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Amyloid-β_{1-43} cerebrospinal fluid levels and the interpretation of *APP*, *PSEN1* and *PSEN2* mutations

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Abstract

Background: Alzheimer's disease (AD) mutations in amyloid precursor protein (*APP*) and presenilins (*PSENs*) could potentially lead to the production of longer amyloidogenic Aβ peptides. Amongst these, $A\beta_{1-43}$ is more prone to aggregation and has higher toxic properties than the long-known $A\beta_{1-42}$. However, a direct effect on $A\beta_{1-43}$ in biomaterials of individuals carrying genetic mutations in the known AD genes is yet to be determined.

Methods: $N = 1431$ AD patients ($n = 280$ early-onset (EO) and $n = 1151$ late-onset (LO) AD) and 809 control individuals were genetically screened for *APP* and *PSENs*. For the first time, $A\beta_{1-43}$ levels were analysed in cerebrospinal fluid (CSF) of 38 individuals carrying pathogenic or unclear rare mutations or the common *PSEN1* p.E318G variant and compared with $A\beta_{1-42}$ and $A\beta_{1-40}$ CSF levels.
The soluble sAPPα and sAPPβ species were also measured for the first time in mutation carriers.

Results: A known pathogenic mutation was identified in 5.7% of EOAD patients (4.6% *PSEN1*, 1.07% *APP*) and in 0.3% of LOAD patients. Furthermore, 12 known variants with unclear pathogenicity and 11 novel were identified. Pathogenic and unclear mutation carriers showed a significant reduction in CSF $A\beta_{1-43}$ levels compared to controls ($p = 0.037$; < 0.001). CSF $A\beta_{1-43}$ levels positively correlated with CSF $A\beta_{1-42}$ in both pathogenic and unclear carriers and controls (all $p < 0.001$). The p.E318G carriers showed reduced $A\beta_{1-43}$ levels ($p < 0.001$), though genetic association with AD was not detected. sAPPα and sAPPβ CSF levels were significantly reduced in the group of unclear ($p = 0.006$; 0.005) and p.E318G carriers ($p = 0.004$; 0.039), suggesting their possible involvement in AD. Finally, using $A\beta_{1-43}$ and $A\beta_{1-42}$ levels, we could re-classify as "likely pathogenic" 3 of the unclear mutations.

(Continued on next page)

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Conclusion: This is the first time that $A\beta_{1-43}$ levels were analysed in CSF of AD patients with genetic mutations in the AD causal genes. The observed reduction of $A\beta_{1-43}$ in *APP* and *PSENs* carriers highlights the pathogenic role of longer Aβ peptides in AD pathogenesis. Alterations in $A\beta_{1-43}$ could prove useful in understanding the pathogenicity of unclear *APP* and *PSENs* variants, a critical step towards a more efficient genetic counselling.

Keywords: Alzheimer's disease (AD), Amyloid-β 1–43 ($A\beta_{1-43}$), Cerebrospinal fluid (CSF), Alzheimer mutations, Oxford Nanopore Technologies (ONT) long-read sequencing

Background

AD is the most common cause of dementia, characterized by progressive cognitive decline and memory loss, accounting for 50 to 75% of all dementia patients [1]. Based on the disease onset, AD is classified into early-onset AD (EOAD, < 65 years) and late-onset AD (LOAD, > 65 years) [2]. Mutations in amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) have been identified as a cause of both EOAD and LOAD [3, 4] explaining 10% of all EOAD and about 2% of LOAD patients [4, 5]. The overproduction and aggregation of amyloid-β (Aβ) in the brain are thought to be the major causal events triggering AD that ultimately lead to neuronal loss [6]. Aβ peptides aggregate in the brain forming amyloid plaques, which together with neurofibrillary tangles (NFTs) of hyper-phosphorylated tau protein are the AD pathological hallmarks [6, 7]. Aβ is generated from cleavages of APP through at least two distinct and mutually exclusive pathways. In the so-called non-amyloidogenic pathway, APP is cleaved by α-secretase and γ-secretase to produce three fragments: a secreted C-terminal fragment (sAPPα), p3 and the APP intracellular domain (AICD) [4]. In the amyloidogenic (pathogenic) pathway, APP is cleaved by β-secretase, followed by γ-secretase cleavage. The cleavage by β-secretase generates a large soluble extracellular secreted domain (sAPPβ) and C99. The latter undergoes additional cleavages by γ-secretase to generate a series of Aβ peptides 39–43 amino acids long, following two different pathways: $A\beta_{1-49} > A\beta_{1-46} > A\beta_{1-43} > A\beta_{1-40}$ and $A\beta_{1-48} > A\beta_{1-45} > A\beta_{1-42} > A\beta_{1-38}$ [8]. Shorter peptides can also be produced, including $A\beta_{1-41}$ from $A\beta_{1-43}$ [9] and $A\beta_{1-34}$ from either $A\beta_{1-42}$ or $A\beta_{1-40}$ [10], which is considered a biomarker of Aβ clearance and AD progression [11]. Pathogenic mutations in *APP* and *PSEN1* and *2* (γ-secretase's catalytic subunits) are known to influence APP metabolism leading to the deposition of Aβ peptides. The most abundant Aβ peptides in the cerebrospinal fluid (CSF) resulting from APP processing are $A\beta_{1-38}$, $A\beta_{1-40}$ and $A\beta_{1-42}$. The latter is considered the most pathological peptide in AD as it is most prone to aggregation into amyloid plaques [12]. $A\beta_{1-42}$ levels have been found to be reduced in CSF of patients with AD [13] as a result of $A\beta_{1-42}$ increased production and subsequent accumulation into plaques [14]. Furthermore, CSF $A\beta_{1-42}$ levels are usually reduced up to decades before the clinical symptoms of dementia appear [15]. Thus, CSF $A\beta_{1-42}$ levels are considered a core biomarker for early AD [16], together with total tau protein (T-tau) (non-AD specific) and tau phosphorylated at threonine 181 (P-tau181) (AD specific) [17]. In addition to Aβ peptides, α and β cleaved soluble APP (sAPPα and sAPPβ) are products of the APP metabolism which also have been investigated as possible AD biomarkers, though with contradicting results [18, 19]. $A\beta_{1-42}$ levels have been used for the pathological classification of AD mutations, both in vivo and in vitro [20]. However, some of the established AD pathogenic mutations do not show altered $A\beta_{1-42}$ levels [21]. For example, in the brain of the *PSEN1* p.R278I knock-in mice, a decrease of $A\beta_{1-40}$ was accompanied by an increase of another Aβ, i.e. species, $A\beta_{1-43}$, which showed higher aggregative properties than $A\beta_{1-42}$ [21]. Interestingly, $A\beta_{1-43}$ was also detected in the brain of sporadic and familial AD patients [22–24] supporting the hypothesis that the generation of relatively long Aβ peptides (>$A\beta_{1-42}$) could explain part of the pathogenic effect of the known deleterious *PSENs* and *APP* mutations [25, 26]. Furthermore, several variants identified in the causal AD genes remain of uncertain significance (VUS) (www.alzforum.org/mutations; AD/FTD Mutation Database [27]), due to lack of functional studies and co-segregation with disease in relatives. Understanding the role of these variants is important for a correct clinical diagnosis, for genetic counselling and for the selection of well-stratified patient groups for clinical trials. The investigation of longer Aβ peptides, including $A\beta_{1-43}$, in CSF of individuals carrying AD mutations or VUS could disclose possible alterations of the Aβ peptide production and explain their possible role in the AD pathogenesis. Recent studies showed that CSF $A\beta_{1-43}$ levels are significantly reduced in individuals with AD and mild cognitive impairment (MCI) [16, 28], as well as in EOAD patients compared to LOAD [29], independently from the mutation status. Studies assessing $A\beta_{1-43}$ levels in CSF of *PSENs* and *APP* mutation carriers are therefore lacking. In the present work, we measured for the first time the $A\beta_{1-43}$ levels in CSF of carriers of *APP* and *PSENs* pathogenic

and VUS mutations and control individuals and we compared these results to $A\beta_{1-42}$ and $A\beta_{1-40}$. We also measured, for the first time in mutation carriers, sAPPα and sAPPβ in relation to $A\beta_{1-43}$, as they are less investigated, but still part of the APP processing.

