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# Genetic variants in PSEN2 and correlation to CSF β-amyloid42 levels in AD

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**Abstract**

Beta-amyloid 42 (Aβ42) concentrations in cerebrospinal fluid (CSF) are significantly decreased in Alzheimer's disease (AD). The aim of this study was to correlate genetic variability in presenilin 2 (PSEN2) in relation to Aβ42 concentrations and to confirm association of apolipoprotein E (APOE) alleles E4/E4 genotype with lower CSF Aβ42. Haplotype analysis of PSEN2 and APOE genotyping were performed in 175 Alzheimer's disease patients, as defined by clinical diagnosis and Aβ42 levels. One distinct haploblock in PSEN2 was detected and the frequent haplotypes were analyzed using 4 tagging single nucleotide polymorphisms (SNPs). We found an association between haplotype 2 and higher CSF Aβ42 concentrations ($p = 0.021$) and lower Aβ42 concentrations in haplotype 5 carriers ($p < 0.001$). APOE E4/E4 carriers had lower Aβ42 levels ($p = 0.009$). Additive regression analysis showed an association of Aβ42 level with APOE genotype ($p = 0.024$), haplotype 4 ($p = 0.064$), and haplotype 5 ($p = 0.04$), whereas gender, age at onset and Mini Mental State Examination (MMSE) remained insignificant. Using CSF Aβ42 as a biomarker we replicated genetic influences in APOE and observed a significant influence of a new haplotype in PSEN2. A better understanding of genetic influences on biomarkers like CSF Aβ42 might help to stratify patients and develop specific treatment strategies.


*Keywords:* Alzheimer's disease; Presenilin2; β-amyloid 42; Haplotype; Cerebrospinal fluid; Apolipoprotein E

## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive cognitive deficit. It is the most common cause of dementia in the elderly (Wimo et al., 2003). In AD, aggregation of β-amyloid (Aβ) peptides of different lengths leads to the formation of extracellular amyloid plaques associated with neuronal death and synaptic loss (St George-Hyslop, 2000). The pathological beta-amyloid 42 (Aβ42) form of the β-amyloid peptide is derived by proteolytic processing from a neuronal membrane protein termed amyloid precursor protein (APP). The proteolysis of APP is catalyzed by β- and subsequently by γ-secretases (Selkoe, 1994) leading to various lengths of Aβ. The most common forms are Aβ40 and Aβ42. The

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longer Aβ42 peptide is more prone to aggregate in vitro and is the type detected in the brains of AD patients (Iwatsubo et al., 1994). Presenilins (PSEN) are the catalytic subunits of the gamma-secretase complex (Wolfe et al., 1999). PSEN1 (chromosomal localization on 14q24.3) and PSEN2 (1q31-q42) are homologous and share 62% amino acid identity. The function of the PSEN1 γ-secretase catalyzing APP (and also Notch) cleavage is better understood than that of PSEN2. The knockout of PSEN1 lead to an embryonic lethal phenotype and dramatically reduced Aβ levels (Palacino et al., 2000; Shen et al., 1997) whereas the knockout of PSEN2 in a mouse produced no specific phenotype and essentially normal levels of Aβ (Herreman et al., 1999).

A large number of mostly rare nonsynonymous mutations were found in the APP, PSEN1, and PSEN2 genes. Some of these variants cause familial AD (FAD) mostly of the early onset type with fast disease progression (De Strooper et al., 1998; Goate et al., 1991). So far 167 PSEN1 nonsynonymous mutations in 364 families and 10 PSEN2 mutations in 18 families have been identified (www.molgen.ua.ac.be/ADMutations/ or www.alzgene.org).

The extracellular level and total brain amount of Aβ42 was increased in transgenic mice brains expressing mutant PSEN1 and PSEN2 (Borchelt et al., 1996; Citron et al., 1997; Duff et al., 1996; Tomita et al., 1997; Xia et al., 1997). Presenilin mutations might cause FAD through a gain of a deleterious function that elevates the amount of extraneuronal Aβ42 (Borchelt et al., 1996; Citron et al., 1997; Duff et al., 1996; Tomita et al., 1997; Xia et al., 1997).

Despite the well characterized associations of some PSEN and APP mutations with FAD, specific mutations have not yet explained the majority of sporadic AD cases showing a wide range in onset age (Bird et al., 1996). Apolipoprotein E (APOE) allele E4 is the only consistently confirmed genetic risk factor for sporadic AD.

