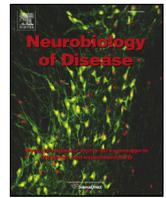




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## Review

## ATP-binding cassette transporter A1: From metabolism to neurodegeneration

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## ABSTRACT

ATP-binding cassette transporter A1 (ABCA1) mediates cholesterol efflux to lipid-free apolipoprotein A-I (apoA-I) and apolipoprotein E (apoE). ABCA1 is an essential regulator of high density lipoproteins (HDLs) and reverse cholesterol transport – a role that determines its importance for atherosclerosis. Over the last 10 years studies have provided convincing evidence that ABCA1, via its control of apoE lipidation, also has a role in Alzheimer's disease (AD). A series of reports have revealed a significant impact of ABCA1 on A $\beta$  deposition and clearance in AD model mice, as well as an association of common and rare ABCA1 gene variants with the risk for AD. Since APOE is the major genetic risk factor for late onset AD, the regulation of apoE level or its functionality by ABCA1 may prove significant for AD pathogenesis. ABCA1 is transcriptionally regulated by Liver X Receptors (LXRs) and Retinoic X Receptors (RXRs) which provides a starting point for drug discovery and development of synthetic LXR and RXR agonists for treatment of metabolic and neurodegenerative disorders. This review summarizes the recent results of research on ABCA1, particularly relevant to atherosclerosis and AD.

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## Introduction

Alzheimer's disease (AD) is a senile dementia characterized by the presence of senile plaques made of amyloid  $\beta$  (A $\beta$ ), neurofibrillary tangles, and cognitive decline. Although the inheritance of  $\epsilon$ 4 allele of APOE

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is the major genetic risk factor for late-onset sporadic form of AD (LOAD), the mechanisms underlying this association remain elusive. It is conceivable that additional genetic factors influence the risk, precipitating the development of dementia.

There is overwhelming data suggesting a link between lipid metabolism and AD (Hanson et al., 2013; Hughes et al., 2014; Reed et al., 2014; Simons et al., 1998). Genetic linkage and association studies have identified genes involved in cholesterol metabolism or transport as AD susceptibility genes (Harold et al., 2009; Jones et al., 2010). Dyslipidemia is a shared risk factor for cardiovascular disease and AD [reviewed in (Reitz, 2013)].

ATP binding cassette transporter A1 (ABCA1) belongs to the large superfamily of ABC transmembrane transporters (Koldamova et al., 2010; Oram and Vaughan, 2006). An important step towards understanding ABCA1 function was the discovery that mutations in its sequence cause Tangier disease (TD) characterized by impaired cellular cholesterol efflux, low levels of HDL particles and inefficient reverse cholesterol transport (RCT). Transcription of ABCA1 is regulated by Liver X Receptors (LXRs), Retinoic X Receptors (RXRs) and Peroxisome Proliferator-activated Receptors (PPARs). ABCA1 regulates cholesterol efflux to cholesterol acceptors, primarily lipid-free apoA-I and apoE but not to large HDL particles. ABCA1 is an essential mediator of HDL generation and loss of its function results in almost complete absence of HDL and apoA-I and a decrease of apoE. The role of ABCA1 as a regulator of HDL level determines its significance for atherosclerosis and cardiovascular disease.

The significance of ABCA1 for AD originates from its effect on apoE lipidation and stability. Experimental and clinical data suggest that apoE is involved in A $\beta$  aggregation, toxicity and clearance [reviewed in (Tai et al., 2014)], therefore it is conceivable to expect that ABCA1 as a modulator of apoE metabolism will have a role in AD pathogenesis. Data from experimental animals demonstrated that *Abca1* deficiency abolishes the lipidation of apoE and increases amyloid plaques in AD model mice (Hirsch-Reinshagen et al., 2005; Koldamova et al., 2005a, b; Wahrle et al., 2005). In contrast treatment of AD model mice with LXR, RXR or PPAR agonists ameliorates AD phenotype (Cramer et al., 2012; Donkin et al., 2010; Fitz et al., 2010; Koldamova et al., 2005b; Terwel et al., 2011; Yamanaka et al., 2012). Additional topic of interest for AD is the effect of ABCA1 on HDL in plasma and HDL-like lipoproteins in brain. Association studies have shown that lower concentration of HDL (Reed et al., 2014) and apoA-I (Merched et al., 2000) correlates with increased risk for AD. The results from Genome Wide Association Studies (GWAS) designed to reveal genetic association of ABCA1 with AD are controversial, however.

In this review, we summarize the results of research exploring the role of ABCA1 in metabolic diseases, mainly atherosclerosis and diabetes, and pathogenesis of LOAD. We will focus on the mechanism of cholesterol efflux and generation of HDL, and how they affect cardiovascular and neurodegenerative diseases.

## ABCA1 mediated the regulation of cholesterol efflux and HDL generation

ABCA1 is a transmembrane protein that transfers phospholipids and cholesterol to lipid free apoA-I or other apolipoproteins for generation of discoidal HDL particles (Oram and Vaughan, 2006). Discoidal HDL particles are composed of 100–200 lipid molecules and are surrounded by two apoA-I molecules (Lund-Katz and Phillips, 2010). A major function of HDLs is to participate in reverse cholesterol transport, a process by which excess cholesterol is removed from the cells and transported to the liver where it is metabolized for excretion (Oram and Vaughan, 2006). ABCA1 is comprised of two halves each having 6 transmembrane domains, and two large extracellular domains connected by intramolecular disulfide bonds that are important for the direct binding to apoA-I (Nagata et al., 2013; Oram and Vaughan, 2006). The mechanism by which ABCA1 translocates cholesterol and phospholipids is not fully

agreed upon but it is established that binding of ABCA1 to lipid-free apolipoproteins is critical for initiating the process of lipid efflux (Westerterp et al., 2014). In contrast to ABCA1, another transporter – ABCG1 mediates cholesterol efflux to HDL particles but not to lipid-free apolipoproteins (Gelissen et al., 2006; Wang et al., 2004).