Methods

Study population

The study population consisted of 1431 AD patients (62.03% [$n = 889$] women, average age at onset (AAO) 73.51 ± 9.81 years, range 29–96 years), ascertained in Belgium through the neurology centres of the clinical partners of the Belgian neurology (BELNEU) consortium. All patients received a diagnosis of possible, probable or definite AD according to the criteria described by the National Institute of Neurological and Communication Disorders and Stroke Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria and the National Institute on Aging–Alzheimer's Association (NIA-AA) [30]. A positive family history of dementia (i.e. at least one first-degree relative affected) was documented in 22.22% ($n = 318$) of the patients. Based on the onset age (< 65 years), 19.57% ($n = 280$) of the patients were classified as having early-onset AD (EOAD, 54.64% [$n = 153$] women, average AAO 58.22 ± 6.14, age range 29–65 years). A Belgian control cohort of 809 individuals (73.54% [$n = 595$] women, average age at inclusion (AAI) 70.12 ± 10.71, 31–96 years) was included in the study. The control individuals were primarily community-dwelling volunteers or spouses of patients. Subjective memory complaints, neurologic or psychiatric antecedents, and a familial history of neurodegeneration were ruled out by means of an interview. Cognitive screening was performed using the Mini-Mental State Examination (cut-off score > 26) [31] and/or the Montreal Cognitive Assessment test (cut-off score > 25) [32]. The spouses of patients were examined at the Memory Clinic of ZNA Middelheim and Hoge Beuken in Antwerp, Belgium. CSF of additional 64 controls individuals (AAI 68.05 ± 5.97), used in this study for comparison purposes, were available at the Reference Centre for Biological Markers of Dementia (BIODEM), Department of Biomedical Sciences, University of Antwerp. These were primarily community-dwelling volunteers enrolled in the BACEi program (ClinicalTrials.gov identifiers: 54861911ALZ1005 (NCT01978548); 54861911ALZ2002 (NCT02260674)) by Janssen Pharmaceutica NV, Beerse, Belgium [33]. Subjective memory complaints, neurologic or psychiatric antecedents, were ruled out by means of an interview. Cognitive screening was performed using the mini-mental state examination (cut-off score > 26). The Clinical Dementia Rating Scale (CDR) score was 0, and AD CSF biomarkers were within the normal range. Genetic screening in these 64 individuals was not performed due to the unavailability of DNA. All research participants or their legal guardian provided written informed consent for participation in genetic and clinical studies. Clinical study protocols and informed consent forms for patient ascertainment were approved by the local medical ethics committees of the collaborating medical centres in Belgium. Genetic study protocols and informed consent forms were approved by the ethics committees of the University Hospital of Antwerp and the University of Antwerp, Belgium.

Mutation screening

Genomic DNA of patients and control individuals was analysed for mutations in *APP*, *PSEN1* and *PSEN2* using a targeted re-sequencing gene-panel as we previously described [34]. We selected non-synonymous variants with a minor allele frequency (MAF) < 1%, including exon-intron boundaries for possible variants affecting splicing. The allelic frequencies of these variants, observed in the patient group, were assessed in the control cohort and Genome Aggregation database (gnomAD, lastly accessed in January 2020) [35]. In addition, *PSEN1* rs17125721 A > G, p.E318G variant (MAF 1.927%, gnomAD), considered a possible risk modifier (https://www.alzforum.org/mutations/psen1-e318g) and Apolipoprotein E (*APOE*) genotypes were analysed. Variants with a read depth below 20X, with Genoqual value below 99 and with an imbalanced wild-type/variant read depth (cut-off > 3) were considered false positives. All the selected variants were validated by Sanger sequencing (BigDye Terminator Cycle sequencing kit v3.1) on the ABI 3730 DNA Analyser (both Applied Biosystems). Sequences were analysed using SeqManII or novoSNP software packages [36]. To screen for *APP* locus duplications, multiplex amplicon quantification (MAQ) was used on 280 EOAD patients, as previously described [37]. Briefly, multiplex PCR amplification of 5 target and 11 reference amplicons was performed using fluorescently labelled primers. The amplification products were size separated on ABI 3730 automatic sequencer using GeneScan-500 LIZ (Applied Biosystems) as internal size standard. Data analysis was performed using the MAQ software (MAQs) package (www.vibgeneticservicefacility.be). One sample carrying the *APP* duplication and four different control samples were used as positive and negative controls, respectively.

CSF biomarker analysis

CSF were available for a subset of 38 mutation carriers: 18 carriers of pathogenic and VUS mutations (MAF < 1%) (AAO 71.58 ± 11.24 years, range 56–85 years) and 20 carriers of *PSEN1* p.E318G variant (AAO 78.09 ± 6.62 years, range 62–87 years) as well as 64 control individuals (AAI 68.05 ± 5.97, cfr. "Study population").

Lumbar puncture, CSF sampling and handling have been performed according to standard protocols [12]. Samples were stored at − 80 °C until analysis. CSF concentrations of $A\beta_{1-42}$ and $A\beta_{1-40}$ were measured using enzyme-linked immunosorbent assay (ELISA) with commercially available single parameter ELISA kits at the BIODEM laboratory as previously described [38]. $A\beta_{1-43}$ levels were measured using the amyloid beta (1–43) (FL) Aβ kit (IBL) and both sAPPα and sAPPβ using sAPPα/sAPPβ kit (Meso Scale Discovery). Concerning T-tau and P-tau181 levels, these were previously measured using hTAU Ag and PHOSPHO-TAU(181P) (INNOTEST), respectively, and the BIODEM laboratory performed the ELISA for the 38 mutation carriers and the Sahlgrenska University Hospital (Sweden) for the 64 control individuals. For this reason, we did not perform statistical analysis on either T-tau or P-tau181 amongst the groups, but we only reported the values of the measurements, which are part of the phenotypic characterization of the cohort. All calibration standards and CSF samples were analysed in duplicate. Only mean values with a coefficient of variation (CV) of the replicates less than or equal to 20% were included in the analysis.

Statistical analyses

Statistical analyses were performed in SPSS version 24, and graphs were made using GraphPad Prism8. A logistic regression analysis was performed in the AD and control cohorts, to determine whether patients had higher probability than controls to carry both the common *PSEN1* p.E318G and *APOE* ε4 genotype. With a similar model, we further tested the prevalence of *APOE* ε4 between EOAD and LOAD cohorts and of *APOE* ε4ε4 between EOAD and LOAD cohorts.

For the CSF biomarker analysis, the mutation carriers were divided in three different groups: carriers of known pathogenic mutations, of VUS and of *PSEN1* p.E318G. First, a Kolmogorov-Smirnov test was performed to check for normal distribution. Since most variables did not follow a normal distribution, non-parametric tests were used. Differences amongst groups were tested using Kruskal-Wallis. Post hoc analysis with pairwise comparisons was carried out (adjusted p value < 0.05). To identify whether an association between two markers was present, the Spearman's rho correlation tests were performed. Correlation coefficients were extracted from each group (controls, known pathogenic, VUS, *PSEN1* p.E318G) separately. After correction for multiple testing (Bonferroni's correction), p values < 0.005 or below were considered to be statistically significant. Receiver operating characteristic (ROC) curves were additionally performed to assess the diagnostic accuracy of the individual biomarkers, and the area under each ROC curve (AUC) was calculated. Finally, explanatory cut-offs were identified as the concentration of specific biomarker that maximizes sensitivity and specificity of the test (Youden's index).

