [14]. SorLA is transported between TGN and endosomal compartments by cargo molecules like Adaptor protein 1 (AP1), Adaptor protein 2 (AP2), Golgi-localized, gamma-ear containing, ADP - ribosylation factor binding (GGA), Phosphofurin acidic cluster sorting protein 1 (PACS1), and the retromer complex [15]. Interactions with these adaptors are important for the implication of sorLA in AD, because deletion of the binding motifs in the cytoplasmic domain for these adaptors led to defective routing and altered APP processing [16, 17, 18, 19]. Importantly, several studies have also found a reduced expression of this sorting receptor in brain of AD patients as compared to healthy non-demented subjects. Besides the role in APP transport and metabolism, reports have also suggested binding of sorLA to ApoE encoded by the risk gene *APOE*, adding further evidence that sorLA is part of a protein network implicated in developing of AD [20]. Even though many studies have been carried out to understand the role of sorLA in progression of AD there are still many unanswered questions left. Thus, understanding the molecular mechanisms of sorLA is instrumental to establish its potential association with AD and may open new avenues for therapeutic interventions for curing the intractable disorder.

In the following, we will provide a summary for the genetic association of *SORL1* with AD, the current knowledge on regulatory mechanisms that determine the expression and cellular levels of sorLA protein, and finally review the existing evidence leading to our model how sorLA contributes to the control of APP trafficking and processing.

SorLA genetics and AD

Table 1. Genetic association studies reporting genetic variants in the SORL1 gene in AD

Year, Author	Population	Investigated SNPs (found to associate)	Citation
2007, Rogaeva *et al*.	North American & European whites, Caribbean Hispanics, African Americans, Israeli Arabs	rs668387, rs689021, rs641120, rs1699102, rs3824968, rs2282649 rs1010159	[23]
2007, Lee *et al*.	African American, Caribbean Hispanic and Non- hispanic whites	rs12285364, rs1784933 rs3824966	[24]
2007, Meng *et al*.	Subjects from TGEN (Translational Genomics Research Institute) database and Mayo clinic	rs1699103, rs11218350, rs10892759, rs1792113, rs726601, rs1784931	[115]
2007, Seshadri *et al*.	Framingham cohorts	rs1131497, rs726601	[41]
2008, Bettens *et al*.	Belgian	rs560573, rs668387, rs689021, rs641120, rs1614735	[25]
2008, Lee *et al*.	Japanese	rs2282649, rs668387	[116]
2008, Li *et al*.	Caucasian	rs2070045	[117]
2009, Cellini *et al*.	Italian	rs661057, rs12364988, rs641120	[26]
2009, Kölsch *et al*.	German	rs2070045, 18ex26, rs3824968, rs1010159	[46]
2009, Kimura *et al*.	Japanese	rs2070045, rs3824968, rs2282649, rs1010159	[118]
2009, Tan *et al*.	Chinese Han	rs3824968, rs1699102, rs2282649	[29]
2009, Beecham *et al*.	Collaborative Alzheimer Project	rs11218342, rs11218343, rs1784919, rs2298814, rs3781835, rs3781838, rs6589885, rs720099, rs7946599	[119]
2009, Feulner *et al*.	German	rs4935774, rs1614735, rs12576704, rs10502262, rs3781835	[120]
2010, Reynolds *et al*.	Swedish	rs668387, rs689021, rs641120, rs2070045, rs3824968, rs2282649	[121]
2010, Ning *et al*.	Chinese	rs2070045, rs3824968, rs2282649	[30]
2011, Cousin *et al*.	French caucasian	rs2282649	[35]
2012, Alexopoulos *et al*.	Non-hispanic caucasians	rs3824968, rs2282649	[122]
2012, Caglayan *et al*.	Whites	rs1699102 , rs2070045	[92]
2012, Guo *et al*.	German	rs661057, rs668387, rs689021, rs641120, rs2070045, 18ex26, rs1699102, rs3824968	[123]
2012, Olgiati *et al*.	Greek - Italian	rs668387, rs689021, rs641120	[124]
2013, Lambert *et al*.	European	rs11218343	[38]
2013, Wen *et al*.	Japanese	rs985421, rs12364988, rs4598682, rs3781834, rs3781836	[28]
2013, Izzo *et al*.	Brazilian	rs641120	[125]
2013, Miyashita *et al*.	Japanese, Koreans and Caucasians	rs4598682, rs17125523, rs3737529, rs3781834	[37]
2014, Jin *et al*.	Chinese Han	rs985421	[27]
2014, Xue *et al*.	Chinese Han	rs2070045	[126]
2014, Beecham *et al*.	National Institute on Aging (NIA) Alzheimer's Disease Centers (ADCs) and ADGC-collaborating studies.	rs11218343	[127]
2015, Louwersheimer *et al*.	German, Non-hispanics, Dutch	rs2070045	[128]
2015, Feng *et al*.	Chinese Han	rs1784933	[129]

The first observation suggesting a possible link between sorLA and dementia was a microarray analysis using lymphoblasts derived from AD patient showing decreased levels of *SORL1* transcripts. This report was further substantiated by immunohistochemistry studies, which demonstrated a decrease in sorLA protein expression in brain from AD patients [21]. This necessitated the need to unravel the function of sorLA in the human brain, as well as identifying the mechanism by which sorLA is reduced in brain and other tissues of AD patients. The landmark finding in this direction was identification that sorLA acts as a neuronal sorting receptor that binds APP *in vitro* and *in vivo* and regulates its trafficking and proteolytic processing into Aβ - the key feature of AD [22].

To understand this further, pioneering work by Rogaeva *et al*. documented strong allelic and haplotypic association of *SORL1* with sporadic AD in four different populations of North American and European whites, Caribbean Hispanics, African Americans and Israeli Arabs [23]. This study encompassing >6000 individuals looked at 29 (numbered 1 to 29 in the following) Single Nucleotide Polymorphisms

(SNPs) distributed along *SORL1* on human chromosome 11 (Figure 1.B). Interestingly, they identified two different sets of haplotypes associated with SNPs rs668387 (SNP 8), rs689021 (SNP 9), and rs641120 (SNP 10) in the 5' end of the gene, and rs1699102 (SNP 22), rs3824968 (SNP 23), rs2282649 (SNP 24), and rs1010159 (SNP 25) in the 3' end of the gene. This was followed by several epidemiological and genetic studies, which further confirmed *SORL1* as a susceptible gene for LOAD in a variety of ethnic populations. One study showed that rs12285364 (SNP 12), near the 5' region and rs1784933 (SNP 26) in the 3' region was associated with AD in a population of African Americans. In the same study rs12285364 (SNP 12) was reported to be associated with AD in a Hispanic population and rs3824966 (SNP 20) in non-Hispanic white individuals [24]. Independent studies confirmed that the five SNPs rs560573, rs668387, rs689021, rs641120, rs1614735 (corresponding to SNP 6, 8, 9, 10, and 27) also showed significant associations with LOAD in a well characterized Belgian population using single SNP analysis [25]. Another study explored influence of *SORL1* through female specific mechanisms in the involvement of rs661057, rs12364988, and rs641120 (SNPs 4, 7, and 10, respectively) with LOAD compared with controls in an Italian population [26]. Recently, a strong association was also established for the *SORL1* gene variant rs985421 with sporadic AD and amnestic mild cognitive impairment in the Han Chinese population [27]. The same SNP (rs985421) also showed a strong association with LOAD together with SNP rs12364988 (SNP 7), rs4598682, rs3781834, and rs3781836 in neuropathologically well-characterized Japanese brain donor subjects [28]. Interestingly, the gene frequency analysis of the data related to this polymorphism shows a marked difference between Asian and European populations [27]. This finding indicates the influence of geographical origin and ethnicity on the distribution of genetic risk factors associated with AD. Additionally, studies on Asian populations showed a positive correlation between SNPs rs2070045, rs3824968, rs2282649 and rs1010159 (SNPs 19 and 22-24) and the risk of AD [29, 30]. However, some studies have only found weak or no association at all for *SORL1* and Alzheimer's disease, adding further evidence to the complicated genetic linkages often encountered when studying multiple gene disorders like dementia [31, 32, 33, 34, 35].

In summary, the literature is extensive and still rapidly increasing (Table 1), and suggestive of a significant association in multiple regions of the *SORL1* gene. These findings point to the existence of different AD-associated haplotypes in different populations, which have lead to a theory of extensive locus and allelic heterogeneity, with disease-associated variants located within multiple different haplotypes [23, 24]. Therefore, a comprehensive meta-analysis was carried out to reexamine the association of known *SORL1* variants with AD in 2011 [36]. From this study, seven markers were consistently found to be significantly associated with AD after correction for multiple testing namely rs661057, rs11218304, rs668387, rs689021, rs641120, rs12285364, and rs2070045 (SNPs 4, 5, 8, 9, 10, 12, and 19, respectively). Furthermore, haplotype analysis revealed that the most significant association was the C-G-C alleles for rs668387-rs689021-rs641120 (SNPs 8-9-10) together with the G allele at rs2070045 (SNP 19) that were also shown to be positively associated with AD in the initial report [23].