According to an earlier model ABCA1 initiates translocation of phospholipids and cholesterol from the cytoplasmic to exofacial leaflet of the plasma membrane which leads to membrane bending and formation of exovesiculated domain to which apoA-I binds with high affinity (Vaughan and Oram, 2003; Vedhachalam et al., 2007). The next step is a spontaneous solubilization of membrane phospholipids and cholesterol in the exovesiculated domain by the bound apoA-I followed by formation of discoidal HDL particles (Vedhachalam et al., 2007; Westerterp et al., 2014). A different model for the formation of discoidal HDL particles was recently proposed by Nagata et al. (2013) (Fig. 1). The authors used a single-molecule fluorescence tracking technique to demonstrate that ABCA1 monomer diffuses freely and translocates lipids on the plasma membrane by an ATP-dependent mechanism (Fig. 1, step 1). Upon reserving sufficient cholesterol and phospholipids ABCA1 undergoes conformational changes and forms dimers. The lipidated ABCA1 dimers interact with the actin filaments in the plasma membrane and are immobilized until lipid-free apoA-I directly binds to the extracellular domains of the ABCA1 dimer (Fig. 1, step 2). In the last step apoA-I or another apolipoprotein accepts the lipids translocated by ABCA1 leading to the formation of discoidal HDL (Fig. 1, step 3). After transferring cholesterol and phospholipids to apoA-I, ABCA1-dimer dissociates into monomers and resumes its function to translocate lipids in an ATP-dependent manner.

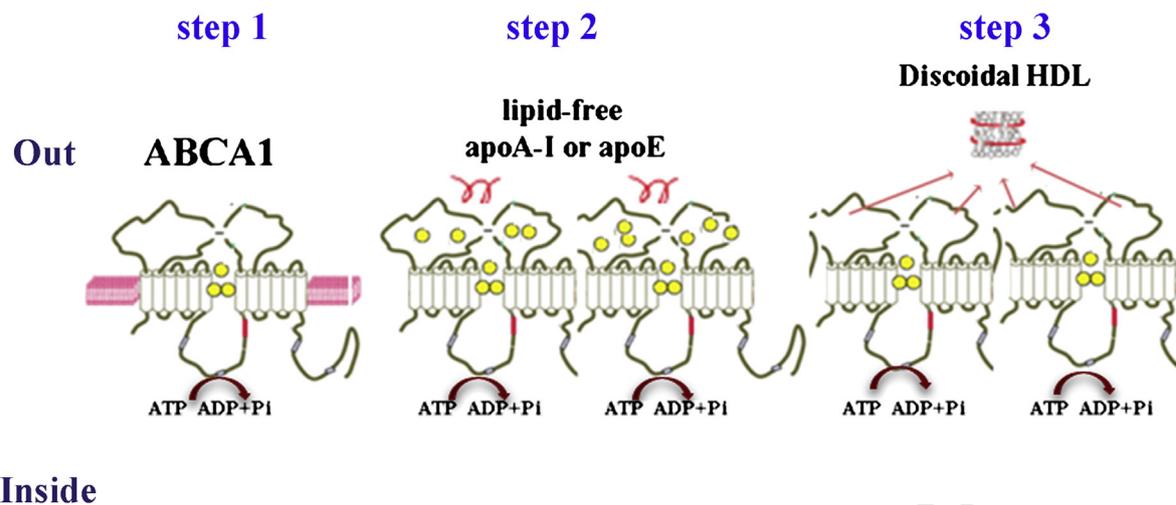
Apolipoproteins that serve as lipid acceptor in ABCA1-mediated cholesterol efflux are mainly apoA-I which comprises 60–70% of the total HDL protein mass and to a lesser extent apoA-II and apoE (Tang and Oram, 2009). Liver is the main peripheral organ for the synthesis of HDL (approximately 70% of plasma HDL) and apolipoproteins, but intestine and other extrahepatic organs may also participate. Discoidal HDLs formed only with apoE are slightly different than HDL containing only apoA-I, in that they are rather quasi-spheroidal (Lund-Katz and Phillips, 2010). When functional ABCA1 is lacking, the apolipoproteins which participate in HDL formation are left un-lipidated and are subjected to degradation in the kidney. Consequently, in patients without functional ABCA1 the levels of apoA-I and apoA-II fall to 1% and 7% of normal values respectively. Since apoE also participates in the formation of other lipoproteins such as very low density lipoproteins (VLDLs), intermediate density lipoproteins (IDLs) and chylomicrons, in patients with nonfunctional ABCA1 this apolipoprotein is partially protected from degradation and its level is decreased only by ~50% (Alaupovic et al., 1981; Mahley et al., 1991).

Transcriptionally, ABCA1 is regulated by nuclear receptors LXRs, RXRs and PPARs. Potential binding sites for other transcription factors in the proximal promoter of *ABCA1* do exist (Santamarina-Fojo et al., 2001), but they have not been interrogated systematically so far. According to a widely accepted model, ligand activated LXR/RXR and PPAR/RXR heterodimers bind to response element upstream of *ABCA1* transcription start site and drive the transcription in response to specific extra- or intracellular signals. The understanding of the interplay between the nuclear receptors and co-regulators involved in the transcriptional control of *ABCA1* and *APOE* is critical for successful development and testing of efficient ligand based therapies for AD (the topic is reviewed by Landreth et al. (2014), in this SI: Nuclear Receptors in Neurodegenerative Diseases, Neurobiol. Dis., <http://dx.doi.org/10.1016/j.nbd.2014.04.001>).