Transcript analysis

To inhibit non-sense-mediated mRNA decay (NMD), 1×10^6 lymphoblast cells of the double mutation carrier (patient 16; *PSEN1* p.G183V, *PSEN1* p.P49L), of the single carrier (sibling of patient 16; *PSEN1* p.G183V) and of 4 non-carriers as negative controls were incubated with 150 mg/mL cycloheximide (CHX) (Sigma-Aldrich) at 37 °C for 4 h. To generate cDNA, total RNA was extracted using the RiboPureTM kit (Life Technologies) followed by a DNase treatment (TURBODNase Kit, Invitrogen). First-strand complementary DNA was synthesized using the SuperScript III First-Strand Synthesis System (Life Technologies) using random hexamer primers. Full-length *PSEN1 t*ranscript sequencing was subsequently performed on a MinION sequencing platform (Oxford Nanopore Technologies (ONT)) based on a modernized cDNA sequencing protocol [39]. Briefly, cDNA amplification of *PSEN1* full transcript was carried out with Platinum® Taq DNA Polymerase (Clontech Laboratories) using exonic primers, designed with Primer3Plus [40], with a 5′ ONT adapter. In a second PCR round, using PCR Barcoding Kit 96 (EXP-PBC096; ONT), sample-specific barcodes were added. Samples were pooled equimolar and processed according to ONT SQK-LSK109 library preparation. Sequencing was performed on a Mk1 MinION (MIN-101B), using FLO-MIN106 flow cells. Base calling and barcode de-multiplexing were performed with Albacore (v2.2.5). Sequencing reads were subsequently aligned using minimap2 [41], with splice-aware parameters. Only full-length *PSEN1* spanning sequencing reads were retained for further analysis. Relative quantifications of splice junctions were calculated by dividing the number of junction-supporting reads by the total number of reads spanning the *PSEN1* transcript in R (R Core Team, 2017).

Results

Mutation screening

The genetic screening identified a total of 41 mutations in 54 individuals (41/1431, 2.86% AD, 13/809, 1.60% controls). The identified variants are listed in Table S1 (Additional file). Fourteen were known pathogenic mutations (4 in *APP*, 10 in *PSEN1*) (www.alzforum.org/mutations, lastly accessed in January 2020) and were identified in 19 patients (19/1431, 1.33%) and 1 control individual (1/809, 0.12%). Specifically, 1.07% (3/280) of EOAD patients carried known pathogenic mutations in *APP* and 4.6% (13/280) in *PSEN1*. Furthermore, in 0.086% (1/1151) and 0.26% (3/1151) of LOAD patients, known pathogenic mutations were identified in *APP* and *PSEN1*, respectively.

In addition, 12 previously reported variants with unclear pathogenicity (5 in *APP*, 2 *PSEN1*, 5 *PSEN2*) were detected in 12 patients (12/1431, 0.84%) and 7 controls (7/809, 0.86%). Three were known benign variants (2 *APP*, 1 *PSEN2*) found in 7 patients (7/1431, 0.49%) and 2 controls (2/809, 0.24%). Lastly, 11 rare variants were novel (5 in *APP*, 1 in *PSEN1*, 5 in *PSEN2*) absent from gnomAD and mutation databases, observed in 8 patients (8/1431, 0.56%) and 3 control individuals (4/809, 0.49%). There was no significant difference between the prevalence of *APOE* ε4 in EOAD (57%) and LOAD (55%) ($p = 0.48$). The prevalence of *APOE* ε4 was higher in EOAD (60%) than LOAD (55%) only on individuals without any *APP* or *PSENs* mutations, but this difference was still not significant ($p = 0.15$). We found a significant difference in the prevalence of *APOE* ε4ε4 between EOAD (20%) and LOAD (9%) when considering all individuals ($p < 0.001$). This difference in the prevalence of *APOE* ε4ε4 (EOAD 20%; LOAD 9%) was similar when running the same model only on individuals without any *APP* or *PSENs* mutations ($p < 0.001$).

APOE genotypes of the mutation carriers are listed in Table S1 (Additional file). Amongst the patients, two were double mutation carriers: patient 16, carrying both *PSEN1* p.G183V and *PSEN1* p.P49L, with LOAD pathology (Figure S1, Additional file), and patient 11, carrying both *APP* p.G625_S628del and *PSEN1* p.P355S. The risk variant *PSEN1* p.E318G was detected in 45 patients (45/1431, 3.14%) and 24 controls (24/809, 2.96%). No significant association was found between *PSEN1* p.E318G and AD, regardless of *APOE* ε4 genotype ($p = 0.29$ and $p = 0.51$, respectively). Furthermore, in the screened cohort, *APP* locus duplications were not detected.

CSF biomarker analysis

The CSF biomarkers were analysed comparing three groups of mutation carriers: (1) known pathogenic, (2) VUS and (3) *PSEN1* p.E318G to control individuals. A significant reduction of CSF $A\beta_{1-43}$ levels was detected in all three carrier groups (all $p < 0.05$; Table 1; Fig. 1). For each carrier of a known pathogenic mutation or a VUS, $A\beta_{1-43}$ levels are shown in the bar plot (Fig. 2). Similarly, $A\beta_{1-42}$ CSF levels were significantly reduced in all three carrier groups (all $p < 0.05$; Table 1; Fig. 3). Comparison of CSF $A\beta_{1-40}$ levels did not show a significant difference amongst all groups ($p = 0.66$).

The $A\beta_{1-43}/A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratios were significantly reduced in all three carrier groups (all $p < 0.05$, Table 1; Figs. 1, 2 and 3). $A\beta_{1-43}/A\beta_{1-42}$ ratio was significantly reduced in the VUS and *PSEN1* p.E318G carrier groups (both $p < 0.001$) (Fig. 1). $A\beta_{1-43}$ significantly correlated with $A\beta_{1-42}$ in all four groups ($r > 0.87$, all $p < 0.001$). $A\beta_{1-43}$ and $A\beta_{1-42}$ were significantly correlated with $A\beta_{1-40}$ in the controls and *PSEN1* p.E318G group (all $r > 0.63$, all $p < 0.001$), while there was a correlation between $A\beta_{1-42}$ with $A\beta_{1-40}$ in the VUS group, but not significant after Bonferroni's correction ($r = 0.65$, $p = 0.015$). Both CSF $A\beta_{1-43}$ and $A\beta_{1-42}$ were able to differentiate the corresponding controls from the group of known pathogenic and *PSEN1* p.E318G (AUCs > 0.9; Table S2, Additional file), while in the VUS group the differentiation was slightly less efficient (AUCs 0.84–0.89; Table S2, Additional file).

CSF sAPPα and sAPPβ levels were significantly reduced in both VUS (both $p < 0.05$) and *PSEN1* p.E318G carriers (both $p < 0.04$) (Table 1; Fig. 4).

$A\beta_{1-43}$ and $A\beta_{1-42}$ showed a positive correlation with both sAPPα and sAPPβ only in the control group (all $r > 0.48$, all $p < 0.001$). $A\beta_{1-40}$ correlated with sAPPα only in the control group ($r = 0.6$, $p < 0.001$) and with

Table 1 Significance levels (adjusted *p* values) of the markers compared amongst groups

	Pathogenic vs *controls*			*VUS* vs *controls*			*p.E318G* vs *controls*		
	Est.	***St.***	***p* value**	**Est.**	***St.***	***p* value**	**Est.**	***St.***	***p* value**
$A\beta_{1-43}$	39.93	2.90	**0.037**	37.36	4.15	**< 0.001**	45.43	5.99	**< 0.001**
$A\beta_{1-43}/A\beta_{1-40}$	42.92	3.12	**0.018**	40.08	4.45	**< 0.001**	46.97	6.20	**< 0.001**
$A\beta_{1-43}/A\beta_{1-42}$	28.45	2.07	0.384	37.37	4.15	**< 0.001**	44.85	5.91	**< 0.001**
$A\beta_{1-42}$	40.32	2.93	**0.033**	32.33	3.59	**0.003**	41.42	5.46	**< 0.001**
$A\beta_{1-42}/A\beta_{1-40}$	43.52	3.14	**0.015**	33.59	3.73	**0.002**	43.87	5.79	**< 0.001**
sAPPα	12.54	0.91	1.0	31.04	3.45	**0.006**	26.90	3.55	**0.004**
sAPPβ	14.06	1.02	1.0	31.32	3.48	**0.005**	21.87	2.89	**0.039**
$A\beta_{1-43}$/sAPPα	37.44	2.73	0.064	21.18	2.35	0.186	38.79	5.12	**< 0.001**
$A\beta_{1-43}$/sAPPβ	36.31	2.64	0.082	21.49	2.39	0.169	41.25	5.44	**< 0.001**