Lately, two independent genome wide association studies (GWAS) identified *SORL1* variants that are significant associated with AD. First, a three stage GWAS was carried out using samples from Japanese, Korean, and Caucasian populations. Four SNPs (rs4598682, rs3781834, rs17125523 and rs3737529) showed significant association for the combined population in the first stage, but in the final stage only SNP rs3781834 showed a significant association with LOAD [37]. Second, a very large GWAS with >70,000 individuals, by Lambert and colleagues, revealed a strong confirmatory signal between SNP rs11218343 and LOAD, providing firm evidence that SORL1 is also associated with AD in the European ancestry [38].

Very interestingly, a recent study also identified familial autosomal dominant mutations in *SORL1* linked to early-onset AD (EOAD), suggesting that such pathogenic mutations may influence AD onset in a similar causative way as the well-known mutations in *APP* and *PSEN* [39]. However, information about the underlying molecular mechanisms how these mutations cause AD is still very limited.

Several studies have tried to identify neuropathological consequences of different *SORL1* variants, however, the physiological effects modulating AD endophenotypes of the sorLA mutations have not yet been firmly established. Magnetic resonance imaging studies showed that variants of *SORL1* are associated with neuropathological measures of neurodegenerative and cerebrovascular disease [36, 40]. Some polymorphisms in *SORL1* are also shown to affect the structural properties of the brain, i.e. general cerebral atrophy, volume of the medial temporal lobe, hippocampal atrophy, and white matter hyperintensities [40, 41, 42]. Interestingly, Assareh and colleagues recently established that these neuroimaging biomarkers associated with *SORL1* polymorphisms are moderated by sex [43]. Independent studies using Kaplan-Meir survival analysis and Cox proportional hazard and linear regression models, also established that the five SNPs rs1784934, rs676759,

Table 2. Reports of sorLA expression decrease in LOAD

Year, Author	Effect /Findings	Method	Citation
2004, Scherzer *et al*.	SorLA shows 25% reduction in AD homogenates	Microarray analysis, WB, IHC	[21]
2006, Dodson *et al*.	SorLA is decreased 30% in sporadic AD	Immunostaining, WB	[47]
2006, Offe *et al*.	Marked reduction in sorLA expression	IHC	[48]
2007, Zhao *et al*.	33 – 56% reduction of sorLA in various brain regions	WB	[49]
2007, Sager *et al*.	55% reduction of sorLA in AD brains	Quantitative IHC	[50]
2009, Ma *et al*.	36% reduction of sorLA in postmortem CSF	WB	[51]
2009, Grear *et al*.	23% reduction of full-length and of total sorLA in AD	qPCR	[90]

rs560573, rs593769, and rs11218313 in male samples, and rs17125558 in female samples, all show significant association with age of AD onset (AAO) [44]. Earlier studies have similarly reported association of SNPs rs536360 and rs556349 [45] and *SORL1*-18ex26 (SNP 21) with the AAO in different populations [46]. Another study using a lifespan approach in four independently collected samples, found correlation between changes in white matter microstructure and expression of certain *SORL1* variants during human neurodevelopmental phases, suggesting that defects in sorLA may start to affect the brain at a very early stage in the human lifespan [4]. Additionally, Sheshadri and colleagues found a strong gene-phenotype association between the SNP rs1131497 and abstract reasoning, starting to shed light on the relation of the *SORL1* gene with cognitive function [41]. All studies taken together provide compelling evidence for a significant association of sorLA variants and AD, but further studies are clearly needed to characterize each variant and identify respective phenotypic outcomes of this gene.

SorLA regulation

The first demonstration of a change in sorLA expression in AD came in 2004. Here, Lah and colleagues applied microarray analysis and found an approximately 2-fold decrease in sorLA transcripts in lymphoblasts from AD subjects compared to control subjects. They also reported a 25% decrease of sorLA protein expression in AD brain homogenates [21]. This report was soon followed by other studies most of which showed a conspicuous decrease of approximately 30-50% in receptor expression in brains from sporadic AD patients as compared to non-demented controls [22, 47, 48, 49, 50, 51], although one study had later difficulties to reproduce its initial findings [52]. Similarly, analysis of cerebrospinal fluid (CSF) from AD patients identified a significant decline of 36% in receptor levels, suggesting that sorLA levels might be a useful biomarker for disease onset [51]. However, conflicting studies later showed an increased level of sorLA in CSF [53], necessitating the need to better understand the correlation between the brain and the CSF levels of the receptor. Reports of a decrease in sorLA expression in AD patients are summarized in Table 2. Taken together further studies are important to validate the levels of sorLA accurately in different regions of brain as well as in different stages of the disease.

The observed decrease of sorLA raised the important question whether receptor decline is directly linked to disease onset, or whether a low sorLA level is a consequence of the disease and ongoing neurodegenerative processes. To address this relevant question, Dodson *et al.* documented that sorLA is unaffected in cases with familial AD [47] indicating non-involvement of the downstream processes controlled by the FAD mutations. There has also been biochemical evidence showing reduced expression of this receptor in cases of mild cognitive impaired subjects making it more likely that the decrease in receptor expression occurs early or even prior to disease onset [50]. This indicates that the loss of the receptor function is a primary event in the progression of AD rather than the consequence of the same.

In line with these findings, studies with transgenic mice with genetically altered sorLA expression have confirmed the causal role of sorLA in the amyloidogenic pathway and accelerating plaque deposition. The first report describing the generation of sorLA deficient mice by Andersen *et al.* showed aggravated processing of endogenous APP accompanied with elevated Aβ peptide generation compared to wild-type animals [22]. Later studies using the same sorLA knockout line but on the PDAPP transgenic background, provided additional evidence that sorLA also determines the processing of the human APP *in vivo*. Additionally, this study provided evidence of stimulation of the ERK pathway and adult neurogenesis, which was strongly correlated to the increased levels of sAPP due to sorLA deficiency in these mice [54]. Similarly, it was also shown that loss of sorLA in mice expressing APP^{SWE} is involved in the early events of the pathogenesis of AD, particularly in the conversion of the precursor molecule to the α-, β-, and γ-secretase derived metabolites, facilitating the forward shift in the onset of AD [55]. The latest study when sorLA deficiency was analyzed in AD10 anti-nerve growth factor mice confirmed that loss of the receptor exacerbates early amyloid pathology of endogeneous APP [56]. Thus, all these studies on the sorLA knockout mice gave a deep insight not only on our understanding of the role of this unique receptor as an APP sorting determinant, but also clearly indicating that sorLA deficiency poses to be an underlying cause and not a consequence of the disease onset.

