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Genetic Report

# Premature termination codon mutations in *ABCA7* contribute to Alzheimer's disease risk in Belgian patients

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ABSTRACT

The ATP-Binding Cassette Subfamily A Member 7 gene (*ABCA7*) was identified as a risk gene for Alzheimer's disease (AD) in genome-wide association studies of large cohorts of late-onset AD (LOAD) patients. Extended resequencing of the *ABCA7* coding regions identified mutations that lead to premature termination codons (PTC) and loss of function of ABCA7. PTC mutations were enriched in LOAD patients and were frequently present in patients with early-onset AD (EOAD). We aimed at assessing the contribution of *ABCA7* PTC mutations to AD in the Belgian population by screening the *ABCA7* coding region in a Belgian AD cohort of 1376 patients, including LOAD and EOAD patients, and in a Belgian control cohort of 976 individuals. We identified a PTC mutation in 67 AD patients (4.9%) and in 18 control individuals (1.8%) confirming the enrichment of *ABCA7* PTC mutations in Belgian AD patients. The patient carriers had a mean onset age of 69.7 ± 9.8 years with a wide onset age range of 42 years (48–90 years). In 77.3% of the families of *ABCA7* carriers, there were AD patients present suggestive of a positive family history of disease, but a Mendelian co-segregation of *ABCA7* PTC mutations with disease is not clear. Overall, our genetic data predict that PTC mutations in *ABCA7* are common in the Belgian population and are present in LOAD and EOAD patients.



## 1. Introduction

Alzheimer's disease (AD) is the most frequent cause of dementia across age groups. Both early-onset AD (EOAD) and late-onset AD (LOAD), defined by an age at onset (AAO) before or after 65 years, have a strong genetic component though much of its heritability and familial clustering remain unexplained. Pathogenic mutations in 3 genes, coding for the amyloid precursor protein (*APP*) and presenilin 1 and 2 (*PSEN1* and *PSEN2*), are linked to autosomal dominant transmission in EOAD families. However, they explain only 5 to 10% of EOAD heritability, leaving a majority of genetic contribution unexplained (Cacace et al., 2016). Heritability in LOAD

*Abbreviations:* AAI, Age at inclusion; AAO, Age at onset; AAD, Age at death; ABC, ATP-Binding Cassette; BCA7, ATP-Binding Cassette Subfamily A Member 7; AD, Alzheimer's disease; APOE, Apolipoprotein E; APP, Amyloid precursor protein; BELNEU, Belgian neurology; DSF, DNA screening facility; EOAD, Early-onset Alzheimer's disease; GWAS, Genome-wide association studies; LOAD, Late-onset Alzheimer's disease; MoCA, Montreal Cognitive Assessment; MMSE, Mini-mental state examination; NBD, Nucleotide binding domain; NMD, Nonsense-mediated mRNA decay; PTC, Premature termination codon; PSEN1, Presenilin 1; PSEN2, Presenilin 2; SNP, Single nucleotide polymorphism; STR, Short tandem repeat; TMD, Transmembrane domain; WES, Whole exome sequencing.

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patients is estimated at 58 to 79% (Gatz et al., 2006). The first risk gene identified in LOAD patients is apolipoprotein E (*APOE*), which contributes with its ε4 alleles to a high risk for AD or lowers onset age of disease (Liu et al., 2013). After a long period, in which no new risk genes were found, numerous multinational genome-wide association studies, using large LOAD patient cohorts and common genetic variants, identified several risk loci and genes, albeit with small effect sizes (Hollingworth et al., 2011; Lambert et al., 2013). One of the prominent risk genes is the *ABCA7*, which belongs to the ATP-binding cassette (ABC) transporter family, a superfamily of highly conserved proteins that are responsible for the transport of various substrates across the membrane (Kaminski et al., 2000).