## Role of ABCA1 in periphery

### Cardiovascular disease

The decrease of cholesterol efflux, HDL, apoA-I and apoE as a result of loss-of-function mutations in ABCA1 affects different metabolic



**Fig. 1.** Mechanism of ABCA1-dependent cholesterol efflux. Step 1: ABCA1 monomer diffuses freely and translocates lipids on the plasma membrane by ATP-dependent mechanism. Step 2: Upon reserving sufficient cholesterol and phospholipids ABCA1 undergoes conformational changes and forms dimers. The lipidated ABCA1 dimers interact with the actin filaments in the plasma membrane and are immobilized until lipid-free apoA-I directly binds to the extracellular domains of ABCA1 dimer. Step 3: apoA-I or another apolipoprotein accepts the lipids translocated by ABCA1 leading to the formation of discoidal HDL. After transferring cholesterol and phospholipids to apoA-I, ABCA1-dimer dissociates into monomers and resumes its function to translocate lipids.

According to the model proposed by Nagata et al.

189 pathways in the periphery and central nervous system (CNS). In atherosclerosis and cardiovascular disease the effects of ABCA1 reflect its role  
190 in HDL formation and RCT. TD is a rare monogenic disorder caused by  
191 loss-of-function mutations in *ABCA1* and characterized by extremely  
192 low HDL level (less than 2% of normal) (Hobbs and Rader, 1999;  
193 Rader and deGoma, 2012). Typical TD patients in addition to very low  
194 HDLs often present with hepatosplenomegaly, peripheral neuropathy  
195 and enlarged yellow tonsils (Hobbs and Rader, 1999; Rader and  
196 deGoma, 2012). TD is inherited as an autosomal recessive trait and  
197 almost all of the patients with clinical phenotype are compound hetero-  
198 zygotes. At present, more than 180 *ABCA1* mutations have been listed in  
199 Human Gene Mutation Database ([http://www.hgmd.cf.ac.uk/ac/gene.  
200 php?gene=ABCA1](http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ABCA1)) and more than 100 of missense, nonsense and  
201 frameshift mutations had been identified in patients with TD and in  
202 subjects with a phenotype of HDL deficiency (Puntoni et al., 2012).  
203 While there is a significantly increased risk of cardiovascular disease  
204 in TD patients, the phenotypic presentation is not always exactly the  
205 same. Some TD patients, for example have a severe form of coronary  
206 heart disease whereas in other, typical symptoms including atheroscle-  
207 rotic lesions are missing (Hovingh et al., 2004). In addition to the  
208 reduced penetrance, variable expressivity of the mutant alleles and alle-  
209 lic interactions are possible explanations for the phenotypic variability  
210 observed in a sub-set of TD patients, where concomitant with HDL,  
211 there is a decrease in LDL level (Hobbs and Rader, 1999). Thus, in  
212 cases of *ABCA1* functional impairment, not well understood environ-  
213 mental and other genetic factors could alleviate the effect of the  
214 otherwise significant functional impairment of cholesterol efflux and  
215 HDL generation.

216 Whereas TD patients are very rare (less than 100 diagnosed in  
217 the world; <http://ghr.nlm.nih.gov/condition/tangier-disease>), *ABCA1*  
218 heterozygosity in the general population is present with a frequency  
219 of 3:1000 and also associates with decreased levels of HDL cholesterol  
220 (Frikke-Schmidt et al., 2008). Most of the published studies report an in-  
221 crease of atherosclerosis in heterozygous patients with missense muta-  
222 tions in *ABCA1* (Bochem et al., 2013; Clee et al., 2000; Frikke-Schmidt  
223 et al., 2005; van Dam et al., 2002). However, not all of the functional muta-  
224 tions in *ABCA1* are associated with an increased risk of cardiovascular  
225 disease (Clee et al., 2000; Frikke-Schmidt et al., 2008). The explanation  
226 of these seemingly controversial results is that the biochemical pheno-  
227 type inherited as an autosomal co-dominant trait in heterozygotes,  
228 and revealed by plasma HDL-C and apoA-I levels, is highly variable –

230 the values can be within the entire range of those measured in normal  
231 individuals and patients with TD. A differential contribution of missense  
232 mutations to the functional defect in cholesterol efflux determining the  
233 biochemical, cellular and clinical phenotypes was recognized early after  
234 the cloning of *ABCA1*. For example, some of the *ABCA1* missense muta-  
235 tions (P1065S, G1216V, N1800H, R2144X) cause only a mild decrease  
236 of cholesterol efflux which in heterozygous state results in a relatively  
237 small reduction of HDL (less than 30% decrease compared to the normal  
238 values) explaining the lack of atherosclerosis (Frikke-Schmidt et al.,  
239 2008). In contrast *ABCA1* mutations which cause more than 50% de-  
240 crease of HDLs were associated with an increased risk for ischemic  
241 heart disease (Bochem et al., 2013; Clee et al., 2000; Frikke-Schmidt  
242 et al., 2005).

243 Recent meta-analysis of GWAS demonstrated that SNPs near *ABCA1*  
244 associate with lower HDL and total cholesterol levels (Teslovich et al.,  
245 2010), but not with cardiovascular risk (Willer et al., 2008). However,  
246 as was discussed in many recent reports, whereas GWAS have a  
247 power to detect common variants of *ABCA1*, most of these variants in  
248 heterozygous patients result only in a small reduction of HDL and are  
249 less likely to affect the risk of cardiovascular disease (Frikke-Schmidt,  
250 2011; Westerterp et al., 2014). In contrast using next generation  
251 sequencing and selecting a population at the extreme end of HDL (or  
252 other quantitative metabolic traits) could help identifying rare variants  
253 of *ABCA1* with large effects on phenotype (cardio vascular disease or  
254 atherosclerosis) (Frikke-Schmidt et al., 2004; Service et al., 2014).

255 Studies with mice lacking *ABCA1* in the whole body demonstrated  
256 dramatic reduction of HDL and apoA-I, a decrease of LDL and foam cell  
257 accumulation in the lungs (McNeish et al., 2000). Recent studies using  
258 targeted approach to delete *Abca1* only in liver showed that expression  
259 of *ABCA1* in this organ causes profound hypo-alpha-lipoproteinemia  
260 and kidney hypercatabolism of apoA-I (Timmins et al., 2005), and mod-  
261 ulates the susceptibility to atherosclerosis (Brunham et al., 2009).