Accordingly, it is imperative to divulge into the molecular

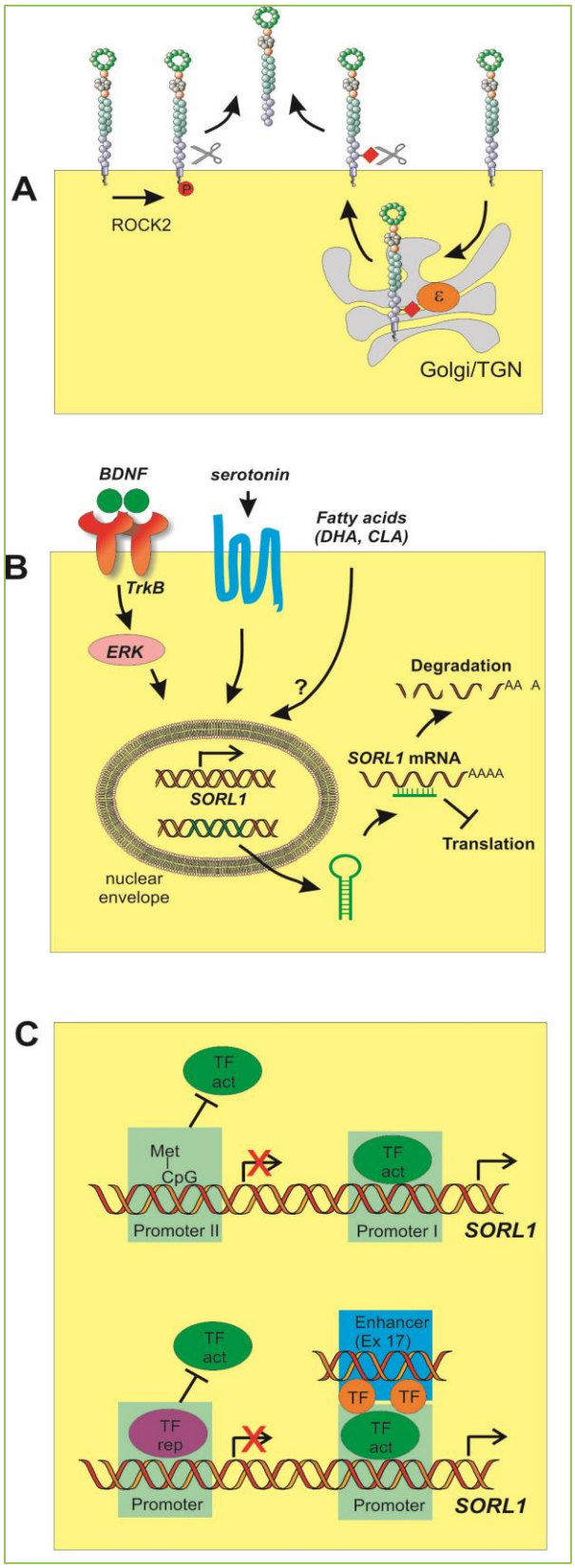

Figure 2. Regulatory pathways controlling sorLA expression. A. Shedding of sorLA leading to loss of neuron-associated receptor activity is regulated by ROCK2-dependent phosphorylation of the cytoplasmic tail, as well as an endocytosis-dependent glycosylation of the ectodomain. **B.** Interactions of extracellular stimuli like BDNF and serotonin with their cognate receptors at the plasmamembrane (ie. TrkB and the serotonin receptor) lead to activation of signaling pathways that enhance *SORL1* expression. Fatty acids like DHA and CLA are also speculated to activate sorLA gene expression by yet unknown mechanisms. Besides the direct role in regulation of *SORL1* transcription, regulation can also occur indirectly by induction of other genes encoding RNA molecules that indirectly leads to changes in *SORL1* mRNA levels, e.g. miRNA or non-coding RNA that initiates degradation of mRNA or leads to alternative splicing of the mRNA. **C.** Regulation at the level of the *SORL1* gene is dependent on binding of transcription factors (TF) that either activates (act) or represses (rep) transcription. Transcription from different promoters (I and II) where the activity is modulated by the methylation state of its CpG islands presents yet another way to control correct timely and spatial sorLA expression. The presence of an enhancer element in exon 17 that assists in transcriptional activity has also been described.

mechanisms, which operate to regulate this pleiotropic receptor. We briefly mentioned that the relation between sorLA levels in brain and in CSF is not clear. However, understanding this correlation is urgently needed if we wish to use the level of sorLA in the CSF as a biomarker for AD in the future. Thus, we need to establish whether low sorLA levels in the brain is associated with elevated or decreased levels of sorLA secreted to the CSF. In one scenario, it could

easily be argued that the more sorLA present in the brain, the more sorLA would also be able to go into the CSF. On the contrary, if sorLA is unable to be shedded from its neuronal cell origin, then we could expect to see very little sorLA in the CSF but an elevated brain profile. Accordingly, it has become very interesting to study the shedding of sorLA [57, 58, 59]. The complex sorting itinerary of sorLA is instrumental to the efficiency of sorLA shedding taking place from the cell surface, and recent studies have identified several post-translational modifications including glycosylation and phosphorylation as regulators of sorLA sorting pathways (Figure 2.A).

SorLA is documented to interact with the Rho-associated coiled-coil containing protein kinase 2 (ROCK2), which in turn has been implicated in controlling the metabolism of APP and Aβ [60]. The binding of ROCK2 to sorLA leads to phosphorylation of the receptor at serine-2206 within the cytoplasmic domain and enhances the shedding of the sorLA ectodomain [61]. Similar, knockdown of this candidate kinase with siRNA decreased sorLA shedding and lead to increased intracellular sorLA [61]. Accordingly, these studies unraveled sorLA as a phosphoprotein, where shedding and consequently the intraneuronal level are regulated through a phosphostate sensitive mechanism.

Glycosylation is another post-translational modification known to regulate the shedding of many transmembrane proteins [62, 63, 64]. The homology of sorLA with LDL receptors, which is a group of proteins known to undergo glycosylation dependent shedding [65], suggests that cleavage and secretion of this receptor may also be regulated by specific glycan structures [57, 66, 67] (Andersen unpublished). Even though the exact physiological significance of the resulting soluble ectodomain is unclear, it may control functions relevant to AD pathogenesis, and influence the level ending up in circulation and/or CSF.

Besides the obvious effect of leaving less functional receptor inside the cell available for sorting activities when sorLA is released from the cell surface, the cleavage of sorLA also generates a C-terminal fragment that can be processed by γ-secretase [57, 68]. This cleavage generates a soluble intracellular domain with a potential function in regulation of gene expression, which may represent a way to also control sorLA transcription itself [69].

Existing literature suggests that genetic factors that may contribute to the regulation of sorLA only can be assigned a minor fraction of 14% [23] indicating involvement of extragenous molecules in the process (Figure 2.B). One such molecule is the brain derived neurotrophic factor (BDNF) found to increase sorLA expression in cultured neurons and *in vivo* [70]. Another example of an extracellular signaling molecule that enhances sorLA expression is serotonin, which is released from the activated platelets and known to act as a mitogenic signal. A recent study demonstrated that serotonin enhances sorLA expression and may consequently cause neointimal hyperplasia by triggering changes in the stages of differentiation of vascular smooth muscle cells [71].

There have also been several reports suggesting effects on sorLA regulation by various fatty acids (FA). These molecules are of particular interest, as the positive effects of Docosahexaenoic acid (DHA), an essential dietary omega-3 polyunsaturated fatty acid (PUFA) on synaptic membrane properties, learning and memory along with reducing the Aβ induced oxidative stress have been suggested by several groups [72, 73]. The link between a lipid regulated diet in form of DHA, and an increase in sorLA levels was demonstrated in a variety of *in vitro* and *in vivo* systems like primary rat neurons, aged non-transgenic mice, and an aged DHA-depleted AD mouse model [74]. However, conflicting data have also been reported, suggesting the opposite function that DHA enriched diets either do not modulate the levels of sorLA in transgenic mouse models of AD [75] or even reduces sorLA expression in AD patients [76]. Accordingly, further studies are needed to settle how sorLA is regulated by DHA. Conjugated linoleic acid (CLA) is another example of a PUFA suggested to influence sorLA expression [77]. Another dietary supplement, citrus pectin increases the endogenous levels of methanol which in turn was shown to upregulate *SORL1* transcription by approximately 1.5-fold in white blood cells of healthy human volunteers after citrus pectin intake [78]. Another study has documented that endothelial sorLA is modulated by shear stress and modified low-density lipoproteins by a p38MAPK dependent mechanism which accelerates atherogenesis [79]. These molecules are all examples of extracellular factors that stimulate sorLA expression, and would obviously be interesting to study further in order to reveal their involvement in the pathways of AD progression (Figure 2.B).

Unfortunately, little is known about the molecular mechanisms how these molecules exert their regulation of sorLA. Besides a direct role in the induction of *SORL1* transcription, it could also be speculated that they work by a more indirect pathway, e.g. activation of other genes that are able to regulate sorLA in classical signaling pathways, or genes that contain precursors at the level of nucleic acid regulators. Currently, microRNAs (miRNA) have been in focus for their role in neurodegenerative diseases [80, 81]. These small, non-coding RNAs that function in complex networks can fine tune protein expression. One of the first studies that demonstrated the abnormal expression of miRNA in AD was by Lukiw and colleagues [82]. Certain

NFκβ sensitive miRNAs, like miRNA-146a, have been shown to be upregulated in AD brains [83]. Thus, it would be interesting to explore the mechanisms leading to the upregulation of these miRNAs, and in particular how they influence expression of *SORL1*.

Another class of RNA molecules that could target expression of sorLA, is the non-coding RNA family that regulates the expression of other protein–coding genes at the level of transcriptional and post-transcriptional processing. Recently, the synthesis of a non-coding RNA, termed 51A, was demonstrated to decrease synthesis of sorLA with subsequent impairment of APP processing and increased amyloid secretion. Interestingly, the 51A molecule was found to be upregulated in post-mortem cerebral cortices from individuals with AD, suggesting a direct role of this non-coding RNA in a *SORL1*-dependent aetiology and/or progression of this disorder [84].

After the discovery that sorLA is linked to dementia, and that low receptor expression is associated with onset of AD, researchers have started to search for regulatory mechanisms at the level of the *SORL1* gene. These studies have included description of gene regulation by promoter methylation, identification of enhancer elements, as well as demonstration of the binding of transcription factors (TF) that function to either activate or repress *SORL1* gene expression (Figure 2.C). Interestingly, splice variants and alternative promoters may represent yet another way to ascertain the complex regulation of *SORL1* expression, although very little information about such control mechanisms has so far been reported.

As one example, the CLA dependent activation of sorLA expression, was shown to involve a pathway dependent of PPAR-γ [77], a TF well known for its function in cellular assimilation of lipids via anabolic pathways. The Hypoxia induced factor-1α (HIF-1α) is another TF that directly induces *SORL1* transcription. Studies using chromatin immune-precipitation (ChIP) showed a direct binding of HIF-1α to a consensus sequence in the proximal *SORL1* promoter just upstream of exon 1 [85]. Other TFs that regulate the temporal and spatial expression of sorLA have not yet been reported. However, looking into the vast number of available ChIP sequencing dataset strongly suggest that *SORL1* transcription is target of multiple factors adding to an emerging picture of a very complex regulation of sorLA activity (Andersen unpublished).