Common variants in *ABCA7* were recognized as susceptibility loci for LOAD in both Caucasian (Hollingworth et al., 2011; Lambert et al., 2013; Naj et al., 2011) and African American cohorts (Cukier et al., 2016; Logue et al., 2011; Reitz et al., 2013). Studies using targeted resequencing, whole exome sequencing (WES) or whole genome sequencing (WGS) identified low frequency and rare genetic variants in *ABCA7* which are significantly associated with AD risk (Aikawa et al., 2018). Steinberg and colleagues established that rare premature termination codon (PTC) mutations are significantly associated with AD in the Icelandic population (Steinberg et al., 2015). Several other independent studies, observed PTC mutations in both EOAD and LOAD patients, conferring intermediate-to-high disease penetrance of the PTC mutations (Allen et al., 2017; Bellenguez et al., 2017; Cuyvers et al., 2015; De Roeck et al., 2017; Del-Aguila et al., 2015; Kunkle et al., 2017; Le Guennec et al., 2016; Vardarajan et al., 2015). Long-read sequencing revealed that PTC mutations lead to loss-of-functional ABCA7 protein by nonsense-mediated mRNA decay (NMD). Some of the PTC mutations partially escaped from NMD by active transcript rescue (De Roeck et al., 2017). Generally, the risk for AD in PTC mutation carriers doubled or more (De Roeck et al., 2019). Additionally, a variable number tandem repeat located in an intron of *ABCA7* is a common high-penetrant risk factor for AD (De Roeck et al., 2018). Expanded variable number tandem repeat (VNTR) alleles increased 4.5-fold the risk of AD by decreasing ABCA7 expression.

Resequencing of *ABCA7* in 772 Belgian patients with predominantly LOAD revealed an increased frequency of loss-of-function PTC mutations in the group of patients compared to the control group (Cuyvers et al. 2015). In the families of the *ABCA7* PTC mutation carriers, the load of AD patients was high. For the *ABCA7* p.E709fs mutation co-segregation with AD was reported in an extended Belgian AD pedigree. The PTC patient carriers presented with a classical AD phenotype and a wide range in onset age (Cuyvers et al., 2015; Van den Bossche et al., 2016). In this study, we aimed at obtaining supplementary data about the frequency of *ABCA7* PTC carriers in Belgian AD patients using a larger cohort of 1376 LOAD and EOAD patients and a Belgian cohort of 976 controls. Additional data could contribute to the elucidation of the PTC mutational spectrum as well as prevalence and penetrance of PTC mutations. A larger cohort of *ABCA7* PTC carriers might also add to a better understanding of the variability of onset age and the risk for AD in sporadic patients and families. Together, the extra data can be helpful in assessing risk and onset age of carriers of an *ABCA7* PTC mutation in a diagnostic setting.

## 2. Methods

### 2.1. Belgian cohorts of AD patients and controls

The members of the Belgian Neurology (BELNEU) consortium are clinical partners from neurology or genetic centers with expertise in neurodegenerative brain disorders. They recruit patients to include in the Belgian AD patient cohort and follow up the patients at their neurology clinic. The Belgian AD cohort included EOAD (n = 491) and LOAD (n = 885) patients. The overall average age was 69.3 ± 10.6 years (range 31–96). All patients received a possible, probable or definite AD diagnosis according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (McKhann et al., 1984) or the National Institute on Aging - Alzheimer's Association (Hyman et al., 2012; McKhann et al., 2011; Montine et al., 2012). The Belgian cohort in this study comprises 1376 AD patients (60.0% [826/1376] women) of which 716 patients were screened previously for *ABCA7* PTC mutations (Cuyvers et al., 2015). Clinical or pathological follow-up of the Cuyvers patient and control cohorts identified patients without AD phenotype and controls who developed memory problems and were excluded. Over the past years new patients and controls were added to the cohorts resulting in extended cohorts of 1376 Belgian patients and 976 controls. In the AD cohort, 79 patients (5.7%, 79/1376) had brain autopsy and neuropathological confirmation of AD brain pathology. We defined AAO based on the age at first reporting of substantial AD symptoms, e.g. cognitive problems, by the patient or a family member or the AAO reported in the first medical record. If AAO data was not available, we used age at inclusion (AAI) of the patient in the AD cohort. To measure the degree of familial AD in the cohort, we labeled patients with a positive family history if at least 1 affected first-degree relative was present in the family. Familial history data was available for 922 patients (67.0%, 922/1376) of which 461 patients had a positive family history (50.0%, 461/922). Genetic screening of *APP, PSEN1* and *PSEN2* was performed in all AD patients and revealed 2 pathogenic *APP* mutations (0.15%) and 9 pathogenic *PSEN1* mutations (0.65%) (Table S1). Overall, we identified known pathogenic mutations in 0.80% of the patients in our AD cohort.

