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## Does the Apolipoprotein E Genotype Influence Amyloid Precursor Protein Sorting by Sortilin-Related Receptor: Implications for Alzheimer's Disease?

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**Abstract.** The amyloid hypothesis of Alzheimer's disease (AD) argues that the cerebral accumulation of amyloid is a primary driver of AD pathology, including amyloid plaque deposition, neurofibrillary tangle formation, synapse loss and neuronal cell death. Many lines of evidence support this hypothesis, and some major players of the cascade have been identified, including the amyloid precursor protein (APP), apolipoprotein E (APOE), the beta-site APP cleaving enzyme (BACE1), and the sortilin-related receptor (SORL1). However, the processes that finally lead to amyloid accumulation are still poorly understood. Recent evidence suggests that SORL1 is able to switch away APP from the amyloidogenic cleavage by BACE1. It has also been suggested that SORL1 activity is influenced by its ligand APOE. Alterations in the three-dimensional structure and in the binding properties of APOE related to the APOE4 genotype may cause changes in the interaction between SORL1 and APOE. These changes could affect the capacity of SORL1 to bind to APP, resulting in a reduced retention of APP by SORL1 in subcellular compartments. It appears promising to investigate possible associations between distinct single nucleotide polymorphisms within the SORL1 gene and the cerebrospinal fluid products of the amyloid cascade, and to test if these associations are modified by the APOE genotype. This may considerably enhance our understanding of the pathological processes leading to AD.

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## 1. Introduction

### 1.1. Alzheimer's disease and amyloid $\beta$

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that predominantly affects the elderly population. It can be classified into the sporadic form with a senile onset in most instances, which accounts for the majority of cases, and into a familial form with a presenile onset, in which causative gene mutations have been identified [1]. The two histopathological hallmarks of both forms of AD consist of the intracellular neurofibrillary tangles, composed of hyperphosphorylated tau protein (p-tau), and the deposition of extracellular amyloid plaques throughout cortical and limbic brain regions. The amyloid deposits of insoluble protein material mainly contain high levels of the 40 and 42 amino acid long amyloid  $\beta$  peptides ( $A\beta$ ).  $A\beta$  is produced through the cleavage of a precursor protein (APP) by the  $\beta$ - and  $\gamma$ -secretases. The accumulation of  $A\beta$  (in particular of  $A\beta_{42}$ ) in the brain initiates a cascade of events, which eventually leads to neuronal dysfunction, neurodegeneration, and dementia [2].

### 1.2. Amyloid precursor protein, the secretases, and amyloid $\beta$

APP is a ubiquitous, large integral membrane protein which is composed of a signal sequence, a large extra-membranous region, a single transmembrane domain and a small cytosolic C-terminal tail. APP cleavage by  $\alpha$ -secretase, the activity of which is primarily found at the cell surface [3], precludes  $A\beta$  generation because it cleaves within the  $A\beta$  sequence, generating a soluble derivative ( $sAPP\alpha$ ) and a shorter C-terminal fragment of 83 aminoacids, which is subsequently cleaved by  $\gamma$ -secretase to form a non amyloidogenic peptide.  $\beta$ -secretase (more recently termed BACE1) has been found to compete with  $\alpha$ -secretase for the first cleavage step of APP. BACE1 is a 501 aminoacid long aspartyl protease, which is predominantly localized in endosomes and in

the trans-Golgi network, but it is also present at the plasma membrane and in a soluble form [4,5,6]. Membrane-bound BACE1 can be endocytosed, processed by sheddases (proteases), or released as a holoprotein into the extracellular space [6]. The extracellular pool of secreted BACE1 can be endocytosed by neurons and therefore contributes to intracellular BACE1 activity [7]. The maximal activity of BACE1 at acidic pH levels is found in endosomal compartments [4]. Degredation by BACE1 occurs at the known  $\beta$  cleavage sites of APP at Asp 11 and Glu 11; the activity site of the enzyme is located on the luminal/extracellular side of the membranes. APP is cleaved into a large soluble fragment ( $sAPP\beta$ ) and a small C-terminal membrane-bound fragment (C99). The latter fragment is subsequently cleaved by  $\gamma$ -secretase, resulting in the  $A\beta$  peptide and a small rapidly degraded cytosolic metabolite. The role of BACE1 as a major player in  $A\beta$  production is confirmed by studies on transgenic APP mice with a BACE1 gene knock-out that showed a significant decrease in  $A\beta$  production compared to APP mice with a functional BACE1 gene [8]. BACE1 levels and activity are also increased in postmortem AD brains [9,10,11,12,13,14] and the CSF of patients with AD [15], and BACE1 levels have been shown to correlate with plaque numbers. The high relevance of BACE1 in the amyloid cascade is also underscored by its association with the CSF products of APP cleavage in AD, including  $A\beta_{40}$  and  $sAPP\alpha/sAPP\beta$  [15]. However, the molecular mechanisms that drive the increase of BACE1 concentration and activity in AD remain unclear.