DNA methylation is one of the important epigenetic markers, which can modify gene regulation pathways instrumental in Alzheimer's disease [86]. The first report of DNA methylation patterns of the *SORL1* promoter came from studies in brain and blood of AD patients. It was shown that DNA methylation of *SORL1* is tissue specific and correlate with observed differences in the expression levels of *SORL1* [87]. Recently, another study showed a strong association between CpG methylation sites in the *SORL1* loci and pathological diagnosis of AD. The authors of this study also found a stronger association of the increased *SORL1* transcript expression with tau tangle density as compared to Aβ accumulation suggesting that this mechanism account for some of the observed changes in sorLA expression during AD [88].

One important function of the observed DNA methylation may be silencing of one promoter allowing expression of sorLA from an alternative promoter. This model may explain the complex expression pattern observed for sorLA, e.g. the tissue and cell specific expression pattern during development of the CNS, although the identification of different transcription start sites have not yet been reported [11, 12, 13, 87, 89].

Besides the use of alternative promoters, other mechanisms of gene regulation include alternative splice variants not containing all the 48 exons present in the *SORL1* gene. The first example of a splice variant of sorLA was identified in 2009, when Grear *et al*. suggested the existence of a receptor variant lacking exon 2 [90]. Besides its function encoding the amino acid sequence of a protein, exons may also act as transcriptional enhancers that regulate gene expression. Recently, this was suggested as an important regulatory mechanism for sorLA expression, as it was found that *SORL1* exon 17 shows enhancer activity (Figure 2.C). Furthermore, this effect is tissue dependent, and showed a clearly different profile in mouse liver and HeLa cells [91].

Finally, recent studies have started to address the question, which of the SNPs in *SORL1* that associate with AD are responsible for the observed decrease in protein expression. A key study that partly answer that question identified two SNPs (rs2070045 and rs1699102*)* that modify the transcript sequence of the gene and converts it from frequent to rare codon usage [92]. Cell culture studies confirmed that this variant reduces the translation efficiency of the receptor, thereby suggesting a molecular mechanism correlating the *SORL1* genotype and receptor expression in human brain.

In summary these studies support a theory of post-translational as well as epigenetic mechanisms underlying the regulation of sorLA, which subsequently regulate APP processing and thereby amyloid deposition.

SorLA as trafficking receptor for APP

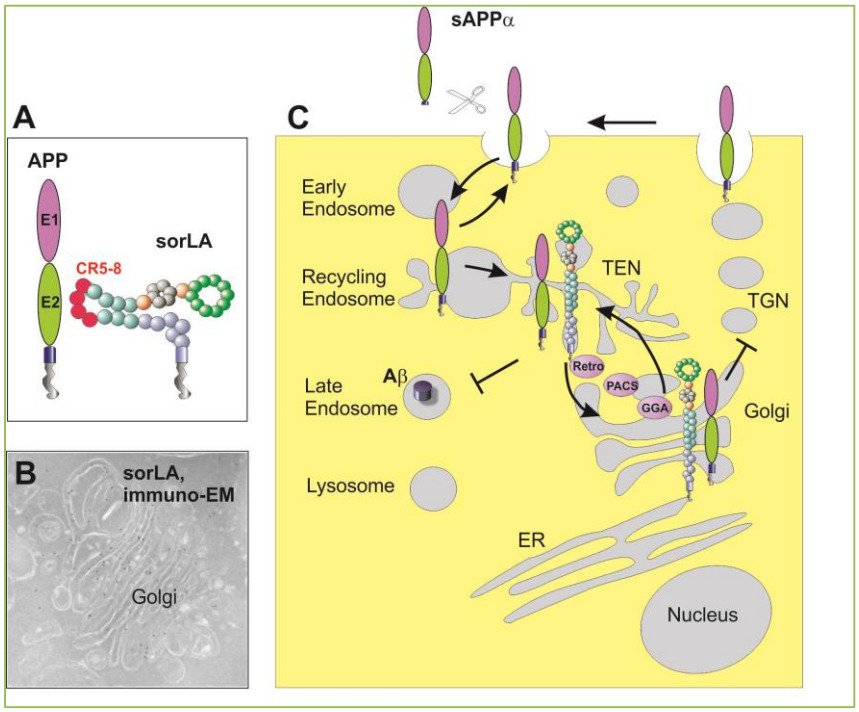

Figure 3. SorLA dependent mechanisms of APP trafficking and processing. A. Schematic model of the suggested sorLA•APP complex formation involving CR-domains 5-8 of sorLA and the E2 domain of APP. **B.** Immuno-electron micrograph showing a high density of sorLA in Golgi bodies of transfected SH-SY5Y cells. **C.** SorLA acts as sorting receptor that traps APP in the Golgi, reducing the speed and number of APP molecules that leave this compartment and enter the amyloidogenic and the non-amyloidogenic processing pathways. SorLA binding to APP in the secretory pathway decreases APP dimerization and enables correct post-translational modifications of the precursor including *O*-glycosylation. These functions requires high receptor density in the Golgi provided by the combined interactions of several retrograde adaptor binding molecules including PACS, GGA, and retromer that bind the cytoplasmic domain of sorLA, ensuring efficient retrograde trafficking of the sorting receptor from early and recycling endosomes to the tubular endosomal network (TEN), trans-golgi-network (TGN), and Golgi.

To understand why neuronal loss of sorLA is linked to amyloidogenic activity, it is mandatory to understand the function of sorLA as a trafficking receptor for APP. APP is a type-1 transmembrane protein widely expressed in neurons and non-neuronal cells playing a pivotal role in AD pathogenesis. APP can undergo several regulated processing steps, leading to the generation of numerous cleavage products with a multitude of described trophic and toxic functions [93, 94]. In the so-called amyloidogenic pathway, APP is cleaved in its ectodomain by β-secretase releasing the soluble APPβ fragment (sAPPβ) and leaving the β-carboxyl-terminal fragment (β-CTF) associated with the membrane. However, this fragment is further cleaved by γ-secretase in the lipid bilayer leading to generation of the Aβ peptide and the APP intracellular domain (AICD). Alternatively, in the non-amyloidogenic pathway, APP is cleaved by α-secretase within the Aβ domain sequence resulting in secretion of an extracellular fragment called sAPPα and APP α-carboxyl-terminal fragment (α-CTF). The α-CTF is processed by γ-secretase to generate AICD and the p3 peptide that is rapidly degraded but importantly inhibits the generation of Aβ.

The delivery of APP to the amyloidogenic and non-amyloidogenic pathways involves intraneuronal trafficking of APP as well as the secretases, which are all transmembrane proteins. Furthermore, the processing pathways show strongly restricted cellular preferences, e.g α-secretase cleaves APP primary in the secretory pathway and at the cell surface. In contrast, the amyloidogenic pathway occurs after APP is internalized from the cell surface and predominates in the endocytic pathway. Thus, transmembrane protein sorting can be postulated as one of the most vulnerable mechanisms in the progression of Alzheimer's disease [95, 96].

Accordingly, the discovery that a sorting receptor like sorLA was decreased in AD brains [21], and genetically

associated with the disease, immediately added focus to studies of sorLA in membrane transport mechanisms.

The first such analysis carried out by Andersen, Willnow and colleagues, showed that sorLA activity in transfected neuronal cells decreases APP proteolysis and thus hinders the production of Aβ [22]. Furthermore, studies using sorLA deficient mice showed that receptor activity also decreases the processing of APP *in vivo*, and thus established sorLA as a key etiological agent in Alzheimer's disease.

At almost the same time, Offe and colleagues showed that sorLA co-localizes with APP in the endocytic compartments and that receptor expression decreases Aβ levels in a dose dependent manner [48], thus providing further evidence for the hypothesis that sorLA is an active determinant of APP processing in neurons.

Yet the exact physiological function of sorLA was still elusive. However, its homology with VPS10p receptors involved in shuttling of proteins between subcellular compartments led to the hypothesis that sorLA influences intracellular transport of APP. To document a role of sorLA in APP trafficking, a direct interaction of the two proteins was shown in both living cells and *in vitro* along with their co-localization in the endosomal and Golgi compartments [22]. From biochemical analysis of the interaction, it was found that the carbohydrate domain of APP is bound by the CR-cluster of sorLA forming a 1:1 stoichiometric complex [97]. The binding site in sorLA was later narrowed down to the central CR-domains (CR5-8) [67] (Figure 3.A).

A second functional binding site in the cytoplasmic domain of APP (C99) and sorLA was also suggested, as the tail of sorLA was sufficient to inhibit APP processing [98]. However, later studies showed that the tail is not required for a direct interaction between sorLA and APP, suggesting an indirect function of the tail domain [67]. It could be speculated that this function involves sorLA-depenent sorting of the secretases (and not APP), as sorLA also modulates the interaction between BACE and APP [98].