The Belgian control cohort used in this study included 976 community-dwelling individuals (63.5% [620/976] women) with a mean AAI of 72.4 ± 8.6 years, of which 726 control individuals were screened previously (Cuyvers et al., 2015). All control individuals were volunteers and underwent a cognitive screening using the mini-mental state examination or the Montreal Cognitive Assessment, cutoff > 25 (Folstein et al., 1975; Nasreddine et al., 2005).

### 2.2. Ethical assurances

All participants or their legal representative signed an informed consent in order to participate in the genetic studies. The ethical committee of the University Hospital Antwerp and the University of Antwerp approved the informed consent and the protocols of the genetic and clinical studies.

### 2.3. Genetic screening of AD patients and controls

The *ABCA7* sequencing data of 716 patients and 726 controls of the previous study were available (Cuyvers et al., 2015). For an additional 486 patients and 250 controls in this study, we sequenced the *ABCA7* coding region by means of targeted resequencing or we extracted the sequences from available WES data of 174 AD patients.

#### 2.3.1. Targeted resequencing of the ABCA7 coding region

We used targeted resequencing of canonical exons and splice sites of *ABCA7* using a custom-designed multiplex PCR MASTR assay (Agilent Technologies, CA, USA), combined with massive parallel sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) (Cuyvers et al., 2015; De Roeck et al., 2017).

**Table 1**
Overview of specific characteristics of the different 14 PTC mutations in the Belgian AD cohort

| cDNA[a] | Predicted protein[b] | No. patient carriers (freq., %) | Mean AAO ± SD | Mean AAD ± SD | Mean DD ± SD | FH + (%) | *APOE* ε4 + (%) |
|---|---|---|---|---|---|---|---|
| c.67-1G>A | p.0 | 3 (0.22) | 62.7 ± 2.3 | 69.7 ± 3.8 | 7.0 ± 6.1 | 100 | 33.3 |
| c.1457delC | p.P486fs | 1 (0.07) | 71 | - | - | 100 | 100 |
| c.1621_1622delGT | p.F542fs | 2 (0.15) | 68.0 ± 7.1 | 76.5 ± 6.4 | 8.5 ± 0.7 | 100 | 100 |
| c.2126_2132del | p.E709fs | 16 (1.16) | 69.7 ± 10.5 | 75.2 ± 12.9 | 7.7 ± 3.4 | 92.3 | 56.3 |
| c.3247C>T | p.Q1083* | 1 (0.07) | 55 | 65 | 10 | - | 0 |
| c.3578-2A>G | p.0 | 2 (0.15) | 63.5 ± 12.0 | - | - | - | 0 |
| c.3641G>A | p.W1214* | 1 (0.07) | 86 | 88 | 2 | 0 | 100 |
| c.4008G>A | p.W1336* | 12 (0.87) | 71.5 ± 11.5 | 75.9 ± 11.3 | 6.9 ± 3.8 | 62.5 | 50.0 |
| c.4202delA | p.Q1401fs | 1 (0.07) | 83 | 86 | 3 | - | 0 |
| c.4208delT | p.L1403fs | 6 (0.44) | 69.8 ± 6.7 | 75.2 ± 6.7 | 5.8 ± 2.6 | 80.0 | 66.7 |
| c.4416 + 2T>G | p.0 | 1 (0.07) | 59 | 73 | 14 | - | 100 |
| c.4690C>T | p.R1564* | 1 (0.07) | 72 | 77 | 5 | - | 100 |
| c.5173_5174insA | p.L1725fs | 1 (0.07) | 60 | 73 | 13 | 100 | 100 |
| c.5570 + 5G>C | p.0 | 19 (1.38) | 70.6 ± 9.6 | 78.1 ± 11.8 | 8.9 ± 5.2 | 66.7 | 68.4 |
| All | | 67 (4.87) | 69.7 ± 9.8 Range: 48–90 | 76.0 ± 10.2 Range: 54–95 | 7.6 ± 4.2 Range: 1–18 | 77.3 | 59.7 |

Key: AAO, age at onset; AAD, Age at death; DD, disease duration; FH, familial history; SD, standard deviation.
* indicates a nonsense mutation.
[a] Coding nomenclature according to NM_019112.3.
[b] Protein nomenclature according to NP_061985.

