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Review

# Alzheimer's and ABC transporters — new opportunities for diagnostics and treatment

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ABSTRACT

Much has been said about the increasing number of demented patients and the main risk factor 'age'. Frustratingly, we do not know the precise pattern and all modulating factors that provoke the pathologic changes in the brains of affected elderly. We have to diagnose early to be able to stop the progression of diseases that irreversibly destroy brain substance. Familiar AD cases have mislead some researchers for almost 20 years, which has unfortunately narrowed the scientific understanding and has, thus, lead to insufficient funding of independent approaches. Therefore, basic researchers hardly have been able to develop causative treatments and clinicians still do not have access to prognostic and early diagnostic tools. During the recent years it became clear that insufficient Aβ export, physiologically facilitated by the ABC transporter superfamily at the brain's barriers, plays a fundamental role in disease initiation and progression. Furthermore, export mechanisms that are deficient in affected elderly are new targets for activation and, thus, treatment, but ideally also for prevention. In sporadic AD disturbed clearance of β-amyloid from the brain is so far the most important factor for its accumulation in the parenchyma and vessel walls. Here, we review findings about the contribution of ABC transporters and of the perivascular drainage/glymphatic system on β-amyloid clearance. We highlight their potential value for innovative early diagnostics using PET and describe recently described, effective ABC transporter-targeting agents as potential causative treatment for neurodegenerative proteopathies/dementias.



**Contents**



*Abbreviations:* AD, Alzheimer's disease; Aβ, amyloid-beta; ABC, ATP binding cassette; BBB, blood–brain barrier; $BP_{ND}$, non-displaceable binding potential; CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; CP, choroid plexus; DLB, dementia with Lewy bodies; FTLD, frontotemporal lobar degeneration; GWAS, genome wide association study; HEK263, human embryonic kidney cell line 263; LC-MS, liquid chromatography-coupled mass spectrometry; LLC, Lewis lung carcinoma cells; LRP1, Lipoprotein related receptor protein 1; MDCK, Madin-Darby canine kidney cells; MSA, multiple systems atrophy; NCL, neuronal ceroid neurolipofuscinose; PD, Parkinson's disease; PET, positron emission tomography; PSP, progressive supranuclear palsy; SNP, single nucleotide polymorphism.

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## Introduction

Life expectancy rises in most countries as prevalence of aging-associated diseases does. This is not only true for AD and PD, but also for other neurodegenerative disorders (Savica et al., 2013; Vann Jones and O'Brien, 2014; Vardarajan et al., 2014). Common to all is the irreversible degeneration of distinct subsets of neurons and the accumulation of aggregated peptides/proteins within the cell body or in their near vicinity. In AD accumulation of Aβ is thought to be the initial pathogenic trigger leading to progressive neuronal dysfunction of the hippocampal formation, temporal and frontal cortex and later spreading to the occipital cortex (Braak and Braak, 1991; Hardy and Allsop, 1991). Reasons for this region specific, temporal pattern are barely known. In fact, also other proteopathies show distinct temporal patterns of neurodegeneration and peptide accumulation (Braak et al., 2003). In PD, α-synuclein accumulates preferentially in neurons of the substantia nigra. In DLB or MSA the same protein affects very different sets of neurons (Braak et al., 2003). Deposits of Tau-proteins or TDP-43 in FTLD appear in the eponymic regions of the brain but in PSP, tauopathy is restricted to distinct nuclei in the basal ganglia. Symptoms of the different diseases highly correlate with the region specific deposition of the respective proteins.

Familiar AD cases were the foundation of AD research for almost 20 years. Focusing research and funding on hypotheses derived thereof led to the understanding that Aβ accumulations arise from pure overproduction or misled processing by cleavage or processing enzymes (Haass and De Strooper, 1999). However, any treatment approach targeting these processes, e.g. γ-secretase for AD treatment, has proven insufficient and has not found its way into the clinics (Doody et al., 2013).

However, especially for AD but as well for PD, MSA, DLB, and PSP, it becomes increasingly clear that the pathologic aggregation of proteins and peptides is due to disturbed clearance mechanisms of the brain's barriers. In this review, we summarize the current knowledge about the contribution of ABC transporters to the clearance of peptides/proteins over the blood–brain barrier, possible roles of the choroid plexus and the potential use of ABC transporters for treatment and diagnostics of various proteopathies of the brain.

