Published in final edited form as:

Neurosci Lett. 2013 November 27; 556: . doi:10.1016/j.neulet.2013.09.058.

ABCA7 expression is associated with Alzheimer's disease polymorphism and disease status

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Abstract

Genome-wide association studies (GWAS) have implicated a series of single nucleotide polymorphisms (SNPs) in Alzheimer's disease (AD) risk. Elucidating the function of these SNPs is critical to identifying the underlying pathways and, potentially, novel therapeutic agents. SNPs within the gene ATP binding cassette A7 (*ABCA7*) reached significance in these studies, warranting investigation into their actions. Here, we analyzed *ABCA7* expression in a set of human brain samples as a function of AD-associated SNPs and AD status. We report that the rs3764650T allele that decreases AD risk is associated with increased *ABCA7* expression. However, *ABCA7* expression is increased in AD individuals. We interpret our findings as suggesting a model wherein increased *ABCA7* expression reduces AD risk and that the increased *ABCA7* observed in AD reflects an inadequate compensatory change.

Keywords

Alzheimer's; *ABCA7*; SNP; phagocytosis; microglia

1. Introduction

AD is defined by short-term memory loss progressing into dementia and neuropathology including senile plaques and neurofibrillary tangles [12, 16, 35, 42]. AD risk increases with age with the disease affecting as many as one out of three people by 85 years of age [5, 9, 11, 15, 29]. Since AD is an impending health crisis, we seek to identify the underlying mechanisms and translate these mechanisms into preventative agents.

GWAS have identified genes in pathways that modulate AD risk, including genetic variants in *ABCA7* (reviewed in [3, 36]). *ABCA7* is part of a large family of transporters expressed in the brain on microglia and neurons and is capable of fluxing phospholipids as well as enhancing phagocytosis of apoptotic cells [17, 22-24, 43]. As both a risk factor in AD and potential drug target, *ABCA7* merits further investigation.

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Here we hypothesized that rs3764650, the primary AD-associated SNP, modulates *ABCA7* expression. In evaluating this hypothesis, we genotyped several SNPs and quantified *ABCA7* expression in 57 human brain samples. We interpret our results as suggesting a model wherein increased *ABCA7* expression reduces AD risk, possibly through enhanced microglial activation and phagocytosis.

2. Materials and methods

2.1. Ethics statement

The work described here was conducted under the approval of the University of Kentucky Institutional Review Board.

2.2. Human autopsy tissue

Human anterior cingulate samples were supplied by the University of Kentucky AD Center Neuropathology Core and have been described previously [28, 46, 47]. Evaluation of AD status was conducted by the AD Center Neuropathology and Clinical Cores using guidelines set forth by the National Institute on Aging Reagan Institute (NIA-RI) that included evaluation of neurofibrillary tangles and neuritic senile plaques [32–34]. Age at death for the cognitively intact, i.e. non-AD donors, was 82.3 ± 1.6 (mean $\pm$ SE, $n = 28$) while AD donors were 81.6 ± 1.1 (mean $\pm$ SE, $n = 29$). The post-mortem interval for non-AD and AD donors was 2.8 ± 0.2 ($n = 29$) hours and 3.4 ± 0.1 ($n = 27$), respectively. By NIA-RI neuropathology criteria, the 28 non-AD individuals included 12 with no AD likelihood, 10 with low likelihood, and six with moderate AD likelihood. Neuropathology in the 29 AD individuals was uniformly robust, i.e., definite AD by NIH-RI criteria [33].

2.3. qPCR

ABCA7 expression levels were determined by quantitative polymerase chain reaction (qPCR) (Bio-Rad, Hercules, CA). Assays contained the sense primer corresponding to sequence in exon 44, i.e., 5'-TCCTTTGGAACAGCCTTTTG-3' and antisense primer corresponding to sequence in exon 45, i.e., 5'-CTGCCCTTGAGATGTTGC-3'. Each 20 μ L reaction mixture contained 20 ng of cDNA, 1 μ M of each primer, and 2x SYBR Green (Quanta Biosciences, Gaithersburg, MD) and was subjected to a qPCR profile of 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 20 s. A standard curve was amplified in parallel to determine the copy number of qPCR product per sample. *ABCA7* copy numbers were normalized to the geometric mean of the reference genes eukaryotic translation initiation factor 4H (*EIF4H*) and ribosomal protein L32 (*RPL32*). Assays were repeated three times.