#### 2.3.2. Whole exome sequencing

We performed library preparation and target enrichment using the SeqCap EZ Exome Library v3.0 kit (Nimblegen Roche, Basel, Switzerland), and sequenced on an Illumina NextSeq platform (Illumina, San Diego, CA, USA). For data processing and variant filtering we used GenomeComb (Reumers et al., 2012).

#### 2.3.3. Sanger sequencing

We validated all PTC mutations using Sanger sequencing technology (BigDye Terminator Cycle Sequencing kit v3.1; analysis on an ABI 3730 DNA Analyzer, Thermo Fisher Scientific, MA, US).

#### 2.3.4. Bioinformatics analyses

Read processing, alignment, variant calling, annotation and downstream filtering of resequencing and WES data was achieved using GenomeComb (Reumers et al., 2012). We considered variants located in segmental duplications, repeated sequences or homopolymer stretches false positives and excluded them from the study. Only variants with a minor allele frequency ≤1% in the Genome Aggregation database v2.1.1 (Lek et al., 2016) and the Exome Variant ServerNHLBI GO Exome Sequencing Project, Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) were retained.

### 2.4. Allele sharing analysis of ABCA7 PTC carriers

We determined haplotypes based on single nucleotide polymorphisms spanning *ABCA7* and 7 short tandem repeats (STR) flanking *ABCA7* as described (Cuyvers et al., 2015; De Roeck et al., 2017). We used multiplex PCR-amplifications of STR markers and separated PCR-products, supplemented with GeneScan 500 LIZ size standard (Thermo Fisher Scientific, MA, USA), using capillary electrophoresis on an ABI 3730 DNA Analyzer (Thermo Fisher Scientific, MA, USA). We assigned STR genotypes using an *in-house* developed LGV v3.01 (https://www.neuromicssupportfacility.be/).

## 3. Results

### 3.1. PTC mutations in Belgian AD patients and control individuals

We acquired sequencing data of *ABCA7* for 1376 AD patients and observed 14 different PTC mutations: 6 frameshift mutations, 4 nonsense mutations and 4 splice mutations, in 67 AD patients (4.9%, 67/1376) (Table 1, Table S2, Fig. 1). Six of the 14 different PTC mutations were also observed in 18 controls (1.8%, 18/976) (Table 2, Table S3, Fig. 1). No other PTC mutations were present in the controls. Previously, we showed that carriers of *ABCA7* p.E709fs are sharing the same disease haplotype (Cuyvers et al., 2015). Consequently, we analyzed haplotype sharing using single nucleotide polymorphismss and STR alleles in carriers of the same *ABCA7* PTC mutation. The data we obtained indicated that carriers of the same *ABCA7* PTC mutation are indeed sharing a common *ABCA7* haplotype suggesting that they have a common ancestor (Figure S1).

### 3.2. Characteristics of ABCA7 PTC mutation carriers

#### 3.2.1. Age-related penetrance curve of the ABCA7 AD carriers

Including all *ABCA7* PTC AD carriers (n = 67), we calculated age-related penetrance (Fig. 2). Together, the PTC carriers have a mean AAO of 69.7 ± 9.8 years with a wide age range of 48–90 years (Table 1). There were no significant differences in AAO between carriers of a frameshift (n = 27), splice site (n = 25) or nonsense (n = 15) PTC mutation (Fig. 3A). Age at death was available for 44 of the 67 *ABCA7* PTC AD carriers. Mean age at death is 76.0 ± 10.2 (54–95) years, with a mean disease duration of 7.6 ± 4.2 (1–18) years (Table 1).