Kruskal-Wallis was performed to assess differences amongst groups. Significance level $p < 0.05$ (adjusted *p* values). *Abbreviations*: *Est.* test estimate, *St.* standard test statistic

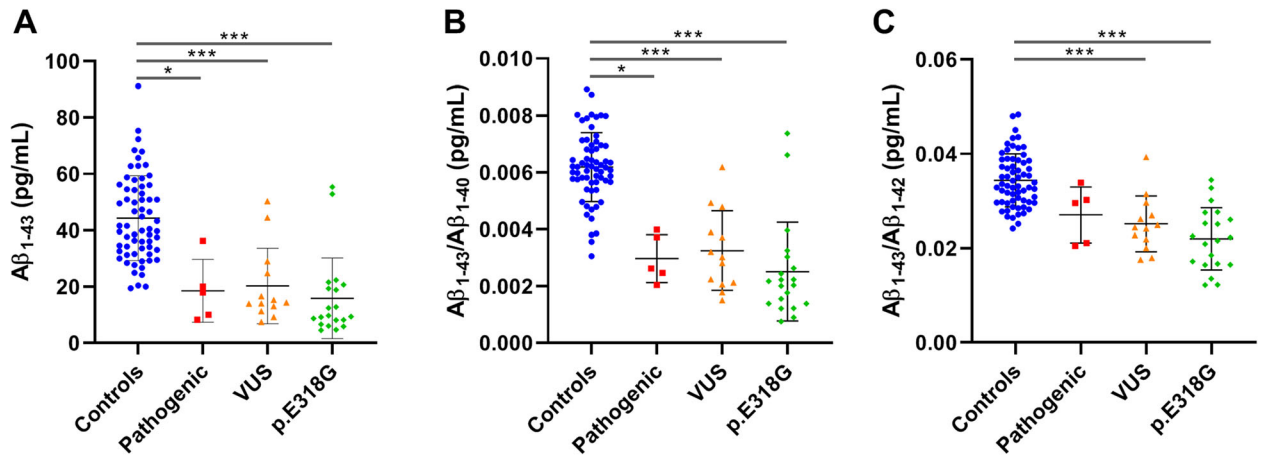

Fig. 1 CSF levels of $A\beta_{1-43}$, $A\beta_{1-43}/A\beta_{1-40}$ and $A\beta_{1-43}/A\beta_{1-42}$ ratios in the three mutation carrier groups compared to controls. Scatter plots show $A\beta_{1-43}$ (**a**), $A\beta_{1-43}/A\beta_{1-40}$ (**b**) and $A\beta_{1-43}/A\beta_{1-42}$ (**c**) CSF levels in controls and in carriers of known pathogenic mutations, of VUS and of *PSEN1* p.E318G. Values of mean ± SD are given. *p* value indicators correspond to the values assessed with Kruskal-Wallis: $*p < 0.05$, $***p < 0.0001$

sAPPβ in the control ($r = 0.65$, $p < 0.001$) and VUS groups ($r = 0.75$, $p = 0.003$). $A\beta_{1-43}$/sAPPα and $A\beta_{1-43}$/sAPPβ CSF levels were significantly lower in *PSEN1* p.E318G carriers compared to controls ($p < 0.001$) (Fig. 4). $A\beta_{1-43}$/sAPPα and $A\beta_{1-43}$/sAPPβ CSF levels also showed a trend to be decreased in carriers of the pathogenic mutations, although this difference was not statistically significant ($p = 0.06$ and $p = 0.08$, respectively) (Table 1; Fig. 4). The AUCs for both sAPPα and sAPPβ were low (AUCs = 0.625; Table S2, Additional file). CSF levels for each marker ($A\beta_{1-43}$, $A\beta_{1-42}$, $A\beta_{1-40}$, sAPPα, sAPPβ) are summarised in Table 2. ROC curve analysis for all biomarkers are listed in Table S2 (Additional file), and the AUC curves are shown in Figures S2-S3 (Additional file).

PSEN1 full transcript analysis

The effect of the *PSEN1* p.G183V and *PSEN1* p.P49L double mutation on PSEN1 alternative transcript generation in patient-derived RNA was examined with ONT MinION sequencing, because of a previously reported effect of this mutation on exon skipping in both

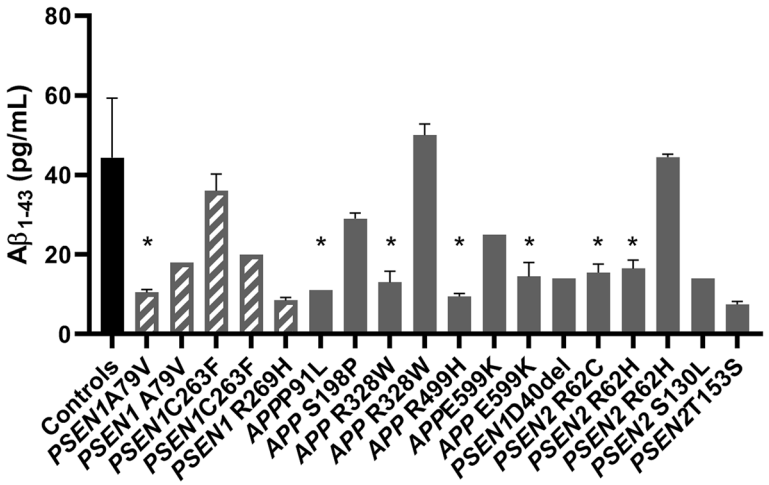

Fig. 2 $A\beta_{1-43}$ CSF levels in carriers of known pathogenic mutations or VUS compared with the control group. The CSF levels of $A\beta_{1-43}$ for each carrier of a known pathogenic mutation (in stripes) or a VUS (in gray) are shown in the bar plots together with the control group (in black). Error bars indicate the SD of the duplicate measurements for the mutation carriers and the average of the values for the controls. The asterisks (*) indicate the carriers of one *APOE* ε4 allele

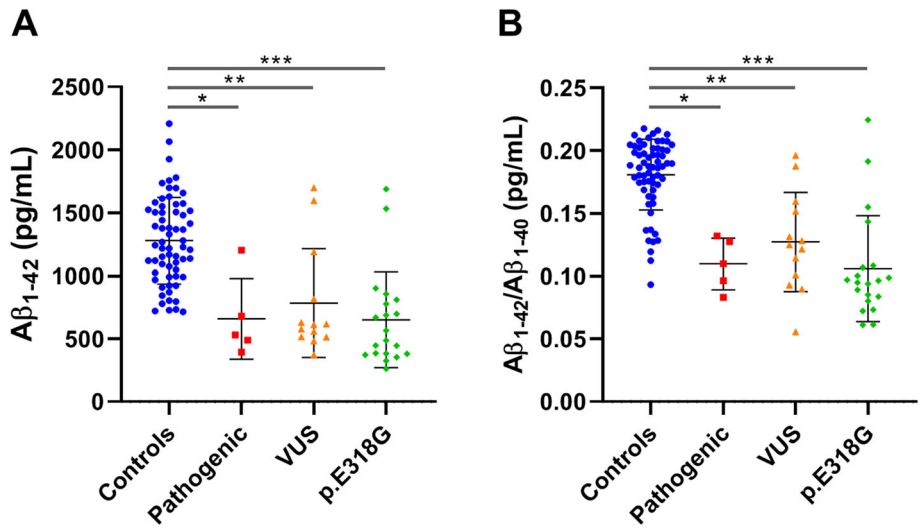

Fig. 3 CSF levels of $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio in the three mutation carrier groups compared to controls. Scatter plots show $A\beta_{1-42}$ (**a**) and $A\beta_{1-42}/A\beta_{1-40}$ (**b**) CSF levels in controls and in carriers of known pathogenic mutations, of VUS and of *PSEN1* p.E318G. Values of mean ± SD are given. *p* value indicators correspond to the values assessed with Kruskal-Wallis: *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$

HEK293 cells [42] and mice brain [43]. Both the double mutation carrier (patient 16; *PSEN1* p.G183V, *PSEN1* p.P49L) and the single *PSEN1* p.G183V carrier show the skipping of exon 6 in 20% of the sequencing reads from the cDNA obtained from the lymphoblast cells treated with CHX and 5% from the untreated (Fig. 5). Exons 6–7 skipping was also detected, but it was a rarer event (Figure S4, Additional file). The analysis of the transcript surrounding the *PSEN1* p.P49L variants did not show splicing alterations (data not shown).