ATP-binding cassette transporters are known since the introduction of p-glycoprotein (ABCB1) by Juliano and Ling (1976). By now, the ABC transporter superfamily comprises 49 human proteins divided into 7 subfamilies that have been designated ABCA to ABCG. They are expressed in every cell type of the brain and mediate the transport of a wide variety of substances. Detailing each family would be beyond the scope of this review, however, there have been comprehensive reviews about the function of ABC transporters as well as their expression within the central nervous system and the BBB (Hartz and Bauer, 2011; Kim et al., 2008; Linton, 2007; Löscher and Potschka, 2005; Pahnke et al., 2008; Schinkel and Jonker, 2003). We have recently started to systematically analyze ABC transporter expression throughout the human brain because this has not been done before. The expression pattern of these transporters differs drastically between different functional areas of the brain, which is not only true for endothelial cells of the BBB but as well for neurons and glia (unpublished data). Currently, the role of ABC transporters in neurodegenerative diseases is mainly attributed to their function or dysfunction at the BBB, which is also the focus of this review. However, it seems to be possible that differential transporter expression also plays a role in the susceptibility of specific brain regions for distinct neurodegenerative diseases. The BBB is a sophisticated system, made up of endothelial cells, pericytes, and neuronal and astrocytic endfeet. This system highly regulates the import and export of nutrients, metabolites and immune cells as well as of xenobiotics. Development and function of the BBB has been nicely reviewed recently (Obermeier et al., 2013)

## ABC transporters and Aβ — 13 years of research

The first report that Aβ interacts with an ABC transporter was published by Lam et al. (2001). They used HEK263 cells, ABCB1-enriched membrane preparations and inside-out vesicles to clearly show that Aβ binds to ABCB1 and is actively transported. One year later, we found first implications for this association in human brains (Vogelgesang et al., 2002). In non-demented elderly amyloid plaques are increasingly recognized near blood vessels without ABCB1 expression but much less next to vessels expressing abundant ABCB1 proteins. In the same year, first evidences pointed to an involvement of ABCA1 in Aβ extrusion from neuronal cells (Fukumoto et al., 2002) which has been reviewed in detail by (Gosselet et al. (2013)(also include review by I. Lefterov in this special issue). In 2004, we presented first evidence for the impact of ABCB1 on CAA (Vogelgesang et al., 2004). CAA first develops in arterioles and spreads to smaller vessels and capillaries only during later stages. When only arterioles were affected ABCB1 expression was high in unaffected capillaries, but as CAA spread to smaller vessels ABCB1 was lost here as well. The age-dependent decline of ABCB1 expression completed the pathologic link between ABCB1 and AD in humans. One year later, Cirrito et al. (2005) published the first mouse study showing impaired Aβ clearance in ABCB1 knockout mice, and also in control mice after treatment with ABCB1 inhibitors. Following these studies a lot more attention was drawn toward the role of ABCB1 in AD. In the following years different *in vivo* approaches confirmed ABCB1 as an important Aβ exporter. Hartz et al. (2010) confirmed the previous findings in mice showing that ABCB1 expression was diminished before plaques were visible and were also able to reduce brain amyloid burden by ABCB1 induction *in vivo*. This has been later again confirmed by Brenn et al. (2011) in a different mouse model and very recently also by Carrano et al. (2014) using material from patients. In 2011, Jeynes et al. analyzed brain tissue of controls and AD patients. ABCB1 positive capillaries were inversely correlated with the presence of neurofibrillary tangles and senile plaques, again confirming our publication from 2004 (Jeynes and Provias, 2011; Vogelgesang et al., 2004). In 2011, our experimental work revealed a strong effect of ABCB1 deficiency in APPPS1 mice and in a mouse model of CAA (Krohn et al., 2011). Interestingly, Qosa et al. (2012) found that rifampicin and caffeine treatment enhanced Aβ clearance via (a possibly combined) action of ABCB1 and/or LRP1. Caffeine intake has been found to reduce cognitive decline in aging men and one study found decreased risk for AD (Maia and de Mendonca, 2002; Ritchie et al., 2007; van Gelder et al., 2007). However, some *in vitro* experiments, designed to investigate ABCB1 functionality in Aβ transport, gave conflicting results. Using MDCK cells transfected with either LRP1 or ABCB1, Nazer et al. (2008) found no effect of ABCB1 on Aβ transport. Kuhnke et al. (2007) showed that ABCB1-transfected LLC cells transported Aβ from the basolateral to the apical compartment. In an immortalized human brain endothelial cell line (hCMEC/D3) ABCB1 inhibition affected only apical to basolateral transport of Aβ (Tai et al., 2009). In bovine brain capillary endothelial cells similar effects were found (Candela et al., 2010; Saint-Pol et al., 2013). Qosa et al. (2014) did mechanistic modeling in murine and human endothelial cells. The inconsistency of *in vitro* and *in vivo* results points to interactions *in vivo* that we do not fully understand, yet. It is conceivable that cell monolayers *in vitro* do not recapitulate certain factors that are apparent *in vivo* but, of course, the differences between epithelial (MDCK) cells, carcinoma cells and endothelial cells must be also taken into account. However, it is unclear how Aβ, produced in the brain (i.e. at the basolateral side of endothelial cells), gets in contact with ABCB1 at the apical side of the blood–brain barrier. Here, LRP1, RAGE, and PrP may contribute to the export system (Pflanzner et al., 2011, 2012).

ABCA1 and ABCB1 are not the only ABC transporters found to be associated with Aβ transport. In 2009, Xiong et al. presented data showing that ABCG2 is up-regulated in AD patients with CAA. Furthermore,