#### 3.2.2. Effect size of the APOE genotypes on AAO of ABCA7 PTC AD carriers

The fraction of AD PTC carriers with at least 1 *APOE* ε4 allele (59.7%, 40/67) is like that of the full AD cohort (58.5%, 790/1350). In the cohort of the *ABCA7* PTC carriers, mean onset age of the *APOE* ε4 homozygotes (n = 9, 66.1 ± 9.7 years) is slightly earlier than for the *APOE* ε4 heterozygotes (n = 31, 69.6 ± 8.1 years) and earlier than for the *APOE* ε4 non-carriers (n = 27, 71.0 ± 11.5 years), though not statistically significant (Fig. 3B).

#### 3.2.3. Family history of ABCA7 PTC AD carriers

Of 44 of the 67 *ABCA7* PTC AD carriers (65.7%, 44/67), we obtained information on familial history of dementia. Family history was positive in 77.3% (34/44). Of the familial carriers, 64.7% (22/34) carried at least 1 *APOE* ε4 allele and 6 (17.6%, 6/34) were homozygous for *APOE* ε4. In contrast, of the *ABCA7* PTC AD carriers with sporadic or unknown familial history data, 54.5% (18/33) carried at least 1 *APOE* ε4 allele and 3 were homozygous for *APOE* ε4 (9.1%, 3/33).

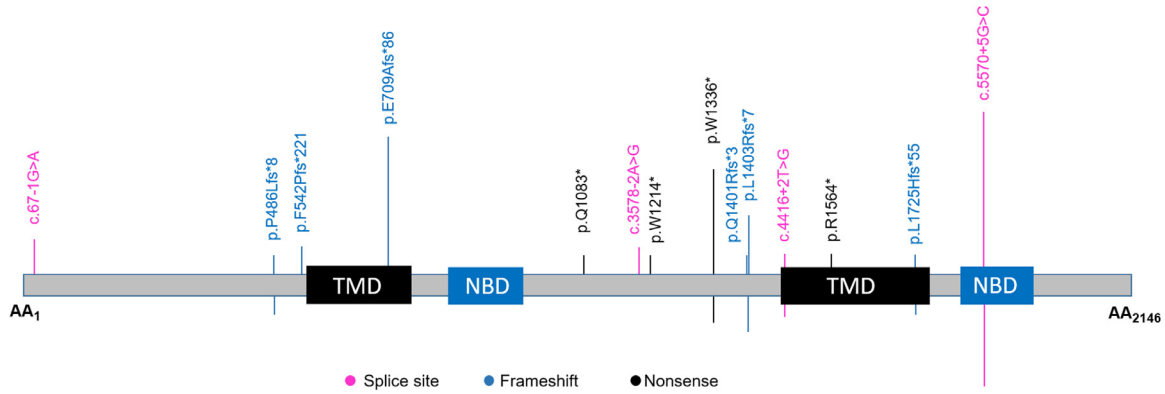


**Fig. 1.** Distribution of rare *ABCA7* PTC mutations across protein domains. On scale, linear representation of *ABCA7* PTC mutations identified in Belgian AD patients (n = 1376) and control individuals (n = 976). The top part of the figure shows the PTC mutations found in patients. The bottom part shows the PTC mutations found in control individuals. ABCA7 encodes a 2146aa long protein consisting of 4 core domains: 2 nucleotide-binding domains (NBD) and 2 transmembrane domains (TMD) (Kaminski et al., 2000).