To determine the mechanism how sorLA activity depresses APP proteolysis, several studies were carried out to characterize the cellular localization of sorLA and to identify the molecules involved in regulation of its cellular trafficking itineraries.

Mutations of the binding sites in the sorLA cytoplasmic domain for GGA1, for PACS1, or for the retromer complex – as well as knockdown studies of these cytosolic adaptors - all lead to a change in the cellular distribution of sorLA along the Golgi-Endosomal pathway in agreement with the known function of these adaptors to convey sorting between these compartments. Based on these data, a model where sorLA is mainly located to the Golgi and endosomes was suggested. However, the exact localization of sorLA in neuronal subpopulations and cell lines is probably dependent on the relative expression of individual adaptor proteins. This may explain why some studies found the majority of sorLA in endosomes [16, 48, 99] whereas other studies showed primarily receptor localization in the Golgi [6, 14, 22, 99, 100, 101] (Figure 3.B).

Importantly, these studies also demonstrated that cells expressing sorLA with targeted deletion of binding sites for GGA, PACS1, and retromer show mis-localization of the sorting receptor itself, and also show changes in the trafficking of APP [16, 18, 19, 100, 102]. In this aspect, the retromer complex is of particular interest as components of this sorting complex is decreased in AD brains [103, 104] and genetically associated with AD [105]. Furthermore, studies using animal models genetically defective in retromer, provide clear evidence for a proximal role of retromer dysfunction in AD [104, 106, 107]. Studies from our laboratory further documented that the FANSHY sequence in the cytoplasmic domain of sorLA is recognized by the VPS26 subunit of the retromer complex, important for proper retrograde trafficking route for the receptor. The lack of this retromer binding results in sorLA distribution into the tubular endosomal network whereby sorLA looses its protective effect against Aβ production [18]. Hence it was shown that sorLA is the functional bridge, connecting APP with retromer as these proteins are unable to associate in the absence of sorLA [17]. These studies provided hope for pharmacological intervention aiming at the retromer-sorLA pathway, and very recently, research by Small and colleagues identified a pharmacological chaperone that can increase the stability and function of retromer and hence protect APP from pathogenic processing [108].

Collectively, the literature convincingly suggests sorLA as an APP sorting determinant by two mechanisms. The main difference between the two models is the cellular site of sorLA activity, and as sorLA continuously traffics between intracellular compartments, the two models are likely not mutually exclusive, and both mechanisms may contribute to the anti-amyloidogenic activity of the receptor. It can act as a retention factor for APP in the Golgi network and inhibit APP release to its usual processing pathway as it traps APP from reaching the cell surface where it can participate in the amyloidogenic and non-amyloidogenic processing in post Golgi bodies [100]. Here, sorLA may also prevent the formation of APP homodimers, which are the preferred substrate for β-secretase [109, 110]. Alternatively, sorLA may also act in the retrograde transport of APP when present in

endocytic compartments, to assist APP escaping amyloidogenic cleavages, and shuttle APP back to the TGN and Golgi vesicles. This intracellular sorting mechanims prevent accumulation of Aβ (Figure 3.C). Both models suggest interaction between sorLA and APP in compartments with slightly acidic pH in line with recent findings that the binding indeed is favored at low pH [67].

Post-translational modifications are also known to influence the sorting and cleavage of APP. Both phosphorylation and glycosylation has been shown to change APP processing [111, 112]. An early study shows that APP is cleaved after maturation by *O*-glycosylation, which occurs during the transport of APP through the Golgi complex [113]. Glycosylation of APP has an impact on the APP processing pathway in determining its route from Golgi to the cell membrane [114]. The role of sorLA in these modifications is not yet clear. However, a novel finding from our group identified that sorLA action in the Golgi may affect APP glycosylations [67]. Mutations of fingerprint residues in sorLA CR-domains 5-7 leads to a novel *O*-linked glycosylation of APP, adding evidence to the model that sorLA retains precursors in the early secretory pathways [67].

Conclusions

Impairment of vesicular transport is a major molecular mechanism underlying neurodegenerative disorders like Alzheimer's disease. Several lines of studies have conclusively shown that sorLA binds to the precursor protein and impacts its intracellular transport and processing. As the receptor deficiency accelerates amyloid accumulation, it is necessary to identify the regulatory mechanisms for sorLA leading to decreased receptor expression in AD. Similarly, extracellular agents could enhance sorLA expression, but this can be a risk until we have the complete functional profile of this receptor. Genetic association studies have uncovered an association of sorLA variants with risk of AD, suggesting that sorLA can be used as genetic risk profiling tool for early diagnosis of the disease. Although much needs to be revealed before using sorLA for clinical intervention it cannot be denied that this pleiotropic receptor holds great promise as a novel diagnostic biomarker and therapeutic tool in AD research.

Conflicting Interests

The authors declare that they have no conflict of interest.

Acknowledgements

The work in our laboratory is financially supported by the Lundbeck Foundation, the Novo Nordisk foundation, the Augustinus Foundation, and the Danish Council for Independent Research.

References

1. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001; 81: 741-766.
2. Schellenberg GD, Montine TJ. The genetics and neuropathology of Alzheimer's disease. Acta Neuropathol 2012; 124: 305-323.
3. Chouraki V, Seshadri S. Genetics of Alzheimer's disease. Adv Genet 2014; 87: 245-294.
4. Felsky D, Szeszko P, Yu L, Honer WG, De Jager PL, Schneider JA, *et al*. The SORL1 gene and convergent neural risk for Alzheimer's disease across the human lifespan. Mol Psychiatry 2014; 19: 1125-1132.
5. Rosenthal SL, Kamboh MI. Late-Onset Alzheimer's Disease Genes and the Potentially Implicated Pathways. Curr Genet Med Rep 2014; 2: 85-101.
6. Jacobsen L, Madsen P, Moestrup SK, Lund AH, Tommerup N, Nykjaer A, *et al*. Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein. J Biol Chem 1996; 271: 31379-31383.
7. Yamazaki H, Bujo H, Kusunoki J, Seimiya K, Kanaki T, Morisaki N, *et al*. Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member. J Biol Chem 1996; 271: 24761-24768.
8. Morwald S, Yamazaki H, Bujo H, Kusunoki J, Kanaki T, Seimiya K, *et al*. A novel mosaic protein containing LDL receptor elements is highly conserved in humans and chickens. Arterioscler Thromb Vasc Biol 1997; 17: 996-1002.
9. Herz J. The LDL receptor gene family: (un)expected signal transducers in the brain. Neuron 2001; 29: 571-581.
10. Hermey G. The Vps10p-domain receptor family. Cell Mol Life Sci 2009; 66: 2677-2689.
11. Hermans-Borgmeyer I, Hampe W, Schinke B, Methner A, Nykjaer A, Susens U, *et al*. Unique expression pattern of a novel mosaic receptor in the developing cerebral cortex. Mech Develop 1998; 70: 65-76.
12. Kanaki T, Bujo H, Hirayama S, Tanaka K, Yamazaki H, Seimiya K, *et al*. Developmental regulation of LR11 expression in murine brain. DNA Cell Biol 1998; 17: 647-657.
13. Motoi Y, Aizawa T, Haga S, Nakamura S, Namba Y, Ikeda K. Neuronal localization of a novel mosaic apolipoprotein E receptor, LR11, in rat and human brain. Brain Res 1999; 833: 209-215.
14. Jacobsen L, Madsen P, Jacobsen C, Nielsen MS, Gliemann J, Petersen CM. Activation and functional characterization of the mosaic receptor SorLA/LR11. J Biol Chem 2001; 276: 22788-22796.
15. Willnow TE, Andersen OM. Sorting receptor SORLA--a trafficking path to avoid Alzheimer disease. J Cell Sci 2013; 126: 2751-2760.
16. Nielsen MS, Gustafsen C, Madsen P, Nyengaard JR, Hermey G, Bakke O, *et al*. Sorting by the cytoplasmic domain of the amyloid precursor protein binding receptor SorLA. Mol Cell Biol 2007; 27: 6842-6851.

17. Fjorback AW, Andersen OM. SorLA is a molecular link for retromer-dependent sorting of the Amyloid precursor protein. Commun Integr Biol 2012; 5: 616-619.

18. Fjorback AW, Seaman M, Gustafsen C, Mehmedbasic A, Gokool S, Wu C, *et al*. Retromer binds the FANSHY sorting motif in SorLA to regulate amyloid precursor protein sorting and processing. J Neurosci 2012; 32: 1467-1480.

19. Herskowitz JH, Offe K, Deshpande A, Kahn RA, Levey AI, Lah JJ. GGA1-mediated endocytic traffic of LR11/SorLA alters APP intracellular distribution and amyloid-beta production. Mol Biol Cell 2012; 23: 2645-2657.