Discussion

Mutation screening

The *APP*, *PSEN1* and *PSEN2* mutation screening in 1431 AD patients and 809 control individuals identified known pathogenic mutations in 5.7% of EOAD (16/280) and 0.3% of LOAD (4/1151) patients and in 0.12% (1/809) of the controls, in line with what it is usually observed [4, 5]. The carrier of the *APP* p.A713T mutation considered with unclear pathogenicity but actually showing segregation with the disease in another study [44] was asymptomatic at the age of inclusion in our cohort (age > 65 years) [34]. This *APP* mutation is known to have a wide onset age range; therefore, it is not surprising to identify asymptomatic carriers [34, 44]. Amongst the known pathogenic mutations, two were also found in AD patients with more than 65 years of onset: the known pathogenic *PSEN1* p.C263F, identified in five patients with onset age range of 53–70, and the *PSEN1* p.G183V, previously identified in a patient with frontotemporal dementia and Pick-type tauopathy [42] (further discussed later in the text). These findings highlight the importance to screen the causal AD genes also in LOAD patients [5]. The genetic screening identified also two double mutation carriers: patient 11 carrying the *APP* p.G625_S628del and the *PSEN1* p.P355S, both VUS, and patient 16 having the *PSEN1* p.G183V and the novel p.P49L.

PSEN1 p.G183V and p.P49L mutation carrier

Patient 16 received a diagnosis of probable AD (age at onset > 65). Clinically, patient 16 showed amnestic presentation without other remarkable signs or symptoms. Single-photon emission computed tomography (SPECT) displayed a moderate hypoperfusion of the bilateral parietal, temporal and frontal lobe, compatible with AD. Magnetic resonance imaging (MRI) showed age-related atrophy and multiple supratentorial lacunary infarcts. Neuropathological examination showed a mild atrophy of the frontotemporal gyri (Figure S1, Additional file), but no FTD symptoms were reported in patient 16. The patient was pathologically diagnosed with definite AD (Montine stage A3B3C3), with an accent on the neurofibrillary pathology (Figure S1). The sibling of patient 16 also carried the *PSEN1* p.G183V, but not the *PSEN1* p.P49L, and was diagnosed with probable AD. Both *PSEN1* mutations therefore segregated independently in the family [45]. The *PSEN1* p.G183V was previously identified in a patient diagnosed with frontotemporal dementia (FTD) and Pick-type tauopathy [42]. The brain lesions in patient 16 were however different from those of the published FTD patient, where severe frontotemporal atrophy and Pick-like pathology were described [42]. There were no intranuclear neuronal inclusions in patient 16 and no signs of Pick's disease. Thus, the Pick's

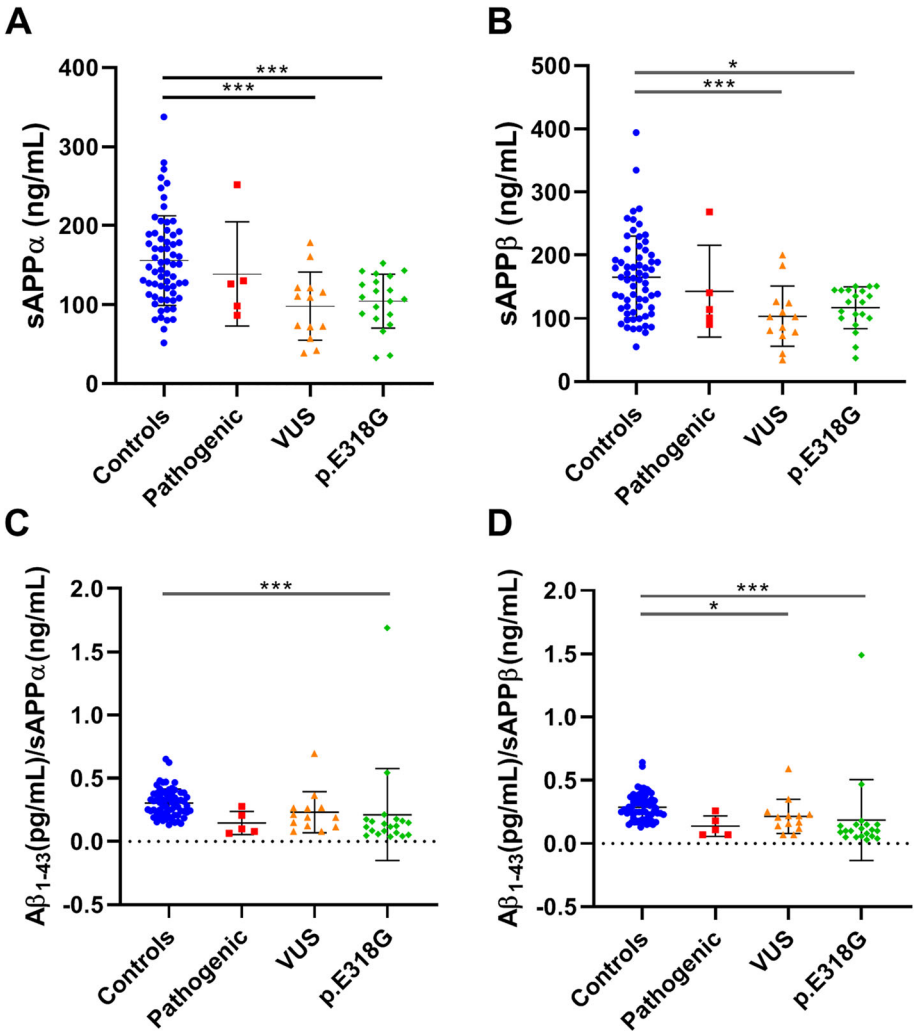

Fig. 4 CSF levels of sAPPα, sAPPβ, $Aβ_{1–43}$/sAPPα and $Aβ_{1–43}$/sAPPβ ratios in the three mutation carrier groups compared to controls. Scatter plots show sAPPα (**a**), sAPPβ (**b**), $Aβ_{1–43}$/sAPPα (**c**) and $Aβ_{1–43}$/sAPPβ (**d**) CSF levels in controls and in carriers of known pathogenic mutations, of VUS and of *PSEN*1 p.E318G. Values of mean ± SD are given. *p* value indicators correspond to the values assessed with Kruskal-Wallis: *$p < 0.05$, ***$p < 0.0001$

pathology detected in the FTD patient [42] is probably independent of this *PSEN1* p.G183V mutation. The *PSEN1* p.G183V is located in the last nucleotide of the exonic splice donor site of exon 6, and experiments in HEK293 cells and mice showed the formation of alternative transcripts with skipping of exons 6 and exon 6–7, which are likely degraded by the non-sense-mediated mRNA decay control mechanism (NMD) [42, 43]. Our PSEN1 transcript analysis with ONT minION sequencing, in patient-derived biomaterials, confirmed that *PSEN1* p.G183V led to the formation of transcripts lacking exons 6 and 6–7, which are indeed degraded by NMD. Only a small amount of these alternative transcripts remains in the cells (≤ 5%) and they unlikely interfere with the wild-type *PSEN1*. The presence of the second *PSEN1* mutation (p.P49L) could drive the disease in patient 16.