**Table 2**
Overview of specific characteristics of control individuals carrying an *ABCA7* PTC mutation

| cDNA[a] | Predicted protein[b] | No. control carriers (freq., %) | Mean AAI ± SD | *APOE* ε4 + (%) |
|---|---|---|---|---|
| c.1457delC | p.P486fs | 1 (0.10) | 66 | 0 |
| c.4208delT | p.W1336* | 2 (0.20) | 70.0 ± 2.8 | 50.0 |
| c.4208delT | p.L1403fs | 3 (0.31) | 68.7 ± 3.5 | 33.3 |
| c.4416 + 2T>G | p.0 | 1 (0.10) | 84 | 0 |
| c.5173_5174insA | p.L1725fs | 1 (0.10) | 82 | 0 |
| c.5570 + 5G>C | p.0 | 10 (1.02) | 73.2 ± 8.5 | 30.0 |
| All | | 18 (1.84) | 72.8 ± 7.7 Range: 62–88 | 27.8 |

Key: AAI, age at inclusion; SD, standard deviation.
* indicates a nonsense mutation.
[a] Coding nomenclature according to NM_019112.3.
[b] Protein nomenclature according to NP_061985.2.

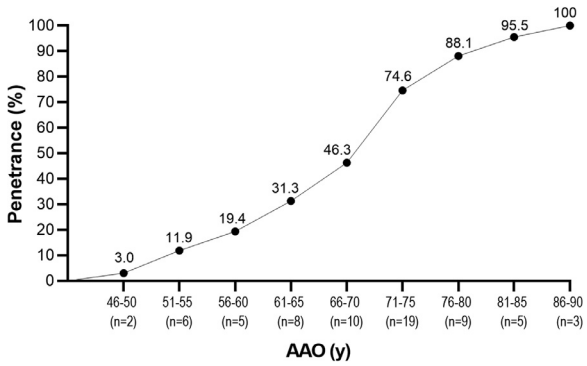


**Fig. 2.** Cumulative penetrance curve. The age-related disease penetrance for all *ABCA7* PTC mutation carriers (n = 67) is shown. We calculated the disease penetrance in age groups of 5 years.

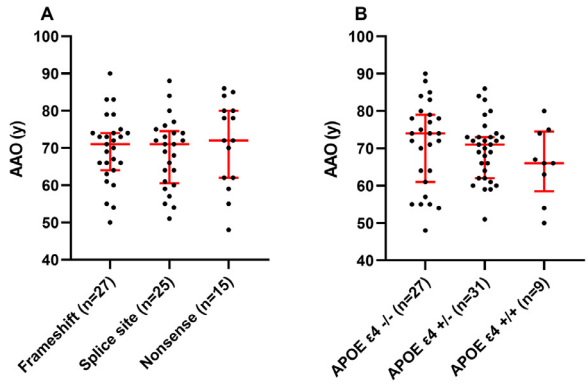


**Fig. 3.** PTC mutation type and *APOE* genotype in relation to AAO of Alzheimer's disease in *ABCA7* PTC mutation carriers. (A) Comparison of AAO between carriers of different types of PTC mutations. Data are presented as median ± interquartile range (IQR). Kruskall-Wallis $p = 0.609$. (B) Genetic modifying effect of *APOE* ε4 on AAO. Data are presented as median ± interquartile range (IQR). Kruskall-Wallis $p = 0.413$.

## 4. Discussion

In *ABCA7*, we identified 14 different PTC mutations in 67 patients of the 1376 patients included in the Belgian AD cohort (4.9%, 67/1376), while in only 11 AD patients (0.8%) a pathogenic mutation was present in *APP* (n = 2, 0.15%) and *PSEN1* (n = 9, 0.65%). The carriers of an *APP* or *PSEN1* mutation were free from an *ABCA7* PTC mutation. In the Belgian cohort of 976 controls, we observed 18 carriers of 1 of 6 different *ABCA7* PTC mutations (1.8%, 18/976), which are also present in AD patients. The location of the 14 *ABCA7* PTC mutations is scattered over the whole length of the ABCA7 protein (Fig. 1). Most of the PTC mutations described in this study were reported before in previous studies, except the nonsense mutation p.Q1083* (Bellenguez et al., 2017; Cuyvers et al., 2015; De Roeck et al., 2017; Le Guennec et al., 2016; Steinberg et al., 2015; Vardarajan et al., 2015). In the Belgian cohort, the splice-site mutation, c.5570+5G>C, is the most frequent *ABCA7* PTC in AD patients (1.4%, 19/1376) and controls (1.0%, 10/976) (Table 1, Table 2). Unfortunately, a comparison with other studies is not feasible since only 3 studies included the c.5570+5G>C splice-site mutation in their counts. Many studies did not include this mutation since it is not a canonical splice site mutation. However, there is proof that this mutation has a LOF effect since the mutated splice-site