20. Yajima R, Tokutake T, Koyama A, Kasuga K, Tezuka T, Nishizawa M, *et al*. ApoE-isoform-dependent cellular uptake of amyloid-beta is mediated by lipoprotein receptor LR11/SorLA. Biochem Biophys Res Commun 2015; 456: 482-488.

21. Scherzer CR, Offe K, Gearing M, Rees HD, Fang G, Heilman CJ, *et al*. Loss of apolipoprotein E receptor LR11 in Alzheimer disease. Arch Neurol 2004; 61: 1200-1205.

22. Andersen OM, Reiche J, Schmidt V, Gotthardt M, Spoelgen R, Behlke J, *et al*. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc Natl Acad Sci U S A 2005; 102: 13461-13466.

23. Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, *et al*. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 2007; 39: 168-177.

24. Lee JH, Cheng R, Schupf N, Manly J, Lantigua R, Stern Y, *et al*. The association between genetic variants in SORL1 and Alzheimer disease in an urban, multiethnic, community-based cohort. Arch Neurol 2007; 64: 501-506.

25. Bettens K, Brouwers N, Engelborghs S, De Deyn PP, Van Broeckhoven C, Sleegers K. SORL1 is genetically associated with increased risk for late-onset Alzheimer disease in the Belgian population. Hum Mutat 2008; 29: 769-770.

26. Cellini E, Tedde A, Bagnoli S, Pradella S, Piacentini S, Sorbi S, *et al*. Implication of sex and SORL1 variants in italian patients with Alzheimer disease. Arch Neurol 2009; 66: 1260-1266.

27. Jin C, Zhang L, Xian Y, Liu X, Wu Y, Zhang F, *et al*. The SORL1 polymorphism rs985421 may confer the risk for amnestic mild cognitive impairment and Alzheimer's disease in the Han Chinese population. Neurosci Lett 2014; 563: 80-84.

28. Wen Y, Miyashita A, Kitamura N, Tsukie T, Saito Y, Hatsuta H, *et al*. SORL1 is genetically associated with neuropathologically characterized late-onset Alzheimer's disease. J Alzheimers Dis 2013; 35: 387-394.

29. Tan EK, Lee J, Chen CP, Teo YY, Zhao Y, Lee WL. SORL1 haplotypes modulate risk of Alzheimer's disease in Chinese. Neurobiol Aging 2009; 30: 1048-1051.

30. Ning M, Yang Y, Zhang Z, Chen Z, Zhao T, Zhang D, *et al*. Amyloid-beta-related genes SORL1 and ACE are genetically associated with risk for late-onset Alzheimer disease in the Chinese population. Alzheimer Dis Assoc Disord 2010; 24: 390-396.

31. Shibata N, Ohnuma T, Baba H, Higashi S, Nishioka K, Arai H. Genetic association between SORL1 polymorphisms and Alzheimer's disease in a Japanese population. Dement Geriatr Cogn Disord 2008; 26: 161-164.

32. Webster JA, Myers AJ, Pearson JV, Craig DW, Hu-Lince D, Coon KD, *et al*. Sorl1 as an Alzheimer's disease predisposition gene? Neurodegener Dis 2008; 5: 60-64.

33. Minster RL, DeKosky ST, Kamboh MI. No association of SORL1 SNPs with Alzheimer's disease. Neurosci Lett 2008; 440: 190-192.

34. Liu F, Ikram MA, Janssens AC, Schuur M, de Koning I, Isaacs A, *et al*. A study of the SORL1 gene in Alzheimer's disease and cognitive function. J Alzheimers Dis 2009; 18: 51-64.

35. Cousin E, Mace S, Rocher C, Dib C, Muzard G, Hannequin D, *et al*. No replication of genetic association between candidate polymorphisms and Alzheimer's disease. Neurobiol Aging 2011; 32: 1443-1451.

36. Reitz C, Cheng R, Rogaeva E, Lee JH, Tokuhiro S, Zou F, *et al*. Meta-analysis of the association between variants in SORL1 and Alzheimer disease. Arch Neurol 2011; 68: 99-106.

37. Miyashita A, Koike A, Jun G, Wang LS, Takahashi S, Matsubara E, *et al*. SORL1 is genetically associated with late-onset Alzheimer's disease in Japanese, Koreans and Caucasians. PLoS one 2013; 8: e58618.

38. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, *et al*. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 2013; 45: 1452-1458.

39. Pottier C, Hannequin D, Coutant S, Rovelet-Lecrux A, Wallon D, Rousseau S, *et al*. High frequency of potentially pathogenic SORL1 mutations in autosomal dominant early-onset *Al*zheimer disease. Mol Psychiatry 2012; 17: 875-879.

40. Cuenco KT, Green RC, Zhang J, Lunetta K, Erlich PM, Cupples LA, *et al*. Magnetic resonance imaging traits in siblings discordant for Alzheimer disease. J Neuroimaging 2008; 18: 268-275.

41. Seshadri S, DeStefano AL, Au R, Massaro JM, Beiser AS, Kelly-Hayes M, *et al*. Genetic correlates of brain aging on MRI and cognitive test measures: a genome-wide association and linkage analysis in the Framingham Study. BMC Med Genet 2007; 8 (Suppl 1): S15.

42. Braskie MN, Ringman JM, Thompson PM. Neuroimaging measures as endophenotypes in Alzheimer's disease. Int J Alzheimers Dis 2011; 2011: 490140.

43. Assareh AA, Piguet O, Lye TC, Mather KA, Broe GA, Schofield PR, *et al*. Association of SORL1 gene variants with hippocampal and cerebral atrophy and Alzheimer's disease. Curr Alzheimer Res 2014; 11: 558-563.

44. Wang K-S, Liu X, Wang L, Briones D, Xu C. Genetic Variants in the SORL1 Gene Are Associated with Age at Onset of Alzheimer Disease: A Survival Analysis. International Journal of Medical Genetics 2014; 2014: 1-6.

45. Lee JH, Chulikavit M, Pang D, Zigman WB, Silverman W, Schupf N. Association between genetic variants in sortilin-related receptor 1 (SORL1) and Alzheimer's disease in adults with Down syndrome. Neurosci Lett 2007; 425: 105-109.

46. Kölsch H, Jessen F, Wiltfang J, Lewczuk P, Dichgans M, Teipel SJ, *et al*. Association of SORL1 gene variants with Alzheimer's disease. Brain Res 2009; 1264: 1-6.

47. Dodson SE, Gearing M, Lippa CF, Montine TJ, Levey AI, Lah JJ. LR11/SorLA expression is reduced in sporadic Alzheimer disease but not in familial Alzheimer disease. J Neuropathol Exp Neurol

2006; 65: 866-872.

48. Offe K, Dodson SE, Shoemaker JT, Fritz JJ, Gearing M, Levey AI, *et al*. The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments. J Neurosci 2006; 26: 1596-1603.

49. Zhao Y, Cui JG, Lukiw WJ. Reduction of sortilin-1 in Alzheimer hippocampus and in cytokine-stressed human brain cells. Neuroreport 2007; 18: 1187-1191.

50. Sager KL, Wuu J, Leurgans SE, Rees HD, Gearing M, Mufson EJ, *et al*. Neuronal LR11/sorLA expression is reduced in mild cognitive impairment. Ann Neurol 2007; 62: 640-647.

51. Ma QL, Galasko DR, Ringman JM, Vinters HV, Edland SD, Pomakian J, *et al*. Reduction of SorLA/LR11, a sorting protein limiting beta-amyloid production, in Alzheimer disease cerebrospinal fluid. Arch Neurol 2009; 66: 448-457.

52. Sager KL, Wuu J, Herskowitz JH, Mufson EJ, Levey AI, Lah JJ. Neuronal LR11 expression does not differentiate between clinically-defined Alzheimer's disease and control brains. PLoS one 2012; 7: e40527.

53. Ikeuchi T, Hirayama S, Miida T, Fukamachi I, Tokutake T, Ebinuma H, *et al*. Increased levels of soluble LR11 in cerebrospinal fluid of patients with Alzheimer disease. Dement Geriatr Cogn Disord 2010; 30: 28-32.

54. Rohe M, Carlo AS, Breyhan H, Sporbert A, Militz D, Schmidt V, *et al*. Sortilin-related receptor with A-type repeats (SORLA) affects the amyloid precursor protein-dependent stimulation of ERK signaling and adult neurogenesis. J Biol Chem 2008; 283: 14826-14834.

55. Dodson SE, Andersen OM, Karmali V, Fritz JJ, Cheng D, Peng J, *et al*. Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease. J Neurosci 2008; 28: 12877-12886.

56. Capsoni S, Carlo AS, Vignone D, Amato G, Criscuolo C, Willnow TE, *et al*. SorLA deficiency dissects amyloid pathology from tau and cholinergic neurodegeneration in a mouse model of Alzheimer's disease. J Alzheimers Dis 2013; 33: 357-371.