Reduced $Aβ_{1–43}$ CSF levels in *APP* and *PSENs* mutation carriers

CSF of 38 *APP*, *PSEN1* and *PSEN2* mutation carriers, both known pathogenic and VUS, was available and used to investigate for the first time the CSF $Aβ_{1–43}$ levels. A significant reduction of CSF $Aβ_{1–43}$ levels was observed in carriers of pathogenic mutations (13/18), of VUS (9/13) and of carriers of *PSEN1* p.E318G (18/20) compared to controls. This reduction was comparable with CSF $Aβ_{1–42}$ levels. CSF $Aβ_{1–43}$ levels positively correlated with $Aβ_{1–42}$ in all mutation carrier groups but correlated with $Aβ_{1–40}$ only in *PSEN1* p.E318G mutation carriers and controls. $Aβ_{1–43}$/$Aβ_{1–40}$ ratio was significantly reduced in all three mutation carrier groups, while $Aβ_{1–43}$/$Aβ_{1–42}$ ratio only in the VUS and *PSEN1* p.E318G groups, as previously shown for AD patients (independently from the mutation status) [9].

Table 2 Summary of CSF marker levels for each mutation carrier

	Individual identifier	Mutation	*APOE*	$A\beta_{1-43}$	$A\beta_{1-42}$	$A\beta_{1-40}$	sAPPα	sAPPβ	T-tau	P-tau181
P	**Patient 12**	*PSEN1* p.A79V	34	**10**	**490**	**3836**	**99**	**90**	NA	NA
	Patient 13	*PSEN1* p.A79V	33	**18**	**531**	**4833**	**87**	**101**	959	136
	Patient 17	*PSEN1* p.C263F	33	**20**	**679**	8165	252	268	458	73
	Patient 21	*PSEN1* p.C263F	33	36	1202	9117	**130**	**141**	281	55
	Patient 24	*PSEN1* p.R269H	33	**8**	**393**	**4063**	**127**	**114**	640	77
V	Patient 10	*APP* p.P91L	34	**11**	**516**	**4026**	**42**	**44**	287	44
	Control 3	*APP* p.S198P	33	29	1190	7838	**111**	**125**	248	47
	Patient 5	*APP* p.R328W	33	50	1598	8134	**72**	**86**	371	76
	Control 4	*APP* p.R328W	34	**13**	**483**	8679	161	184	NA	NA
	Patient 6	*APP* p.R499H	34	**9**	**513**	**4486**	**74**	**78**	440	51
	Patient 7	*APP* p.E599K	34	**14**	**816**	8065	179	200	> 1200	225
	Patient 8	*APP* p.E599K	33	25	**630**	5184	**116**	**110**	633	104
	Patient 29	*PSEN1* p.D40del	33	**14**	**618**	6641	**122**	**103**	615	82
	Patient 31	*PSEN2* p.R62C	34	**15**	**613**	6823	**57**	**73**	NA	NA
	Patient 32	*PSEN2* p.R62H	33	44	1699	9048	**122**	**127**	407	59
	Patient 34	*PSEN2* p.R62H	34	**17**	**561**	**4269**	**110**	**103**	783	82
	Patient 36	*PSEN2* p.S130L	33	**14**	**578**	**4631**	**73**	**81**	340	54
	Patient 40	*PSEN2* p.T153S	33	**7**	**373**	**2340**	**39**	**34**	212	32

List of carriers of *APP* and *PSENs* known pathogenic (P) and VUS (V) mutations with CSF available. Notes: all carriers had AD diagnosis except control 3 and control 4, who were controls at the moment of inclusion. Control 4 developed vascular dementia at follow-up (AAO 75 years). Patients in bold are EOAD patients (AAO range 56–65 years). Values for $A\beta_{1-43}$, T-tau and P-tau181 are in pg/mL. Underlined values and values in bold, for $A\beta_{1-43}$, $A\beta_{1-42}$, $A\beta_{1-40}$, sAPPα and sAPPβ, are respectively higher and lower levels of the markers based on the exploratory cut-offs (Table S2, Additional file). Normal cut-offs: T-tau < 297 pg/mL; P-tau181 < 57 pg/mL

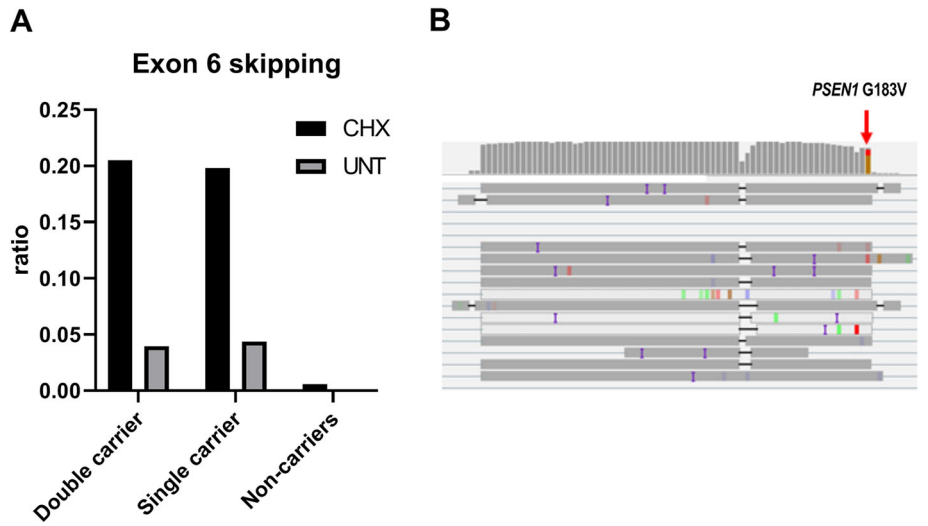

Fig. 5 Transcript analysis of PSEN1 in patient 16. The bar graph shows the relative quantifications of exon 6 in the double carrier (patient 16, *PSEN1* p.G183V, p.P49L), single carrier (*PSEN1* p.G183V) and non-carrier lymphoblast cells CHX treated (CHX) and untreated (UNT). Relative quantifications of splice junctions were calculated by dividing the number of junction-supporting reads by the total number of reads spanning the PSEN1 transcript. The quantifications for both CHX and UNT of the non-carriers are reported as averages (values of SD for CHX ± 0.001052869 and for UNT ± 0.000671837) (**a**). Visualization of the PSEN1 exon 6 cDNA MinION reads from Integrative Genomic Viewer software (IGV). Sequencing reads of PSEN1 cDNA of the double and single carriers confirm exon 6 skipping due to the *PSEN1* p.G183V mutation (**b**)

While longer Aβ peptides (e.g. $Aβ_{1–42}$ and $Aβ_{1–43}$) promote aggregation and neurotoxicity, $Aβ_{1–40}$ appears to act protectively [46]. Moreover, CSF $Aβ_{1–40}$ levels did not differ significantly between AD patients and controls in a meta-analysis [19]. Therefore, we speculated that the positive correlation of $Aβ_{1–43}$ with $Aβ_{1–42}$, but not with $Aβ_{1–40}$ (except in *PSEN1* p.E318G carriers), further supports the involvement of $Aβ_{1–43}$ in AD. It is however still unclear why $Aβ_{1–40}$ would be unaltered and lacking of correlation with $Aβ_{1–43}$ in the mutation carrier groups, as it is related to the process of $Aβ_{1–43}$. It is plausible that a possible decrease of $Aβ_{1–40}$ in CSF would only be visible or measurable during the earlier stages of the disease when mostly all neurons are still intact, rather than in later stages (when it is normally measured), when Aβ production is higher but less neurons are preserved. Alternatively, it is possible that the cleavage of the C99 fragment by γ-secretase may be heavily impaired at the third cleavage step when $Aβ_{1–40}$ is generated from $Aβ_{1–43}$, as suggested by Kakuda et al. [47] for the pathogenic *PSEN1* p.R278I, which would result in negligible levels of $Aβ_{1–40}$ and unusually high levels of $Aβ_{1–43}$. Furthermore, a recent study found a correlation between Aβ peptide length and plaque load ($Aβ_{1–43} > Aβ_{1–42} > Aβ_{1–40}$), indicating that longer Aβ peptides have an increased tendency towards accumulation in the brain [23], thus explaining their lower CSF levels. Further analysis of the shorter Aβ species $Aβ_{1–41}$, $Aβ_{1–37}$ (from $Aβ_{1–43}$) $Aβ_{1–38}$ (from $Aβ_{1–42}$) and $Aβ_{1–34}$ (from either $Aβ_{1–43}$ or $Aβ_{1–42}$) are however needed to better clarify the role of longer Aβ peptides in AD and the pathogenic events linked to Aβ process, degradation and clearance in the presence of *APP* and *PSENs* mutations [9–11].