#### Type 2 diabetes mellitus

262 It was reported that mice with selective ablation of *ABCA1* in pancre-  
263 atic  $\beta$ -cells had markedly impaired glucose tolerance and defective insu-  
264 lin secretion but normal insulin sensitivity (Brunham et al., 2007).  
265 The results of the study suggested that dysfunctional *ABCA1* contributes  
266 to the development of type 2 diabetes through increased cholesterol  
267 levels in pancreatic  $\beta$ -cells.  
268

Studies in a limited number of TD patients demonstrated impaired insulin secretion from pancreatic  $\beta$ -cells suggesting, in support to the studies in genetically modified mice, that ABCA1 may be involved in insulin secretion (Koseki et al., 2009; Vergeer et al., 2010). A highly frequent non-synonymous variant of ABCA1 (R230C) was identified in a Mexican population and shown to associate with obesity and type 2 diabetes (Villarreal-Molina et al., 2008). Also in a Mexican population it was reported that diabetic patients harboring this mutation needed a higher dose of glyburide to control glucose level (Aguilar-Salinas et al., 2013). However, a recent study examining several genetic variants in ABCA1 and ABCG1 did not find an association with an increased risk of type 2 diabetes (Schou et al., 2012).

## ABCA1 and Alzheimer's disease

The role of ABCA1 in amyloid deposition, clearance and memory deficits in experimental animals has been demonstrated and confirmed by the results of studies conducted in different laboratories. First, studies from our and other groups have demonstrated that the lack of ABCA1 increases amyloid deposition and cognitive decline in different APP transgenic mice accompanied by significant decrease in the levels of soluble apoE (Hirsch-Reinshagen et al., 2005; Koldamova et al., 2005a; Lefterov et al., 2009; Wahrle et al., 2005). Second, it was shown that treatment with LXR and RXR ligands which increases global *Abca1* expression in mice significantly ameliorates amyloid pathology (Cramer et al., 2012; Fitz et al., 2010; Jiang et al., 2008; Koldamova et al., 2005b). Third, transgenic mice overexpressing *Abca1* in brain have less amyloid plaques (Wahrle et al., 2008). Although similar data in humans are missing, the results of a recent case control genetic study with the application of advanced sequencing technologies clearly indicated that rare ABCA1 gene variants, some of them associated with higher levels of HDL, may have protective effect against AD (Lupton et al., 2014).

## Physiological function of ABCA1 in central nervous system (CNS)

ABCA1 is expressed in all brain cell types and regulates cholesterol efflux to lipid-free apolipoproteins (Koldamova et al., 2003). ABCA1 mediates cholesterol efflux from astrocytes and microglia, brain capillary endothelial cells and pericytes to lipid-free apolipoproteins (Koldamova et al., 2003), (Kim et al., 2007; Panzenboeck et al., 2002; Saint-Pol et al., 2012). Since the blood brain barrier (BBB) is not penetrable for cholesterol and lipoproteins from periphery (Dietschy and Turley, 2001) brain HDL and ABCA1 do not contribute to RCT. In the brain apoE is the main apolipoprotein that serves as an acceptor of the lipids translocated by ABCA1. ApoE-containing HDL lipoproteins were identified in astrocytes conditioned media, brain interstitial fluid (ISF) and cerebrospinal fluid (CSF) (Fitz et al., 2010; Pitas et al., 1987; Yu et al., 2010). ApoE-containing HDL lipoproteins isolated from astrocytes conditioned media are discoidal and do not contain cholesterol esters. In CSF apoE-containing HDL are spherical in shape and have the size of plasma HDL containing a core of cholesterol esters (Koch et al., 2001; Yu et al., 2010). ApoA-I-containing lipoproteins are also detected in CSF and are smaller than apoE-HDL (Pitas et al., 1987). ApoA-I passes BBB from the circulation in lipid-free form but there are some reports suggesting apoA-I expression in brain capillary endothelial cells (Panzenboeck et al., 2002). However, using a RNA-seq approach we were not able to detect an appreciable mRNA expression of *ApoA-I* in mouse brain or to detect apoA-I protein in ISF (Koldamova & Lefterov unpublished data).

The normal physiological role of ABCA1 in the brain is currently unknown however one can presume that it is to maintain cholesterol transport from glial cells (mainly astrocytes) to neurons. Previous studies have reported that cholesterol is needed for neurite outgrowth and repair, and synaptic vesicle formation and regeneration (Pfrieger and Barres, 1997). Recently, we examined the neurite architecture of pyramidal hippocampal neurons of *Abca1*<sup>ko</sup> mice (Fitz and Koldamova, unpublished data). Our data revealed a significant decrease in neurite

length and number of neurite segments in CA1 region of hippocampus of *Abca1*<sup>ko</sup> mice when compared to WT mice suggesting the importance of disruption of ABCA1 for neurite degeneration in the brain.

In a similar way to periphery, the lack of functional ABCA1 affects apoE lipidation and stability leading to a significant decrease of apoE in the brain of *Abca1*<sup>ko</sup> mice (Hirsch-Reinshagen et al., 2004; Wahrle et al., 2004). The effect of ABCA1 is apoE selective, since the levels of apoJ, another lipoprotein secreted by astrocytes, do not change regardless of the absence of functional ABCA1. Mice with a targeted disruption of brain *Abca1* had a significant reduction of apoE in CNS and a very low level of apoE-HDL in CSF (Karasinska et al., 2009).

## Genetic variation in ABCA1 and Alzheimer's disease

### Common genetic variants and risk for AD

The very first indication that there might be a link between AD and lipid metabolism came with the discovery of APOE as a major risk factor for LOAD (Corder et al., 1993; Poirier et al., 1993; Saunders and Roses, 1993). Recent GWAS have identified genes involved in cholesterol metabolism or transport as AD susceptibility genes, providing strong support for the association (Harold et al., 2009; Jones et al., 2010).