leads to intron retention generating a PTC (De Roeck et al., 2017; Steinberg et al., 2015). The Belgian *ABCA7* PTC carriers have a high familial load compared to the patients in the Belgian AD cohort (77.3% vs. 50.0%), signifying that genetic screening of *ABCA7* in AD patients with a familial history might be considered in a diagnostic context. However, clear co-segregation patterns with disease were mostly lacking. Carriers of the same PTC mutation shared a common disease haplotype spanning the entire *ABCA7* gene suggesting a genetic relatedness indicating a common ancestor (Figure S1).

It is expectable that PTC mutations lead to loss of functional protein through NMD or protein truncation and degradation. Previously, we investigated NMD efficiency in carriers of a c.67-1G>A, p.E709fs, p.W1336*, p.L1403fs, or c.5570+5G>C mutation and reported a wide variability between the different mutations. We also investigated occurrence of transcript rescue events in carriers of p.E709fs, p.W1336* and p.L1403fs and noted a wide variability in transcript rescue between carriers of different *ABCA7* PTC mutations and between carriers of the same PTC mutation (Table S4) (De Roeck et al., 2017). Reduction of *ABCA7* brain expression on mRNA or protein level was studied for c.67-1G>A, p.E709fs, p.W1214*, p.L1403fs and c.5570+5G>C and varied between the different PTC mutations (Allen et al., 2017; Cuyvers et al., 2015; De Roeck et al., 2017) (Table S4). It is possible that variability in NMD-efficiency, transcript rescue or protein expression contributes to the pathogenic character and age-related penetrance of the separable PTC mutations. Wide ranges in onset age are present in both carriers of a different or the same PTC mutation, hinting towards the influence of modifiers. The deletion c.2126_2132del leads to a frameshift mutation and a truncated protein, p.E709fs, and is present in 1.2% of the Belgian AD patients and absent in control individuals. This mutation has the lowest reported NMD-efficiency and shows a potential rescue transcript due to alternative splicing, which is possibly accountable for the wide range of AAO (50–90 years) among the carriers (Table S4) (De Roeck et al., 2017). In a Belgian family, p.E709fs appeared to co-segregate with AD in an autosomal dominant manner (Cuyvers et al., 2015; Van den Bossche et al., 2016). Nevertheless, p.E709fs is present in non-Belgian control populations and has a frequency of 0.15% in the Genome Aggregation database non-neuro database (accessed March 2020), which could be indicative for a population-specific effect. Otherwise, the wide AAO range of p.E709fs observed in AD carriers does not exclude that control carriers of p.E709fs might still develop disease at later age. Several studies using in vitro and in vivo models have shown that *ABCA7* deficiency may lead to a decreased microglial A$\beta$ clearance and an increased *APP* processing, therefore exacerbating A$\beta$ accumulation in the brain (Aikawa et al., 2018).

The mean AAO of the 67 AD patients carrying a PTC mutation is 69.7 $\pm$ 9.8 years which is like the mean AAO of the entire Belgian AD cohort (69.3 $\pm$ 10.6 years). In the *ABCA7* PTC AD carriers the AAO is highly variable between carriers of a different PTC mutation as well as between carriers of the same PTC mutation with a range from 48 to 90 years. Analysis of a possible influence of *APOE* $\varepsilon$4 alleles on AAO of the 67 *ABCA7* PTC carriers was not significant. The frequency of the *APOE* $\varepsilon$4 allele in the *ABCA7* PTC carriers is like in the complete AD cohort (59.7% vs. 58.5%). Likewise, Steinberg et al. did not detect a statistical interaction between the AAO of *ABCA7* PTC carriers and *APOE*. Nonetheless, further investigation into co-occurrence with *APOE* - and other potential genetic, epigenetic and/or environmental modifiers - remains of high interest, as they are both involved in the lipid metabolism pathway leading to AD. Of note, the *ABCA7* carriers with a positive family history were more likely to carry an $\varepsilon$4 allele, suggesting that the combination of different genetic risk factors can contribute to the familial occurrence. Despite the seemingly reduced penetrance of *ABCA7* mutations compared to established causal mutations, they could have a larger overall contribution to the familial clustering observed in AD, as they are more frequent. However, the presence of *ABCA7* PTC mutations in control individuals is non-negligible and identification of modifiers affecting the penetrance of these mutations is essential to assess the pathogenicity of individual *ABCA7* mutations. Since the age range of the control carriers (AAI range of 26 years [62–88]) falls within the wide onset age range of the PTC carriers in our study (AAO range 42 years [48–90]) we cannot exclude that control carriers may develop AD later in life.