The production of longer Aβ peptides (> $Aβ_{1–42}$), including $Aβ_{1–43}$, in the pathogenesis of AD is receiving increased attention [23, 25, 26, 29, 48]. Studies showed that the *PSEN1* p.L435F produces primarily $Aβ_{1–43}$, which was detected in the brain plaques [49] and induced pluripotent stem cell (iPSC) neurons [50] of patients carrying this mutation. Another work suggested that the *APP* mutations affect the endopeptidase activity of γ-secretase, leading to $Aβ_{1–42}$ formation, while *PSEN1* mutations inhibit its carboxypeptidase activity, releasing multiple longer peptides including $Aβ_{1–42}$ and $Aβ_{1–43}$ [25]. Unfortunately, the number of mutations we investigated was not sufficient to make similar observations.

Based on the CSF biomarker analyses performed, some of the *PSENs* VUS mutations analysed showed biomarker levels comparable to the known pathogenic mutations: for instance, the *PSEN1* p.40del, which was described for the first time in an EOAD patient with prominent frontal features and no family history of dementia [51]. This variant was considered "most likely benign" or causing a small increase in risk based on its frequency in gnomAD [52]. However, an in vitro assay, testing the mutant *PSEN1* ability to cleave the APP-C99 fragment, revealed a robust decrease in $Aβ_{1–42}$ production and undetectable levels of $Aβ_{1–40}$ [8]. Our data is in favour of an involvement of *PSEN1* p.40del in Aβ alteration, showing decreased CSF levels of both $Aβ_{1–43}$ and $Aβ_{1–42}$ and unaltered $Aβ_{1–40}$ levels in the patient carrier, and also in line with a recent study showing increased $Aβ_{1–42}$ and unaltered $Aβ_{1–40}$ in the medium collected from mice neuro-2A cells transfected with the plasmid containing the *PSEN1* p.40del mutation [53]. The *PSEN2* p.S130L variant was first reported in an Italian family, but segregation with disease could not be determined [54]. Another study described a carrier of this variant who had an autopsy-confirmed AD diagnosis [55]. The pathogenicity of *PSEN2* p.S130L is currently unclear, since it did not affect $Aβ_{1–42}$ levels or $Aβ_{1–42}/Aβ_{1–40}$ ratio in in vitro experiments [56]. According to an algorithm proposed by Guerreiro et al. [57], this variant has been considered "probably pathogenic." In line with this classification, we found a significant reduction of both $Aβ_{1–43}/Aβ_{1–40}$ and $Aβ_{1–42}/Aβ_{1–40}$ ratios in the patient carrier, pointing out to a possible pathogenicity of *PSEN2* p.S130L. Finally, the carrier of the novel *PSEN2* p.T153S variant also had reduced CSF levels of $Aβ_{1–43}$ and $Aβ_{1–42}$, comparable to the known pathogenic mutations. In light of these data, we could consider those mutations as "likely pathogenic", since they seem to affect or interfere with Aβ production. Furthermore, it is important to note that the three mutation carriers were all *APOE* ε4 negative, meaning that the detected Aβ alteration is likely due to the mutations themselves without an effect of *APOE*. For the *APP* variants, instead, we could not drive conclusions on the variants pathogenicity. In fact, all the *APP* variants analysed were located outside the pathogenic exons 16–17, encoding for the Aβ domain. Furthermore, two carriers of *APP* variants were considered controls at the moment of inclusion: control 3 carried the *APP* p.S198P variant and CSF analysis did not reveal Aβ alterations; control 4, carrying the *APP* p.R328W and having altered CSF marker levels, developed vascular dementia at follow-up. Of note, control 3 but not control 4 was *APOE* ε4 negative. We would like to stress the importance to further investigate a possible interference of the *APP* variants outside the Aβ domain in AD. There was variability of $Aβ_{1–43}$ CSF levels between the carriers of the same mutations (*PSEN1* p.A79V, *PSEN1* C263F, *APP* R328W, *APP* E599K, *PSEN2* R62H, Fig. 2; Table 2). We initially hypothesized an effect of *APOE* ε4 on the decreased $Aβ_{1–43}$ CSF levels as already demonstrated for Aβ [58]. This seemed a valid hypothesis for all mutations but one (*PSEN1* p.C263F, Fig. 2; Table 2). However, a recent study showed a reduced influence of *APOE* on $Aβ_{1–43}$ aggregation in cerebrovascular cells [48].

$A\beta_{1-43}$ CSF levels in *PSEN1* p.E318G carriers

To better understand the role of *PSEN1* p.E318G on AD pathology, we investigated the CSF $A\beta_{1-43}$ levels in carriers, detecting a reduction. This *PSEN1* variant is suggested to be an AD risk modifier as it was associated with high levels of P-tau181P and T-tau [59, 60]. Moreover, carriers of both *APOE* ε4 and *PSEN1* p.E318G variant were reported to have a higher risk of developing LOAD than *APOE* ε4 carriers without the *PSEN1* variant [59, 60]. In our study, however, the *PSEN1* p.E318G variant did not show an association with AD regardless of *APOE* ε4 genotype, which is in line with the study of Hippen et al. [61]. A recent analysis of CSF $A\beta_{1-42}$, T-tau and P-tau181 in asymptomatic p.E318G carriers of a large LOAD Italian family did not reveal any changes in CSF markers [62]. The levels of $A\beta_{1-43}$ were not measured and therefore not allowing a direct comparison.

Soluble species sAPPα and sAPPβ

We also detected reduced levels of sAPPα and sAPPβ in the three carrier groups analysed compared to controls, but statistical significance was not detected in the group of the known pathogenic mutations. Our results are different from other studies where no difference in sAPPα and sAPPβ levels was observed between patients and controls [19]. Of note, in these studies, the mutation status of the patients was not described [19].

We report that both sAPPα and sAPPβ positively correlated with $A\beta_{1-43}$ and $A\beta_{1-42}$ only in the control group but not in the mutation carriers. This result is in line with another study where the correlation between $A\beta_{1-42}$ and sAPPα or sAPPβ in AD patients was not significant [63]. We cannot explain why both sAPPα and sAPPβ levels would be reduced in the mutation carriers and correlate with the control group. In fact, sAPPα and sAPPβ should not be affected by the presence of AD mutations, which affect γ-secretase activity, while sAPPα and sAPPβ are respectively generated by α- and β-secretases in the earlier steps, before Aβ production. However, the data of CSF sAPPα and sAPPβ levels in AD are unclear [19] and there are no studies so far that have measured CSF sAPPα and sAPPβ in AD mutation carriers. The possible involvement of sAPP fragments in AD is still not fully understood [18], and the interpretation of the results remains therefore challenging. It is suggested that sAPPα enhances neurogenesis and has a neuro-protective role, and it is involved in neurotransmission and synaptic plasticity [64]. A recent study, however, showed that all fragments of sAPP acted as agonists of a specific GABA receptor (GABAB-R1a) and would therefore explain their role in neurotransmission and plasticity [65]. In fact, a fragment derived from sAPPα inhibited synaptic transmission in mouse hippocampus. Additional studies in carriers of AD mutations are essential to fully understand the involvement of sAPP in AD.