2.4. Genotyping

DNA samples were genotyped for rs3764650, rs3752246, rs4147914, rs3752229, rs7247084, rs757232, rs2279796, rs7251792, 12710103, and rs4147930 by using Taqman FAM and VIC dye-labeled probes (Life Technologies, Foster City, CA) by qPCR (Bio-Rad, Hercules, CA).

2.5. Statistical analysis

The extent that *ABCA7* mRNA levels were associated with AD-associated SNPs and/or AD-status was determined by using linear regression analysis (SPSS v.19 (IBM, Somers, NY)). A dominant allelic model was assumed, i.e. individuals homozygous for the rs3764650 minor G allele were grouped with those heterozygous for the SNP and compared with individuals homozygous for the rs3764650 major T allele. The model also included age at death and sex as covariates.

3. Results

The *ABCA7* SNP rs3764650 [T/G] is associated with AD risk [3]. To assess the hypothesis that *ABCA7* expression is modulated by this SNP, we quantified *ABCA7* copy number and then normalized these values to the geometric mean of two housekeeping genes [7, 27]. As depicted in Figure 1, *ABCA7* expression appears reduced in individuals with the AD-risky G allele and increased in those with AD. To evaluate this apparent trend, we used linear modeling that included rs3764650, AD status, age, and sex. Table 1 shows that *ABCA7* expression was significantly associated with rs3764650 genotype and AD status. Overall, we interpret these results as suggesting that *ABCA7* expression is reduced with the minor rs3764650G allele and increased with AD.

Several *ABCA7* SNPs have been associated with *ABCA7* expression in brain or monocytes by others [2, 45]. To evaluate whether these SNPs are associated with *ABCA7* expression in our brain samples, we genotyped the samples for these SNPs and performed linear regression analysis with each individual SNP. Overall, the SNPs showed variable association with *ABCA7* expression with rs3764650 having the lowest p value (Table 2). We hypothesized that if these other SNPs were associated with expression only through rs3764650, the linkage disequilibrium between each SNP and rs3764650 would correlate with the strength of the SNP's association with *ABCA7* expression. We found a robust correlation in this analysis (Figure 2). We interpret this positive relationship as supporting the concept that these other SNPs are associated with *ABCA7* expression only through rs3764650.

4. Discussion

Our primary findings are that *ABCA7* expression is decreased in individuals that carry the AD-risky rs3764650G allele. Also, *ABCA7* expression is increased with AD. The most parsimonious interpretation of our results is that the AD-risky rs3764650G allele decreases *ABCA7* prior to AD onset, i.e., increased *ABCA7* expression would reduce AD risk. From these results we propose that increased *ABCA7* expression during AD is insufficient to block disease progression. The mechanistic underpinnings of *ABCA7* action are unclear but likely involve lipid regulation or phagocytosis.

Our finding that *ABCA7* expression is increased in AD is consistent with a prior report from Karch et al that *ABCA7* expression was increased in individuals with increased cognitive decline [20]. Our finding that *ABCA7* expression is reduced with the AD-risky rs3764650G allele has not been reported previously. Instead, Allen et al identified several other SNPs that were associated with *ABCA7* expression [2]. We evaluated these SNPs and found that their association with *ABCA7* expression appears to reflect their linkage disequilibrium with rs3764650. However, Karch et al examined rs3764650 directly and did not detect an association between this SNP and *ABCA7* expression. Several technical and analytical differences between Karch et al and this study may underlay the differences in our findings. Technical differences include that we used SYBR-green based qPCR and normalized to the geometric mean of *RPL32* and *EIF4H* while Karch et al used TaqMan-based qPCR and normalized relative to *GAPDH*. Additionally, we used a dominant model for rs3764650 while Karch et al used an additive model; we re-analyzed our data with an additive model and found a less robust significance for SNP association with *ABCA7* expression ($p=0.011$ for additive model as opposed to $p=0.0035$ for dominant model). Karch et al examined 112 brain samples while our study included 57 samples; a post-hoc power analysis of the SNP association with expression here was 85.3%. A greater number of samples from non-AD individuals with the rs3764650G allele would provide greater insight on rs3764650G effects on *ABCA7* expression in the absence of pathology. In summary, the normalization genes