The identification of ABCA1 as a principal/master regulator of RCT and possibly a major risk factor for atherosclerosis in case of mutations or polymorphic variants, known to influence intracellular cholesterol efflux and HDL levels in periphery, reinvigorated the search for association of disturbed cholesterol metabolism in the CNS and the development of AD.

The establishment of association of non-synonymous common genetic variation in ABCA1 with altered lipoprotein levels and a modified risk for Coronary Artery Disease (CAD) (Clee et al., 2000) was followed by more than a dozen of targeted Genetic Association Studies (GAS) to test the hypothesis if common SNPs within the sequence of ABCA1 gene are related to the risk for AD (Cascorbi et al., 2013; Chu et al., 2007; Jiang et al., 2012; Katzov et al., 2004; Khorram Khorshid et al., 2011; Reynolds et al., 2009; Rodriguez-Rodriguez et al., 2007; Shibata et al., 2006; Sun et al., 2012; Sundar et al., 2007; Wahrle et al., 2007; Wang and Jia, 2007; Wavrant-De Vrieze et al., 2007; Wollmer et al., 2003). The association of higher levels of HDL-C in elderly individuals and lower risk of LOAD further rationalized studies to test the combination of ABCA1 variants and other cholesterol-related genes (Cascorbi et al., 2013; Xiao et al., 2012). R219K (rs2230806), I883M (rs4149313), and R1587K (rs2230808) are the non-synonymous variants most extensively investigated since they translate into amino acid changes and have been shown to associate with the risk for CAD. Altogether, the results of those studies were inconsistent with lack of reproducibility even in same ethnic groups and contradicting conclusions ranged from no effect, increasing or decreasing the risk for AD (detailed comments on earlier studies published before 2010 are available in (Koldamova et al., 2010)). A meta-analysis based on those studies — 6214 patients and 6034 controls, concluded that the data so far does not support a model for association between ABCA1 and AD suggesting among other reasons, insufficient or low number of patients and controls investigated so far (Wang et al., 2013; Xiao et al., 2012). There have been two recent reports not included in the meta-analysis. Cascorbi et al. (2013) used cerebellar brain tissue for genotyping from 71 histopathologically verified AD cases and 81 non-demented controls and found no connections between rs2230806 or rs1800977 and amyloid deposition in brain. Xiao et al. (2012) investigated possible associations of common non-synonymous polymorphic markers in several lipid related genes including ABCA1R219K, with susceptibility to AD and plasma lipid levels (104 AD patients and 104 controls, Han Chinese). The results demonstrated association of significantly higher levels of HDL cholesterol and apoA-I, in the carriers of KK genotype and K allele, and a decreased AD risk.

Like in many other association studies the reasons for the controversial results in the studies discussed above are not clear: size of the sample, differences in genetic background, ethnicity and race, as well

as significant differences in minor allele frequencies in many of those studies obviously exist. However, the results of all major GWAS on LOAD risk associations published during the last 5 years, and numerous meta-analysis reports based on those, demonstrate that it is very difficult or even impossible to overcome the problems listed above and the discovery of new significant associations of variants within ABCA1 (or perhaps any other gene) is unlikely (Ridge et al., 2013). Insufficient, or complete lack of understanding of the functional relationship of the majority of genes identified by GWAS and LOAD (APOE is perhaps example #1) adds strongly to this rather disappointing conclusion. However, recent pathway analyses (Jones et al., 2010) based on association signals identified in 2 large GWAS (Harold et al., 2009; Lambert et al., 2009) identify sets of genes and physiological processes related to cholesterol metabolism and innate immune response and demonstrate that these processes are etiologically relevant and suitable targets for novel and existing therapeutic approaches. It is quite remarkable that while ABCA1 had not been identified in either of the GWAS, the gene is listed in the lipid related processes as a gene with a SNP significantly enriched at  $p < 0.0001$ . There are two subsets of genes (Table 5 in (Jones et al., 2010)), that deserve a particular attention and provide further insight into ABCA1 role in LOAD: a) the first subset is composed of 3 genes: ABCA1, APOE and APOA-I. The significance of the proteins in the pathogenesis of LOAD has been underlined numerous times in this review; b) the second subset is represented by the following 6 genes: APOE, APOC1, APOC2, APOC4, and ABCG1. All of those are transcriptionally regulated by LXR/RXR heterodimers and experimental therapeutic approaches have been successfully applied numerous times in in vitro and in vivo systems. Regardless of some controversies, the application of synthetic RXR agonists remains promising and is being evaluated in preclinical and Phase I clinical trials (Cramer et al., 2012; Fitz et al., 2013; Price et al., 2013; Tesseur et al., 2013; Veeraraghavalu et al., 2013). There are no such other groups of genes within the lipid-related processes identified by Jones et al. (2010). Overall, the representation of identified genes involved in cholesterol/lipid metabolism is impressive, lending further credibility to and suggesting a role for ABCA1, as well as other cholesterol transporters and APOE gene cluster in AD pathogenesis. These results also provide solid foundation for the development of relevant targeted therapeutic approaches.

#### 333 Rare functional variants and AD pathology

334 A differential contribution of missense mutation to the biochemical,  
335 cellular and clinical phenotypes was recognized early after the cloning  
336 of ABCA1. There are two notable examples of ABCA1 mutations highly  
337 relevant to our understanding of its association to AD risk and AD  
338 pathogenesis.

339 N935S mutation was identified in a patient with extremely low  
340 levels of HDL, but without accelerated development of premature ath-  
341 erosclerosis and with signs of severe dementia and amyloid depositions  
342 in the brain at age of 60 (Walter et al., 1994a,b). In vitro experiments  
343 with an immortalized cell line derived from the patient's fibroblasts  
344 demonstrated the dominant negative effect of the mutant protein and  
345 the influence of ABCA1 mediated disturbance of cholesterol efflux on  
346 APP processing: the cells responded to the LXR ligand (T0901317) treat-  
347 ment with increased A $\beta$  secretion (Koldamova et al., 2005b).