### 1.3. Apolipoprotein E and amyloid $\beta$

Among the many factors that influence the complex interactions between APP and BACE1, APOE appears to have an outstanding role. APOE is involved in the distribution and metabolism of lipids in many organs and cell types [16] and is the primary cholesterol transporter in the brain [17]. In humans, the

APOE coding gene shows polymorphism with three different alleles (E2, E3, and E4) that result in six different genotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4). These genotypes impact on the three-dimensional structure and the lipid binding properties of the isoforms. E2 and E3 preferentially bind high density lipoproteins (HDL) and E4 binds very low density lipoproteins (VLDL) [16]. Although APOE4 carrier status is the strongest known genetic risk factor for sporadic AD [16], the underlying pathomechanisms are still highly disputed. An interaction between the APOE4 allele and CSF BACE1 activity was demonstrated. Ewers et al. [18] reported that the APOE4 allele was associated with higher BACE1 activity in patients with MCI and AD. The authors suggest an influence of the APOE genotype on A $\beta$  production via modulation of cholesterol levels, because high cholesterol concentrations have been linked to increased secretase activity and up-regulation of A $\beta$  generation [19-21], which might explain this interaction. Another possible interpretation is that APOE4 might increase APP endocytosis in a cholesterol independent way, since it has greater affinity for A $\beta$  than APOE2 and APOE3. This means that it may lead to an increased exposure of BACE1 to APP within the cell compartments (*i.e.*, endosomes), enhancing the amyloidogenic processing of APP. A third possibility is that increased BACE1 activity in APOE4 carriers may partially reflect APOE4-regulated A $\beta$  production within the vasculature or a role of APOE4 in the accumulation and clearance of A $\beta$  at the blood-brain barrier. However, the mechanisms involved in the interaction between BACE1 and APOE remain unclear.

#### **1.4. The sortilin-related receptor and the regulation of the amyloid pathways**

The sorting of APP and BACE1 into particular sub-cellular compartments such as the Golgi apparatus and endosomes has gained great interest, since certain cleavage steps

predominantly occur at specific locations. SORL1 has been associated with APP sorting, and a growing body of evidence highlights its role in the production of A $\beta$ . SORL1 is a 250 kDA type 1 receptor with an affinity to bind ligands of classical low density lipoprotein (LDL) family members such as APOE [22-25]. SORL1 is diffusely expressed throughout the brain [26] and can perform different functions depending on the environmental setting. This includes both mechanisms as a cell surface lipoprotein receptor, which binds APOE-rich lipoproteins and mediates their uptake [24], and as an intracellular sorting receptor [27,28] that engages in the Golgi apparatus-endosome transport. However, in contrast to other lipoprotein receptors of the same family, most SORL1 activity is found in intracellular compartments [29], where it seems to be crucially involved in APP and BACE1 sorting and their interactions. According to recent evidence, SORL1 promotes the retention of APP in subcellular compartments less favorable for BACE1 processing and thereby reduces the extent of proteolytic breakdown into both amyloidogenic and non-amyloidogenic products [30]. The co-expression of SORL1 and APP not only results in the formation of complexes but also in SORL1 dependent translocation of APP and concomitant drastic decrease in A $\beta$  production [27,28,31]. On the other hand, in a mouse model of AD with SORL1 gene knockout, A $\beta$  generation was significantly elevated [27,32]. Furthermore, SORL1 appears to interfere with the interactions between APP and BACE1 in the Golgi apparatus, directly inhibiting BACE1 activity on APP [28]. Multiple single nucleotide polymorphisms (SNPs) of the SORL1 gene were most recently associated with the risk of AD in several studies [33-38]. These variants are likely to be in intronic regulatory sequences which may govern cell type-specific or tissue-specific expression of SORL1 and affect the risk for development of AD by altering the physiological role of SORL1 in the processing of APP holoprotein. AD-associated haplotypes in SORL1

may result in a reduction of SORL1 transcription; this notion is corroborated by reduced SORL1 expression in carriers of AD risk haplotypes [33].

## 2. Hypothesis

Despite great efforts to unravel the mechanisms causing AD, there are still many open questions. At present, the amyloid hypothesis of AD is a highly promising field of research and several major players have been identified. APOE, BACE1, SORL1, and APP (including its cleavage products) all apparently play major roles, and interactions between them have been assumed. However, no comprehensive study has been conducted so far taking into account all aforementioned variables; therefore, important associations could have been missed. Since the expression of SORL1 can be increased by binding to its ligands (*i.e.*, APOE) [24,39], we hypothesize that the alterations in the three-dimensional structure and the binding properties of APOE related to the APOE4 genotype cause changes in the interaction between SORL1 and APOE. These changes may affect the capacity of SORL1 to bind to APP, resulting in a reduced retention of APP through SORL1 in subcellular compartments. As a consequence, APP is more exposed to BACE1 processing. The identification of such interactions would have major implications not only for the understanding of the pathological processes of AD but also on future therapeutic developments. APOE-mimetic compounds are in development that could enhance SORL1 activity due to the improved binding by an APOE3-like conformation of the applied lipoprotein [40]; furthermore, agents are in pipeline that could alter the structure of APOE4 to an APOE3-like conformation, which again could improve SORL1 activity [41]. Based on these considerations, further research on interactions between SORL1, APOE, BACE1, and the amyloid cascade are warranted. A study design capable of investigating these interactions would include patients with AD with

information on APOE and SORL1 genotypes, CSF BACE1 activity, and levels of CSF products of the amyloid cascade such as A $\beta$ 42 and sAPP $\alpha$ /sAPP $\beta$ . It could then be tested if (1) SNPs (or haplotypes) within the SORL1 gene are associated with the CSF products of the APP cleavage, taking into account inter-individual differences in the BACE1 activity; and (2) to test whether the APOE genotype modifies the association between the SORL1 SNPs (haplotypes) with the CSF products of the APP cleavage, again accounting for inter-individual differences in BACE1 activity. Based on the predictions of the hypothesis presented in this paper, (1) a significant association between SORL1 SNPs (haplotypes) and the CSF proteins can be expected; and (2) the APOE genotype will have a modifying effect on this association; more precisely, beneficial effects of distinct SORL1 SNPs (haplotypes) will be weakened by the APOE4 allele.

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