57. Hampe W, Riedel IB, Lintzel J, Bader CO, Franke I, Schaller HC. Ectodomain shedding, translocation and synthesis of SorLA are stimulated by its ligand head activator. J Cell Sci 2000; 113: 4475-4485.

58. Hermey G, Sjogaard SS, Petersen CM, Nykjaer A, Gliemann J. Tumour necrosis factor alpha-converting enzyme mediates ectodomain shedding of Vps10p-domain receptor family members. Biochem J 2006; 395: 285-293.

59. Tsukamoto S, Takeuchi M, Kawaguchi T, Togasaki E, Yamazaki A, Sugita Y, *et al*. Tetraspanin CD9 modulates ADAM17-mediated shedding of LR11 in leukocytes. Exp Mol Med 2014; 46: e89.

60. Lane RF, Gatson JW, Small SA, Ehrlich ME, Gandy S. Protein kinase C and rho activated coiled coil protein kinase 2 (ROCK2) modulate Alzheimer's APP metabolism and phosphorylation of the Vps10-domain protein, SorL1. Mol Neurodegener 2010; 5: 62.

61. Herskowitz JH, Seyfried NT, Gearing M, Kahn RA, Peng J, Levey AI, *et al*. Rho kinase II phosphorylation of the lipoprotein receptor LR11/SORLA alters amyloid-beta production. J Biol Chem 2011; 286: 6117-6127.

62. Ullrich S, Munch A, Neumann S, Kremmer E, Tatzelt J, Lichtenthaler SF. The novel membrane protein TMEM59 modulates complex glycosylation, cell surface expression, and secretion of the amyloid precursor protein. J Biol Chem 2010; 285: 20664-20674.

63. Chun YS, Park Y, Oh HG, Kim TW, Yang HO, Park MK, *et al*. O-GlcNAcylation Promotes Non-Amyloidogenic Processing of Amyloid-beta Protein Precursor via Inhibition of Endocytosis from the Plasma Membrane. J Alzheimers Dis 2015; 44: 261-275.

64. Karabasheva D, Cole NB, Donaldson JG. Roles for trafficking and O-linked glycosylation in the turnover of model cell surface proteins. J Biol Chem 2014; 289: 19477-19490.

65. Iijima H, Miyazawa M, Sakai J, Magoori K, Ito MR, Suzuki H, *et al*. Expression and characterization of a very low density lipoprotein receptor variant lacking the O-linked sugar region generated by alternative splicing. J Biochem 1998; 124: 747-755.

66. Fiete D, Mi Y, Oats EL, Beranek MC, Baenziger JU. N-linked oligosaccharides on the low density lipoprotein receptor homolog SorLA/LR11 are modified with terminal GalNAc-4-SO4 in kidney and brain. J Biol Chem 2007; 282: 1873-1881.

67. Mehmedbasic A, Christensen SK, Nilsson J, Ruetschi U, Gustafsen C, Poulsen AS, *et al*. SorLA Complement-type Repeat Domains Protect the Amyloid Precursor Protein against Processing. J Biol Chem 2015; 290: 3359-3376.

68. Bohm C, Seibel NM, Henkel B, Steiner H, Haass C, Hampe W. SorLA signaling by regulated intramembrane proteolysis. J Biol Chem 2006; 281: 14547-14553.

69. Nyborg AC, Ladd TB, Zwizinski CW, Lah JJ, Golde TE. Sortilin, SorCS1b, and SorLA Vps10p sorting receptors, are novel gamma-secretase substrates. Mol Neurodegener 2006; 1: 3.

70. Rohe M, Synowitz M, Glass R, Paul SM, Nykjaer A, Willnow TE. Brain-derived neurotrophic factor reduces amyloidogenic processing through control of SORLA gene expression. J Neurosci 2009; 29: 15472-15478.

71. Nagayama D, Ishihara N, Bujo H, Shirai K, Tatsuno I. Effects of serotonin on expression of the LDL receptor family member LR11 and 7-ketocholesterol-induced apoptosis in human vascular smooth muscle cells. Biochem Biophys Res Commun 2014; 446: 906-910.

72. Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, *et al*. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 2005; 25: 3032-3040.

73. Dyall SC, Michael GJ, Michael-Titus AT. Omega-3 fatty acids reverse age-related decreases in nuclear receptors and increase neurogenesis in old rats. J Neurosci Res 2010; 88: 2091-2102.

74. Ma QL, Teter B, Ubeda OJ, Morihara T, Dhoot D, Nyby MD, *et al*. Omega-3 fatty acid docosahexaenoic acid increases SorLA/LR11, a sorting protein with reduced expression in sporadic Alzheimer's disease (AD): relevance to AD prevention. J Neurosci 2007; 27: 14299-14307.

75. Perez SE, Berg BM, Moore KA, He B, Counts SE, Fritz JJ, *et al*. DHA diet reduces AD pathology in young APPswe/PS1 Delta E9 transgenic mice: possible gender effects. J Neurosci Res 2010; 88: 1026-1040.

76. Vedin I, Cederholm T, Freund-Levi Y, Basun H, Garlind A, Irving GF, *et al*. Effects of DHA-rich n-3 fatty acid supplementation on

gene expression in blood mononuclear leukocytes: the OmegAD study. PLoS one 2012; 7: e35425.

77. McCarthy C, O'Gaora P, James WG, de Gaetano M, McClelland S, Fitzgerald DJ, *et al*. SorLA modulates atheroprotective properties of CLA by regulating monocyte migration. Atherosclerosis 2010; 213: 400-407.

78. Shindyapina AV, Petrunia IV, Komarova TV, Sheshukova EV, Kosorukov VS, Kiryanov GI, *et al*. Dietary methanol regulates human gene activity. PLoS one 2014; 9: e102837.

79. Bajari TM, Winnicki W, Gensberger ET, Scharrer SI, Regele H, Aumayr K, *et al*. Known players, new interplay in atherogenesis: Chronic shear stress and carbamylated-LDL induce and modulate expression of atherogenic LR11 in human coronary artery endothelium. Thromb Haemost 2014; 111: 323-332.

80. Long JM, Lahiri DK. Advances in microRNA experimental approaches to study physiological regulation of gene products implicated in CNS disorders. Exp Neurol 2012; 235: 402-418.

81. Maciotta S, Meregalli M, Torrente Y. The involvement of microRNAs in neurodegenerative diseases. Front Cell Neurosci 2013; 7: 265.

82. Lukiw WJ. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. Neuroreport 2007; 18: 297-300.

83. Lukiw WJ, Andreeva TV, Grigorenko AP, Rogaev EI. Studying micro RNA Function and Dysfunction in Alzheimer's Disease. Front Genet 2012; 3: 327.

84. Ciarlo E, Massone S, Penna I, Nizzari M, Gigoni A, Dieci G, *et al*. An intronic ncRNA-dependent regulation of SORL1 expression affecting Abeta formation is upregulated in post-mortem Alzheimer's disease brain samples. Dis Model Mech 2013; 6: 424-433.

85. Nishii K, Nakaseko C, Jiang M, Shimizu N, Takeuchi M, Schneider WJ, *et al*. The soluble form of LR11 protein is a regulator of hypoxia-induced, urokinase-type plasminogen activator receptor (uPAR)-mediated adhesion of immature hematological cells. J Biol Chem 2013; 288: 11877-11886.

86. Mastroeni D, Grover A, Delvaux E, Whiteside C, Coleman PD, Rogers J. Epigenetic mechanisms in Alzheimer's disease. Neurobiol Aging 2011; 32: 1161-1180.

87. Furuya TK, da Silva PN, Payao SL, Rasmussen LT, de Labio RW, Bertolucci PH, *et al*. SORL1 and SIRT1 mRNA expression and promoter methylation levels in aging and Alzheimer's Disease. Neurochem Int 2012; 61: 973-975.

88. Yu L, Chibnik LB, Srivastava GP, Pochet N, Yang J, Xu J, *et al*. Association of Brain DNA Methylation in SORL1, ABCA7, HLA-DRB5, SLC24A4, and BIN1 With Pathological Diagnosis of Alzheimer Disease. JAMA Neurol 2015; 72:15-24..

89. Lu H, Liu X, Deng Y, Qing H. DNA methylation, a hand behind neurodegenerative diseases. Front Aging Neurosci 2013; 5: 85.

90. Grear KE, Ling IF, Simpson JF, Furman JL, Simmons CR, Peterson SL, *et al*. Expression of SORL1 and a novel SORL1 splice variant in normal and Alzheimers disease brain. Mol Neurodegener 2009; 4: 46.

91. Birnbaum RY, Patwardhan RP, Kim MJ, Findlay GM, Martin B, Zhao J, *et al*. Systematic dissection of coding exons at single nucleotide resolution supports an additional role in cell-specific transcriptional regulation. PLoS Genet 2014; 10: e1004592.

92. Caglayan S, Bauerfeind A, Schmidt V, Carlo AS, Prabakaran T, Hubner N, *et al*. Identification of Alzheimer disease risk genotype that predicts efficiency of SORL1 expression in the brain. Arch Neurol 2012; 69: 373-379.