Mechanisms leading to decreased concentrations of Aβ42 in cerebrospinal fluid (CSF) of sporadic AD patients have not been clarified yet. Deposition of the peptide in the plaques was suggested. However, this hypothesis cannot fully explain the picture as a selective decrease of the concentration of Aβ42 in the CSF of patients with Creutzfeldt-Jakob disease as well as patients with bacterial meningitis has been shown without development of any amyloid plaques at all (Sjogren et al., 2001; Wiltfang et al., 2003). Nevertheless, taken as a diagnostic biomarker for AD, cerebrospinal Aβ42 discriminates AD from elderly controls at quite high sensitivity and specificity (sensitivity between 78% and 85% and specificity between 81% and 84%) (Blennow et al., 2001; Galasko et al., 1998; Hulstaert et al., 1999; Lewczuk et al., 2004; Wiltfang et al., 2005) and has been shown to inversely correlate with Aβ deposition in the brain in AD (Fagan et al., 2006).

It is currently unknown whether genetic polymorphisms in PSEN1 and PSEN2 contribute to incidence and clinical course of sporadic AD. It is also not known whether PSEN polymorphisms are correlated with Aβ42 concentrations in CSF.

According to the variations of Aβ-peptide levels in different laboratories (Lewczuk et al., 2004), in our laboratory a cutoff 600 pg/mL for the differential diagnostic question (AD vs. nondemented control) was established (Schöttle, 2008). By defining an "Aβ42 CSF-positive" phenotype (cutoff below 600 pg/mL), we aimed at identifying novel genetic modifiers for AD. As Aβ42 levels predominantly rely upon the activity of the active sites of γ-secretase, presenilin 1 and 2, we therefore tested the hypothesis whether rare frequent genetic haplotypes in PSEN2 are associated with decreased Aβ42 concentrations in CSF of AD patients compared with controls.

## 2. Methods

### 2.1. Patient samples

From 1999 to 2008, all patients who were diagnosed with probable AD at the Memory Clinic of the Neurology University Hospital of Ulm participated in the study. Diagnosis was verified according to National Institute of Neurological and Communication Disorders Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria and the Diagnostic and Statistical Manual of Mental Disorders, 4th Ed. (DSM-IV) (McKhann et al., 1984).

The patients gave written informed consent to participate in the study and to have their clinical data documented together with results from genetic analyses in genes potentially relevant to AD. The study was approved by the university ethical review board (No. 2001/67).

Before diagnosis, subjects underwent a comprehensive clinical examination including their medical history, family history, a neurological and psychiatric examination, a routine blood analysis, a lumbar puncture with CSF analysis for Aβ42 level, morphological imaging (magnetic resonance or computed tomography [CT]), APOE genotyping, and a comprehensive neuropsychological examination, including Alzheimer's disease Assessment Scale-cognitive subscale (ADAS-cog). The degree of dementia was evaluated by the Mini Mental State Examination (MMSE).

Subjects with AD diagnosis were included in the AD group if the CSF Aβ42 concentration was below the level of 600 pg/mL. CSF collection and preanalytical processing were performed using a standardized protocol in a clinical routine setting as previously described (Brettschneider et al., 2006). Aβ42 measurement was performed using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) (Innogenetics, Ghent, Belgium) according to the manufacturer's instructions.

### 2.2. Genetic analyses

DNA was isolated from its blood using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to

the manufacturer's protocols. DNA quantification was performed on a GeneQuantpro RNA/DNA Calculator (Biochrom, Cambridge, UK) measuring the absorbance value at 260 nm.

APOE genotypes of all the individuals and brain samples were determined by the restriction of a polymerase chain reaction (PCR) product with the enzyme HhaI as described earlier (Wenham et al., 1991).

### 2.3. Haplotype analysis of PSEN2 gene

Haplotype analyses for PSEN2 was performed in order to discriminate potentially functional haplotypes. Linkage disequilibrium (LD) maps containing 30 polymorphisms for the PSEN2 gene were generated from HapMap genotyping data (www.hapmap.org, release 23 by March 2008) for the CEPH (Utah residents with ancestry from northern and western Europe) (abbreviation: CEU) population using Haploview 3.32 software (Haploview, www.broad.mit.edu/mpg/haploview). The term "CEPH" stands for the Centre d'Etude du Polymorphisme Humain, the organization that collected these samples in 1980.