348 The second example is a compound heterozygous mutation  
349 (D1099Y and F2009S) identified in a subject with severe HDL cholester-  
350 ol deficiency (Ho Hong et al., 2002). The patient had no history or  
351 clinical manifestation of CAD and no other cardiovascular disease risk  
352 factors, except for low HDL cholesterol. There were no clinical signs of  
353 TD either. The patient developed and died of complications related  
354 to cerebral amyloid angiopathy (CAA). These two examples point to  
355 the significance of rare functional variants of ABCA1 which can be asso-  
356 ciated with AD risk, most probably operating through HDL cholesterol  
357 levels, although other mechanisms, influencing APP processing cannot  
358 be excluded.

359 Recently, the contribution of rare non-synonymous gene variants to  
360 the risk of common diseases, including TREM2, APP, ABCA1, and AD has  
361 been demonstrated by deep re-sequencing. Lupton et al. (2014) used  
362 pooled DNA samples from 311 cases and 367 controls for next genera-  
363 tion sequencing to identify low frequency, non-synonymous ABCA1  
364 variation. They found a significantly higher proportion of rare ABCA1  
365 variants in control individuals, compared to AD cases. The interpretation  
366 of the results, according to the authors was suggestive of a protective  
367 effect. Importantly, the number of non-synonymous alleles of previous-  
368 ly identified rare variant E1172D, known to be associated with very high  
369 HDL-C levels, was more than twice higher in the control compared to  
370 the case samples. While the number of these studies is small and the  
371 protocol is still relatively expensive, the potential of those studies to  
372 reveal new gene-level associations that further explain the phenotypic  
373 variance in AD beyond the variants identified in GWAS, is obvious.

#### 374 ABCA1 and the concept of pleomorphic risk loci

375 In 2011 A. Singleton and J. Hardy suggested that common disease,  
376 common variant (CDCV) and multiple rare variant (MRV) hypotheses  
377 are not mutually exclusive and these two ideas can be brought together  
378 as a general hypothesis for disease susceptibility (Singleton and Hardy,  
379 2011). They coined the term “pleomorphic risk loci” (PRL) and outlined  
380 the steps necessary to conduct, including targeted resequencing, as to  
381 understand and delineate, in the context of the hypothesis, benign,  
382 risk and protective rare variants. The technological advent of NGS plat-  
383 forms, relatively easy to work out protocols to generate sequencing  
384 libraries and the availability of free open source bioinformatics software  
385 now makes it possible to test the hypothesis. A candidate PRL would  
386 influence the disease through different biological effects, accomplished  
387 by several, distinct disease-related mechanisms that coexist at the  
388 same locus and on a single gene. The total number of mutations in  
389 ABCA1 is more than 180, and the majority of those in heterozygous  
390 form (excluding compound heterozygotes with Tangier disease) deter-  
391 mine a biochemical phenotype distinguished by low, or even very low  
392 HDL levels. Epidemiological studies provide enough evidence that in a  
393 subset of patients those levels may underlie an increased risk for AD.  
394 The non-synonymous rare variants already identified in patients with  
395 amyloid depositions in brain, amyloid angiopathy and early cognitive  
396 decline provide additional support. On the other side, the application  
397 of newest sequencing technologies with samples from patients with  
398 AD and atherosclerosis in case-control studies demonstrates that  
399 there are rare, yet protective ABCA1 gene variants. It is possible to pre-  
400 dict, that the majority of those 180 mutations within the sequence of  
401 the entire ABCA1 will be benign with no detectable effect relevant to  
402 any disease. We are predicting soon that we will have a better idea if  
403 ABCA1 is a PRL.

#### 404 ABCA1 and AD model mice

405 In APP transgenic mice ABCA1 deficiency increases parenchymal amy-  
406 loid plaques and CAA (Hirsch-Reinshagen et al., 2005; Koldamova et al.,  
407 2005a; Wahrle et al., 2005). In these mice the level of soluble apoE and  
408 apoA-I in brain was significantly reduced while insoluble apoE was not  
409 changed. In contrast, transgenic mice overexpressing *Abca1* in brain  
410 have less amyloid plaques. It was also demonstrated that overexpression  
411 of ABCA1 resulted in apoE-containing particles with a larger size than  
412 normal, while the total level of apoE was decreased (Wahrle et al., 2008).

413 Haplodeficiency of *Abca1* was examined in APP mice expressing  
414 mouse apoE and human apoE3 or E4 isoforms. In old APP23 mice ex-  
415 pressing mouse apoE absence of one copy of *Abca1* deteriorates cogni-  
416 tive deficits in correlation with an increased level of soluble oligomers  
417 but not with amyloid plaques (Lefterov et al., 2009). In a recent study  
418 Fitz et al. demonstrated that *Abca1* haplodeficiency had a differential ef-  
419 fect on the phenotype of mice expressing apoE3 or apoE4 (Fitz et al.,  
420 2012). The lack of one copy of *Abca1* significantly aggravates memory  
421 deficits, A $\beta$  plaques and clearance in APP/E4 but not in APP/E3 mice