93. O'Brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 2011; 34: 185-204.

94. Haass C, Kaether C, Thinakaran G, Sisodia S. Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med 2012; 2: a006270.

95. Small SA, Gandy S. Sorting through the cell biology of Alzheimer's disease: intracellular pathways to pathogenesis. Neuron 2006; 52: 15-31.

96. Sannerud R, Annaert W. Trafficking, a key player in regulated intramembrane proteolysis. Semin Cell Dev Biol 2009; 20: 183-190.

97. Andersen OM, Schmidt V, Spoelgen R, Gliemann J, Behlke J, Galatis D, *et al*. Molecular dissection of the interaction between amyloid precursor protein and its neuronal trafficking receptor SorLA/LR11. Biochemistry 2006; 45: 2618-2628.

98. Spoelgen R, von Arnim CA, Thomas AV, Peltan ID, Koker M, Deng A, *et al*. Interaction of the cytosolic domains of sorLA/LR11 with the amyloid precursor protein (APP) and beta-secretase beta-site APP-cleaving enzyme. J Neurosci 2006; 26: 418-428.

99. Klinger SC, Glerup S, Raarup MK, Mari MC, Nyegaard M, Koster G, *et al*. SorLA regulates the activity of lipoprotein lipase by intracellular trafficking. J Cell Sci 2011; 124: 1095-1105.

100. Schmidt V, Sporbert A, Rohe M, Reimer T, Rehm A, Andersen OM, *et al*. SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1. J Biol Chem 2007; 282: 32956-32964.

101. Chen Y, Peng Y, Che P, Gannon M, Liu Y, Li L, *et al*. alpha(2A) adrenergic receptor promotes amyloidogenesis through disrupting APP-SorLA interaction. Proc Natl Acad Sci U S A 2014; 111: 17296-17301.

102. Burgert T, Schmidt V, Caglayan S, Lin F, Fuchtbauer A, Fuchtbauer EM, *et al*. SORLA-dependent and -independent functions for PACS1 in control of amyloidogenic processes. Mol Cell Biol 2013; 33: 4308-4320.

103. Small SA, Kent K, Pierce A, Leung C, Kang MS, Okada H, *et al*. Model-guided microarray implicates the retromer complex in Alzheimer's disease. Ann Neurol 2005; 58: 909-919.

104. Small SA, Petsko GA. Retromer in Alzheimer disease, Parkinson disease and other neurological disorders. Nat Rev Neurosci 2015; 16: 126-132.

105. Vardarajan BN, Faber KM, Bird TD, Bennett DA, Rosenberg R, Boeve BF, *et al*. Age-specific incidence rates for dementia and Alzheimer disease in NIA-LOAD/NCRAD and EFIGA families: National Institute on Aging Genetics Initiative for Late-Onset Alzheimer Disease/National Cell Repository for Alzheimer Disease (NIA-LOAD/NCRAD) and Estudio Familiar de Influencia Genetica en Alzheimer (EFIGA). JAMA Neurol 2014; 71: 315-323.

106. Muhammad A, Flores I, Zhang H, Yu R, Staniszewski A, Planel E, *et al*. Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration, and Abeta accumulation. Proc Natl Acad Sci U S A 2008; 105: 7327-7332.

107. Wen L, Tang FL, Hong Y, Luo SW, Wang CL, He W, *et al*. VPS35 haploinsufficiency increases Alzheimer's disease neuropathology. J Cell Biol 2011; 195: 765-779.

108. Mecozzi VJ, Berman DE, Simoes S, Vetanovetz C, Awal MR, Patel VM, *et al*. Pharmacological chaperones stabilize retromer to limit APP processing. Nat Chem Biol 2014; 10: 443-449.

109. Lao A, Schmidt V, Schmitz Y, Willnow TE, Wolkenhauer O. Multi-compartmental modeling of SORLA's influence on amyloidogenic processing in Alzheimer's disease. BMC Syst Biol 2012; 6: 74.

110. Schmidt V, Baum K, Lao A, Rateitschak K, Schmitz Y, Teichmann A, *et al*. Quantitative modelling of amyloidogenic processing and its influence by SORLA in Alzheimer's disease. EMBO J 2012; 31: 187-200.

111. Georgopoulou N, McLaughlin M, McFarlane I, Breen KC. The role of post-translational modification in beta-amyloid precursor protein processing. Biochem Soc Symp 2001; 67: 23-36.

112. Suzuki T, Nakaya T. Regulation of amyloid beta-protein precursor by phosphorylation and protein interactions. J Biol Chem 2008; 283: 29633-29637.

113. Tomita S, Kirino Y, Suzuki T. Cleavage of Alzheimer's amyloid precursor protein (APP) by secretases occurs after O-glycosylation of APP in the protein secretory pathway. Identification of intracellular compartments in which APP cleavage occurs without using toxic agents that interfere with protein metabolism. J Biol Chem 1998; 273: 6277-6284.

114. McFarlane I, Georgopoulou N, Coughlan CM, Gillian AM, Breen KC. The role of the protein glycosylation state in the control of cellular transport of the amyloid beta precursor protein. Neuroscience 1999; 90: 15-25.

115. Meng Y, Lee JH, Cheng R, St George-Hyslop P, Mayeux R, Farrer LA. Association between SORL1 and Alzheimer's disease in a genome-wide study. Neuroreport 2007; 18: 1761-1764.

116. Lee JH, Cheng R, Honig LS, Vonsattel JP, Clark L, Mayeux R. Association between genetic variants in SORL1 and autopsy-confirmed Alzheimer disease. Neurology 2008; 70: 887-889.

117. Li Y, Rowland C, Catanese J, Morris J, Lovestone S, O'Donovan MC, *et al*. SORL1 variants and risk of late-onset Alzheimer's disease. Neurobiol Dis 2008; 29: 293-296.

118. Kimura R, Yamamoto M, Morihara T, Akatsu H, Kudo T, Kamino K, *et al*. SORL1 is genetically associated with Alzheimer disease in a Japanese population. Neurosci Lett 2009; 461: 177-180.

119. Beecham GW, Martin ER, Li YJ, Slifer MA, Gilbert JR, Haines JL, *et al*. Genome-wide association study implicates a chromosome 12 risk locus for late-onset Alzheimer disease. Am J Hum Genet 2009; 84: 35-43.

120. Feulner TM, Laws SM, Friedrich P, Wagenpfeil S, Wurst SH, Riehle C, *et al*. Examination of the current top candidate genes for AD in a genome-wide association study. Mol Psychiatry 2010; 15: 756-766.

121. Reynolds CA, Hong MG, Eriksson UK, Blennow K, Johansson B, Malmberg B, *et al*. Sequence variation in SORL1 and dementia risk in Swedes. Neurogenetics 2010; 11: 139-142.

122. Alexopoulos P, Guo LH, Tsolakidou A, Kratzer M, Grimmer T, Westerteicher C, *et al*. Interrelations between CSF soluble AbetaPPbeta, amyloid-beta 1-42, SORL1, and tau levels in Alzheimer's disease. J Alzheimers Dis 2012; 28: 543-552.

123. Guo LH, Westerteicher C, Wang XH, Kratzer M, Tsolakidou A, Jiang M, *et al*. SORL1 genetic variants and cerebrospinal fluid biomarkers of Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci 2012; 262: 529-534.

124. Olgiati P, Politis A, Albani D, Rodilossi S, Polito L, Ateri E, *et al*. Association of SORL1 alleles with late-onset Alzheimer's disease. findings from the GIGAS_LOAD study and mega-analysis. Curr Alzheimer Res 2012; 9: 491-499.

125. Izzo G, Forlenza OV, Santos Bd, Bertolucci PH, Ojopi EB, Gattaz WF, *et al*. Single-nucleotide polymorphisms of GSK3B, GAB2 and SORL1 in late-onset Alzheimer's disease: interactions with the APOE genotype. Clinics (Sao Paulo) 2013; 68: 277-280.

126. Xue X, Zhang M, Lin Y, Xu E, Jia J. Association between the SORL1 rs2070045 polymorphism and late-onset Alzheimer's disease: interaction with the ApoE genotype in the Chinese Han population. Neurosci Lett 2014; 559: 94-98.

127. Beecham GW, Hamilton K, Naj AC, Martin ER, Huentelman M, Myers AJ, *et al*. Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. PLoS Genet 2014; 10: e1004606.

128. Louwersheimer E, Ramirez A, Cruchaga C, Becker T, Kornhuber J, Peters O, *et al*. The influence of genetic variants in SORL1 gene on the manifestation of Alzheimer's disease. Neurobiol Aging 2015; 36: 1605 e13-20.

129. Feng X, Hou D, Deng Y, Li W, Tian M, Yu Z. SORL1 gene polymorphism association with late-onset Alzheimer's disease. Neurosci Lett 2015; 584: 382-389.