## 5. Conclusions

Taken together, deleterious PTC mutations in *ABCA7* are relatively frequent in Belgian AD patients (4.9%) in contrast to Belgian controls (1.8%). Our data confirm an enrichment of the Belgian AD cohort with *ABCA7* PTC mutations compared to the Belgian control cohort. Analysis of the extended Belgian patient and control cohorts, showed similar results as in the study from Cuyvers et al., who reported a frequency of 5.1% PTC mutations in patients and 1.8% in control individuals, confirming the high frequency of ABCA7 PTC mutations in the Belgian population. The Belgian frequency of *ABCA7* PTC carriers is somewhat higher than in other studies of Caucasian patients. We reported 3.66% of *ABCA7* PTC mutations in AD patients and 1.33% in controls in a European EOAD cohort (De Roeck et al., 2017). Two studies in a French EOAD + LOAD cohort and a French EOAD cohort, reported a PTC mutation frequency of 3.09% in EOAD + LOAD patients versus 1.96% in controls and 4.13% in EOAD patients versus 1.34% in controls (Bellenguez et al., 2017; Le Guennec et al., 2016).

Our data confirm that PTC mutations in *ABCA7* could have a significant contribution to the genetic architecture of AD. The PTC mutation carriers have an increased familial load, signaling that *ABCA7* could explain a proportion of the familial clustering observed in AD patients. Our results indicate that screening of *ABCA7* is of high interest in Belgian AD patients, and in particular familial AD patients who tested negative for causal mutations in Alzheimer genes *APP, PSEN1* and *PSEN2*. *ABCA7* could represent an important genetic subtype of AD, thus improving genetic diagnosis, risk prediction and development of targeted therapeutics.

However, further elucidation of the pathogenic nature, age-related penetrance, and segregation patterns of individual mutations - and possible modifiers thereof - remains of high priority for the implementation of *ABCA7* screening in clinical practice and genetic counselling.

**BELNEU consortium: (collaborators)**

The following members of the BELNEU consortium have contributed to this paper by selecting Alzheimer patients to include into the Belgian AD cohort for genetic studies: Johan Goeman, Roeland Crols (General Hospital Network Antwerp, Antwerp); Anne Sieben, Tim Van Langenhove, Bart Dermaut (University Hospital Ghent, Ghent); Olivier Deryck, Bruno Bergmans (General Hospital Sint-Jan Brugge, Bruges); Jan Versijpt (University Hospital Brussels, Brussels); Bernard Hanseeuw (University Hospital Saint-Luc, Brussels); Boudewijn Michielsens (General Hospital Sint-Jozef, Malle). The authors are also thankful for the support of the personnel of the VIB CMN Neuromics Support Facility, and of the DNA Screening Facility and the Human Biobank of the VIB NBD group.

## Disclosure statement

The authors have no conflicts of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2021.04.023.

## CRediT authorship contribution statement

**Liene Bossaerts:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Elisabeth Hens:** Investigation, Writing – original draft, Visualization, resources, Validation. **Bernard Hanseeuw:** Investigation, resources, Validation. **Rik Vandenberghe:** Investigation, resources, Validation. **Patrick Cras:** Investigation, resources, Validation. **Peter P. De Deyn:** Investigation, resources, Validation. **Sebastiaan Engelborghs:** Investigation, resources, Validation. **Christine Van Broeckhoven:** Conceptualization, resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

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