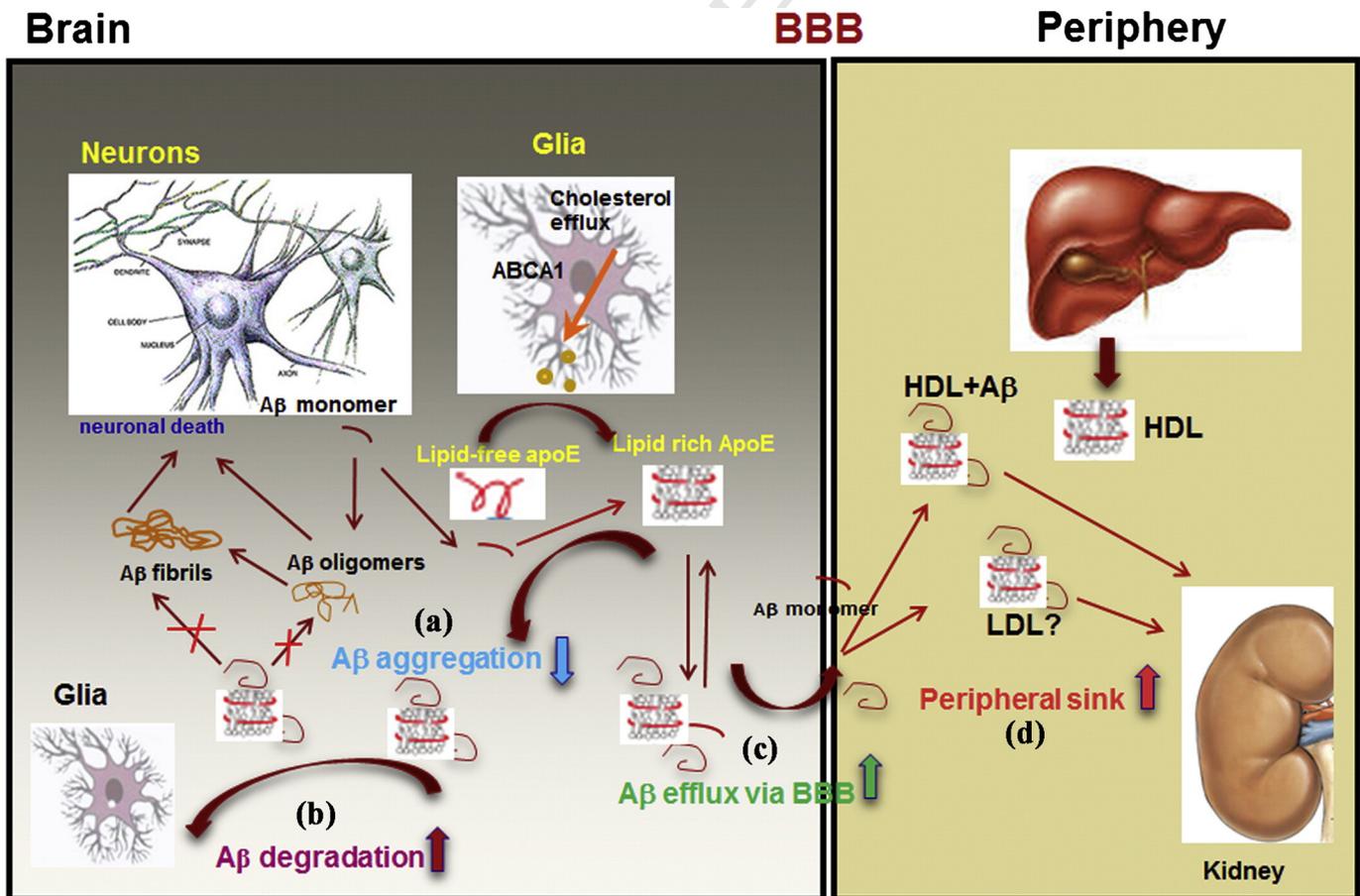
(Fitz et al., 2012) suggesting that apoE4 confers less resistance to additional genetic defects. Interestingly, the same study also found a correlation between HDL in plasma and amyloid load in brain implying a causative connection between peripheral lipoproteins and A $\beta$  load in CNS.

A plausible hypothesis to explain the above observations could be an effect of ABCA1 on AD pathogenesis (if any), intertwined with its role in cholesterol efflux and generation of HDL in brain and periphery. In CNS the role of ABCA1 is generally attributed to its effect on apoE lipidation and stability that ultimately controls apoE level. Numerous data suggest that apoE affects A $\beta$  aggregation and clearance in isoform-dependent manner, however, the molecular mechanism underlying the effect is still not sufficiently clarified [reviewed in (Kanekiyo et al., 2014; Tai et al., 2014)]. It is a matter of debate if APOE4 isoform is deleterious or less protective with evidence supporting both claims [reviewed in (Kanekiyo et al., 2014; Kim et al., 2009; Mahley and Huang, 2012)].

Clinical data demonstrate that apoE protein level in plasma and CSF is lower in APOE4 than in APOE3/APOE2 carriers. A cross sectional study by Gupta et al. demonstrated that plasma total apoE and apoE4 levels were significantly lower in patients with AD and they further decrease with the increase of A $\beta$  load as assessed by PET (Gupta et al., 2011). A recent large-scale case-control study of CSF samples demonstrated that apoE protein levels in CSF positively associated with CSF A $\beta$ 42 levels independent of APOE4 genotype (Cruchaga et al., 2012). These data suggest that apoE levels in CSF or plasma may be causatively related to AD. In addition to total apoE level, apoE lipidation could also affect interaction with A $\beta$  and consequently A $\beta$  aggregation [reviewed in (Tai et al., 2014)]. In vitro studies have shown binding of synthetic A $\beta$  to apoE isolated from cell conditioned media (LaDu et al., 1994) CSF

(Wisniewski et al., 1993) and plasma (Strittmatter et al., 1993). Using solid-phase binding assays we also found that synthetic A $\beta$ 40 binds avidly to apoE2 ( $K_D = 15$  nM) and apoE4 ( $K_D = 19$  nM) (Koldamova et al., 2001). However, a recent study demonstrated that apoE binding to A $\beta$  is not essential for A $\beta$  metabolism (Verghese et al., 2013) clearly advocating for more research on this topic. Published studies are also conflicting in regard to the effect of apoE has on A $\beta$  aggregation. In vivo, lack of APOE in APP transgenic mice was shown to dramatically reduce amyloid plaques (Bales et al., 1997), confirmed by more recent studies (Bien-Ly et al., 2012; Kim et al., 2011). In vitro studies are more contradicting with some demonstrating that apoE inhibits A $\beta$  aggregation (Wood et al., 1996a,b) and others that it promotes A $\beta$  aggregation (Castano et al., 1995; Hashimoto et al., 2012).