Limitations

A potential limitation of this study is the small cohort size used to analyse $A\beta_{1-43}$ and the other markers in CSF of *APP* and *PSENs* mutation carriers, despite the genetic screening was performed in a relatively large AD population. As we know, *APP* and *PSENs* mutation carriers are extremely rare [4], as well as the availability of their biomaterials. Therefore, a replication study was not performed. The availability of a larger control cohort allowed us to reach satistical significance when analysing $A\beta_{1-43}$ CSF levels. Unfortunately, age at onset, gender and *APOE* genotype could not be used as covariates in the analysis, because the number of mutation carriers was not sufficient for the statistical test. Our pilot study is nevertheless relevant because it highlights the involvement of $A\beta_{1-43}$ in AD and adds missing information regarding specific mutations to very recent studies published during these last 2 years [23, 25, 48]. As CSF $A\beta_{1-43}$ has never been studied before in CSF of *APP* and *PSENs* mutation carriers, the results need to be interpreted with caution and more studies are needed to replicate these findings in larger cohorts. Analysis of the shorter Aβ peptides (e.g. $A\beta_{1-34}$, $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-41}$) could provide a more comprehensive interpretation of our results. Lastly, in our study, it was not possible to assess the difference between *PSEN1* and *APP* mutations in affecting γ-secretase and Aβ generation [25]; therefore, this aspect warrants further follow-up, also in terms of interpretation of novel and VUS mutations in the AD genes.

Conclusions

In this study, CSF levels of the long Aβ peptide $A\beta_{1-43}$ were investigated for the first time in carriers of known pathogenic and unclear (VUS) *APP* and *PSENs* mutations. We observed a significant reduction of CSF $A\beta_{1-43}$ levels and a positive correlation with $A\beta_{1-42}$ in all mutation carrier groups. We suggested the re-classification of three VUS into "likely pathogenic", as their biomarker levels were comparable to the known pathogenic mutations. We added important information on the debatable genetic modifier *PSEN1* p.E318G and we were able to clarify the role of *PSEN1* p.G183V, using ONT long-read sequencing, considered so far pathogenic, but probably not involved in AD.

From a clinical perspective, our data could prove useful. A recent study showed that Aβ43 was cleared more than Aβ42 in plaques of patients treated with Aβ immunotherapy [23]. These are important data that open new possibilities for personalized medicine in patients with AD, who have a high $A\beta_{1-43}$ load in the brain. For

these reasons, $A\beta_{1-43}$ could be considered an added AD biomarker together with the others already in use. Despite the small study cohort, the data presented here corroborate previous findings on $A\beta_{1-43}$ [21, 25, 26, 29, 49], suggesting a possible involvement of longer Aβ peptides in the AD pathophysiology.

CSF levels of sAPPα and sAPPβ were also analysed for the first time in AD mutation carriers, where their levels were reduced compared to controls. Based on the recent analysis on sAPP fragments as regulators of (GABA) neurotransmission, further investigation in AD is important to study more in detail this pathway to open new venues for therapy strategies.

Finally, functional work using patient biomaterials can prove valuable to better understand the pathogenicity of unclear AD mutations. This will be useful for patient stratification for clinical trials, genetic counselling and therapy development.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13195-020-00676-5.

Additional file 1: Table S1. List of the identified *APP*, *PSEN1* and *PSEN2* mutations. **Table S2.** Diagnostic accuracy of the different markers and ratios to discriminate between controls and mutation carriers, measured by ROC curve analysis. **Table S3.** Descriptive features of *PSEN1* p.E318G mutation carriers with CSF. **Figure S1.** Neuropathology of Patient 16. Right lateral (A) and right medial (B) hemispheres showing brain atrophy. Atrophy can be observed also at the ventricles and thalamus (C). The 4G8 staining shows amyloid plaques in the hippocampus CA4 (D). Classic neurofibrillary tangles are present in the hippocampal CA4 (AT8 stain) (E). **Figure S2.** Area under the curve (AUC) calculated for the three mutation carrier groups compared to controls of $A\beta_{1-43}$, $A\beta_{1-43}/A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{1-42}/A\beta_{1-40}$, $A\beta_{1-40}$ and $A\beta_{1-43}/A\beta_{1-42}$. AUC are calculated for the know pathogenic (red), VUS (orange) and *PSEN1* p.E318G (green) mutation carrier groups compared to the control group. The AUC values and the ones for sensitivity and specificity are listed in Table S2. **Figure S3**. Area under the curve (AUC) calculated for the three mutation carrier groups compared to controls of sAPPα, sAPPβ, $A\beta1_{-43}$/sAPPα and $A\beta_{1-43}$/sAPPβ. AUC are calculated the know pathogenic (red), VUS (orange) and *PSEN1* p.E318G (green) mutation carrier groups compared to the control group. The AUC values and the ones for sensitivity and specificity are listed in Table S2. **Figure S4**. Transcript analysis of PSEN1 in Patient 16. The bar graph shows the relative quantifications of exon 6-7 in the double carrier (Patient 16; *PSEN1* p.G183V, *PSEN1* p.P49L), the single carrier (sibling of Patient 16; *PSEN1* p.G183V), and 4 non-carriers lymphoblast cells CHX treated (CHX) and untreated (UNT). Relative quantifications of splice junctions were calculated by dividing the number of junction-supporting reads by the total number of reads spanning the PSEN1 transcript. The quantifications for both CHX and UNT of the non-carriers are reported as averages (values of SD for CHX ± 0,001052869 and for UNT ± 0,000671837).

Abbreviations

AD: Alzheimer's disease; AAI: Age at inclusion; AAO: Age at onset; APOE: Apolipoprotein E; APP: Amyloid precursor protein; AUC: Area under the curve; Aβ: Amyloid-β; CJD: Creutzfeldt-Jakob disease; CSF: Cerebrospinal fluid; CV: Coefficient of variation; DLB: Dementia with Lewy bodies; ELISA: Enzyme-linked immunosorbent assay; EOAD: Early-onset Alzheimer; FTD: Frontotemporal dementia; gnomAD: Genome Aggregation database; iPSC: Induced pluripotent stem cells; LOAD: Late-onset Alzheimer; MAF: Minor allele frequency; MAQ: Multiplex amplicon quantification; MCI: Mild cognitive impairment; MRI: Magnetic resonance imaging; NINCDS-ADRDA: National Institute of Neurological and Communication Disorders and Stroke Alzheimer's disease and Related Disorders Association criteria; NFTs: Neurofibrillary tangles; NIA-AA: National Institute on Aging–Alzheimer's Association; NMD: Non-sense-mediated mRNA decay control mechanism; P-tau181: Tau phosphorylated at threonine 181; PSEN1: Presenilin 1; PSEN2: Presenilin 2; ROC: Receiver operating characteristic; sAPPα: α cleaved soluble amyloid precursor protein; sAPPβ: β cleaved soluble amyloid precursor protein; SPECT: Single-photon emission computed tomography; STR: Short tandem repeat; T-tau: Tau protein; VaD: Vascular dementia; VUS: Variants of uncertain significance

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Authors' contributions

FP, CVB and RC conceived the study. FP performed the experiments and the analysis. MB performed the ELISA experiments. ADR performed the Oxford Nanopore experiments and analysis. AS and JJM provided pathological information of the patients. MB, MT and SE provided the CSF samples. EH, RV, SE and PDD provided clinical information on the patients. KS and JVDZ provided advice and support. FP, CVB and RC drafted the manuscript. All co-authors reviewed the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

All data relevant to the study are included in the research paper or as supplementary information. Additional information will be shared by the corresponding authors upon reasonable request.

Ethics approval and consent to participate

Clinical study protocols and informed consent forms for the participation in research for patient ascertainment were approved by the local medical ethics committees of the collaborating medical centres in Belgium. Genetic study protocols and informed consent forms were approved by the ethics committees of the University Hospital of Antwerp and the University of Antwerp, Belgium.

Consent for publication

Not applicable.

Competing interests

MT reports personal fees (current employment) from Janssen Research & Development, a Division of Janssen Pharmaceutica NV, Beerse, Belgium, and owns stock/stock options in the company.

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