may be the most likely variable underlying our differing results, although additional unknown variables are also possible.

Two primary mechanisms may underlie the actions of *ABCA7* in AD. First, *ABCA7* may modulate AD risk through its ability to transfer phospholipids to apolipoproteins such as apoE, apoJ and apoA-1 [1, 43]. This is suggestive that *ABCA7* may act similarly to *ABCA1*, which has been shown to transfer cholesterol to apoE and modulate amyloid-beta ($A\beta$) levels [6, 8, 19, 23, 25, 44]. For example, *ABCA1* deficient mice have decreased levels of lipidated apoE and increased $A\beta$ deposits [26]. However, a key difference between *ABCA1* and *ABCA7* is that apoE levels are not altered in *ABCA7* deficient mice [23]. Additionally, *ABCA1* deficiency causes Tangiers disease in humans and a robust phenotype in mice but *ABCA7* polymorphisms do not associate with lipid homeostasis and *ABCA7* deficiency has only a modest effect on lipid homeostasis in mice [10, 20, 21, 23, 31]. Overall, the actions of *ABCA7* on lipid homeostasis may be modest relative to *ABCA1*. A second mechanism whereby *ABCA7* may modulate AD risk is through microglial phagocytosis [17, 23]. Indeed, *ABCA7* is a homolog of *ced-7*, the *C. elegans* gene critical for apoptotic cell engulfment [17]. Here, we note that ectopic *ABCA7* expression increases microglial phagocytosis of multiple substrates, including apoptotic cells, $A\beta$, and synthetic substrates *in vitro* [17, 23, 39–41]. Consistent with this possibility, *ABCA7* deficient mice have increased $A\beta$ deposition *in vivo* and rs3764650G in humans has been associated with increased neuritic plaque burden [23, 37, 38]. In summary, (i) *ABCA7* increases phagocytosis activity *in vitro*, (ii) *ABCA7* deficiency increases $A\beta$ in a murine model, and (iii) the rs3764650G allele that correlates with reduced *ABCA7* expression is associated with increased neuritic plaques in human. Hence, we propose that the most likely interpretation of current results is that *ABCA7* modulates AD risk through phagocytosis.

This interpretation is consistent with two recent genetic findings that have implicated microglial function in AD risk. First, *TREM2* increases microglial activation; a rare, apparently inhibitory, *TREM2* allele is associated with robustly increased AD risk [14, 18]. Second, *CD33* reduces microglial activation; a *CD33* allele that reduces functional *CD33*, apparently by modulating exon 2 splicing, is associated with decreased AD risk [4, 13, 30]. These results overall suggest that increased microglial activation reduces AD risk. Hence, the observation that *ABCA7* expression enhances microglial activation coupled with our finding that an AD-protective *ABCA7* allele is associated with increased *ABCA7* expression generates a consistent overall model wherein increased microglial activation, especially phagocytosis, is AD-protective (Figure 3). *TREM2* and *CD33* modulate phagocytosis by signaling through Syk and a Syk phosphatase, SHP1, respectively. The signaling pathway whereby *ABCA7* modulates phagocytosis is unclear but may involve the ERK signaling [17].

In summary, our primary findings are that *ABCA7* expression is decreased with the AD-risky rs3764650G allele and increased with AD. The increase in *ABCA7* expression in AD could reflect that *ABCA7* contributes to AD risk. However, a parsimonious model is that the *ABCA7* induction in AD is in response to the disease because *ABCA7* expression was also increased with the AD-protective rs3764650T allele. Hence, we interpret our data overall as suggesting a model wherein a SNP-driven, presumably long-term, increase in *ABCA7* expression reduces AD risk; increased *ABCA7* expression during AD is insufficient to block disease progression.