Our hypothesis is that ABCA1 controls over the generation of apoE (or apoA-I)-containing HDL in brain and plasma affects A $\beta$  metabolism on several levels (see Fig. 2). In the brain: (a) by decreasing A $\beta$  aggregation and preventing its conversion into toxic oligomers (Lefterov et al., 2009) or plaques (Hirsch-Reinshagen et al., 2005; Koldamova et al., 2005a; Wahrle et al., 2005); (b) by maintaining A $\beta$  in soluble state and facilitating A $\beta$  clearance by glia or its degradation by extracellular proteases (Jiang et al., 2008); (c) by facilitating A $\beta$  removal from the brain through BBB and drainage into CSF [discussed by Saint-Pol et al. (Gosselet et al., 2013; Saint-Pol et al., 2012)]. A recent randomized clinical trial tested the effect of diet in patients with mild cognitive impairment and demonstrated the significance of apoE and A $\beta$  lipidation to impact cognitive decline (Hanson et al., 2013). However, the role of ABCA1 in maintaining plasma levels of HDL, apoA-I and apoE may be as important for AD as its CNS functions. Since A $\beta$  binds HDL and other plasma lipoproteins (Koudinov et al., 1998; LaDu et al., 1995, 1999



**Fig. 2.** A simplified model explaining the role of ABCA1 in A $\beta$  deposition and clearance on both sides of the BBB. In the brain ABCA1 mediates apoE lipidation that can affect A $\beta$  metabolism on several levels: (a) decreases A $\beta$  aggregation and prevents its conversion into toxic oligomers or fibrils; (b) enables A $\beta$  clearance by glia or degradation by extracellular proteases; (c) facilitates A $\beta$  removal from the brain through BBB or drainage into CSF. In plasma, ABCA1 is essential for HDL level that could affect A $\beta$  clearance by the mechanism of "peripheral sink" (d).

2012), it is possible that circulating lipoproteins could affect A $\beta$  balance on both sides of BBB and increase its clearance by the mechanism of “peripheral sink” (see (d) on Fig. 2). The rarity of TD patients and death from CAD at age earlier than LOAD normally develops, preclude studies examining an association of TD and AD and testing various aspects of the hypothesis as outlined above (Pervaiz et al., 2012; Shahim et al., 2013). Some of the genetics studies as already discussed, however, are in agreement with a concept that higher level of HDL is protective against dementia and AD, or as measured by Global PiB index in a recent study, lower HDL and higher LDL levels are both associated with cerebral amyloidosis (Dias et al., 2014; Reed et al., 2014; Xiao et al., 2012).

## 591 Therapeutic implications

592 The fact that both ABCA1 and apoE are under the transcriptional control of LXR and RXR transcription factors offers an attractive potential for targeted therapeutic interventions. These approaches are rationalized by the molecular mechanism of nuclear receptor activation (see Landreth et al. (2014), in this SI: Nuclear Receptors in Neurodegenerative Diseases, *Neurobiol. Dis.*, <http://dx.doi.org/10.1016/j.nbd.2014.04.001>), the discovery that LXR $\alpha/\beta$  have a role in AD pathogenesis and other forms of neurodegeneration (Andersson et al., 2005; Kim et al., 2008; Wang et al., 2002; Zelcer et al., 2007), and positive results of experimental treatment in AD mice. Numerous studies reported data confirming the beneficial effect of LXR agonists on amyloid plaques and cognitive performance in APP mice (Donkin et al., 2010; Fitz et al., 2010, 2014; Jiang et al., 2008; Katz et al., 2009; Koldamova et al., 2005b; Riddell et al., 2007; Terwel et al., 2011; Vanmierlo et al., 2011). More recently, Cramer et al. reported that FDA approved RXR agonist Bexarotene decreases plaque load, increases A $\beta$  clearance and ameliorates cognitive deficits in APP expressing mice (Cramer et al., 2012). Although the effect on amyloid deposition, as reported, has not been confirmed in follow-up studies (Price et al., 2013; Veerarghavalu et al., 2013), other laboratories reported a significant cognitive improvement even without change in plaque load (Fitz et al., 2013; Tesseur et al., 2013). The effect on memory was challenged in a recent study (LaClair et al., 2013). Yet, recent experimental and clinical data demonstrated that Bexarotene has beneficial effects on other brain disorders such as Parkinson disease (McFarland et al., 2013) and schizophrenia, and is safe for a chronic use (Lerner et al., 2008, 2013).

618 One confounding factor for the use of LXR and RXR agonists in patients is the increase of triglycerides in plasma (Lilley et al., 2013; Tontonoz and Mangelsdorf, 2003). Interestingly, the only published clinical trial with LXR agonist reported adverse effects on CNS and not an increase of serum triglycerides (Katz et al., 2009). Second, because of the uncertainty regarding the molecular mechanisms underlying the association of apoE4 and the increased risk of AD, there is skepticism in the AD scientific community if the effect of LXR and RXR agonists will be equally beneficial for APOE3 and APOE4 carriers. If studies from transgenic mice are any indication, Bexarotene decreases A $\beta$  oligomers (Fitz et al., 2013) and restores cognitive deficits with similar efficiency in both APOE3 and APOE4 expressing mice (Boehm-Cagan, in press; Fitz et al., 2013). It is obvious that additional research is needed to answer the question if AD therapies based on LXR and RXR activation are worth pursuing.

## 633 Conclusion

634 The significance of ABCA1 for cardiovascular disease is determined by its role as essential regulator of cholesterol efflux and HDL generation. Likewise the probable implication of ABCA1 in AD pathogenesis streams from its main physiological function namely to control the level of lipidated apoE in CNS and plasma HDL level subsequently affecting A $\beta$  metabolism.

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