Acknowledgments

The authors acknowledge the NIH (National Institutes of Health) for funding of this work (P01-AG030128 (SE), P30-AG028383 (DWF), P20-GM103436 (DWF) as well as the University of Kentucky Alzheimer's Center for tissue (P30-AG028383).

Abbreviations

GWAS	Genome-wide association studies
SNP	Single nucleotide polymorphism
AD	Alzheimer's disease
ABCA7	ATP binding cassette A7
qPCR	quantitative polymerase chain reaction
Aβ	Amyloid-beta
EIF4H	eukaryotic translation initiation factor 4H
RPL32	ribosomal protein L32

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Highlights

- GWAS have identified an AD-associated SNP within *ABCA7*.
- We investigated the extent that this AD-associated SNP modulates *ABCA7* expression.
- The rs3764650G allele, which increases AD risk, was associated with decreased *ABCA7* expression.
- *ABCA7* expression was increased with AD status.
- We propose a model wherein increased *ABCA7* expression reduces AD risk with the increase observed in AD reflecting a poor compensatory response.

Figure 1. *ABCA7* expression as a function of rs3764650 genotype and AD status
ABCA7 expression is decreased in individuals carrying rs3764650G and increased with. Expression was evaluated as a function of the AD-associated SNP rs3764650 in a dominant model, i.e., rs3764650 GT and GG individuals were compared against TT individuals.

Figure 2. SNP linkage disequilibrium with rs3764650 correlates with SNP association with *ABCA7* expression

The r^2 for the relationship between SNP linkage disequilibrium and association with *ABCA7* expression was 0.93.

Figure 3. ABCA7, TREM2 and CD33 modulate microglial function to alter AD risk
This model proposes that enhanced microglial phagocytosis due to increased ABCA7 or TREM2 function, or reduced CD33 function, leads to decreased AD risk.

Table 1

Rs3764650 and AD status are associated with *ABCA7* expression. Linear regression statistical analysis included AD status, rs3764650 genotype, age at death and sex. The adjusted R^2 for the model was 0.285. Age and sex were not significantly associated with *ABCA7* expression ($p=.44$ and $p=.26$, respectively).

Standard Beta		
Parameter	Coefficient	P-Value
rs3764650	-0.375	3.5×10^{-3}
AD Status	0.502	1.1×10^{-5}

Table 2

Relationship between SNP linkage disequilibrium with rs3764650 and association with *ABCA7* expression. The linkage disequilibrium between rs3764650 (r^2) and each of the other SNPs was determined by using SNAP (<http://www.broadinstitute.org/mpg/snap/ldsearch.php>). The association between each individual SNP and *ABCA7* expression was determined by linear regression analyses that included AD status, age, sex and the SNP in a dominant model. The rationale for including each SNP is noted in the comment column.

SNP	r^2 with rs3764650	Standard Beta Coefficient	P-Value	Comment (reference)
rs3764650	1.000	−0.402	0.001	AD GWAS [15, 31]
rs4147914	0.584	−0.296	0.021	Exonic SNP
rs3752229	0.541	−0.296	0.028	Associates with <i>ABCA7</i> expression in brain [2]
rs7251792	0.245	0.161	0.209	Associates with <i>ABCA7</i> expression in monocytes [43]
rs3752246	0.230	−0.231	0.066	Non-synonymous exonic SNP [31]
rs757232	0.173	−0.170	0.174	Associates with <i>ABCA7</i> expression in brain [2]
rs4147930	0.166	−0.190	0.138	Exonic SNP
rs2279796	0.036	0.123	0.354	Associates with <i>ABCA7</i> expression in monocytes [43]
rs7247087	0.007	0.169	0.171	Associates with <i>ABCA7</i> expression in brain [2]
rs12710103	0.006	0.037	0.772	Associates with <i>ABCA7</i> expression in monocytes [43]