The normalized disequilibrium coefficient D' and logarithm of the odds (LOD) were used as parameters to assess the probability of 2 loci to be inherited together and the level of recombination between them. The maximum value of D' which is 1 indicates the complete LD between 2 loci. The LOD value reflects the statistical significance for LD, with a LOD value of 2 indicating that odds are 100 to 1 in favor of linkage.

Red and pink squares on the D′/LOD scheme correspond to statistically significant LD between the pair of single nucleotide polymorphisms (SNPs) (LOD $>$ 2) with the maximum pairwise D′ value for the red squares (D′ $=$ 1). White squares indicate pairwise D′ values lower than 1 with no statistically significant evidence of LD (LOD $<$ 2). Blue squares signify pairwise D′ values of 1 but without statistical significance (LOD $<$ 2). Haplotype block is depicted as a black triangular comprising SNPs inherited together. Only SNPs with the minor allele frequency higher than 5% are depicted on the LD plot.

### 2.4. Selection of tagging SNPs for PSEN2 gene

The squared correlation coefficient $r^2$ is a measure of statistical association between 2 loci indicating the power to detect an effect at 1 locus by typing another one, whereas D′ value provides the information about the history of recombinations. Therefore $r^2$ coefficients above 0.8 were used for tagging SNPs selection. Polymorphisms were selected based on the highest minor allele frequency and exonic location.

### 2.5. Genotyping of PSEN2 tagging SNPs

Predefined and validated assays for TaqMan real-time PCR were used for the genotyping of PSEN2 tagging SNPs (Applied Biosystems, Mannheim, Germany, Catalogue number: rs2073489 [C_340454_1_], rs11405 [C_340278_20], rs6759 [C_9633596_20], and rs1800680 [C_9633614]). Each reaction consisted of 20 ng genomic DNA template, 6 μL 2xTaqMan Universal PCR Master Mix, No AmpErase UNG®, and 0.3 μL 40xTaqMan SNP Genotyping Assay. TaqMan, Real-Time PCR was performed under conditions described in the manufactured protocol: a preread run (allelic discrimination) at 60 °C, an amplification run (absolute quantification) containing 10 minutes at 95 °C, 40 cycles of 92 °C for 15 seconds, and 60 °C for 1 minute, and a postread run (allelic discrimination) at 60 °C.

### 2.6. Statistical analysis

Statistical analysis was performed using the SPSS statistical package version 12.0 (SPSS, Inc., Chicago, IL, USA). The fit of genotype frequencies to the Hardy-Weinberg equilibrium was tested by a $\chi^2$ test. Differences in genotype frequencies in different subgroups were examined using a Pearson $\chi^2$ test. One-way analysis of variance (ANOVA) test was used to assess the effects of genetic variants on Aβ42 levels.

One-way analysis of variance was also used to evaluate the strength of associations of Aβ42 concentrations with haplotype 2 and 5, APOE E4 allele, gender, and age at disease onset. Additionally, effects of haplotypes were assessed under an additive model, further including the APOE genotype in the statistical model. Using a similar model based on the number of haplotypes per individual, the association of the 5 different haplotypes with Aβ42 levels was explored simultaneously, after choosing the most common haplotype 1 as the reference haplotype. A value of $p < 0.05$ was chosen as the cutoff level for statistical significance.

Table 1
Clinical characteristics of the patients diagnosed with Alzheimer's disease

| Patient clinical characteristics | |
|---|---|
| Total patients, *n* | 175 |
| Gender | |
| Female, *n* | 106 |
| Male, *n* | 69 |
| Age at onset, years | 72.7 ± 6.5 |
| Mean CSF Aβ42 level, pg/mL | 407 ± 105 |
| MMSE score | 22.3 ± 5.4 |
| Family history | |
| Relatives with AD | 12 (8%) |
| Relatives with cognitive decline | 29 (19%) |
| APOE genotype allele, number of cases | |
| x/x | 47 |
| x/4 | 74 |
| 4/4 | 28 |

Key: Aβ42, beta-amyloid 42; AD, Alzheimer's disease; APOE, apolipoprotein E; CSF, cerebrospinal fluid; MMSE: Mini Mental State Examination; x, any other than APOE E4 allele.

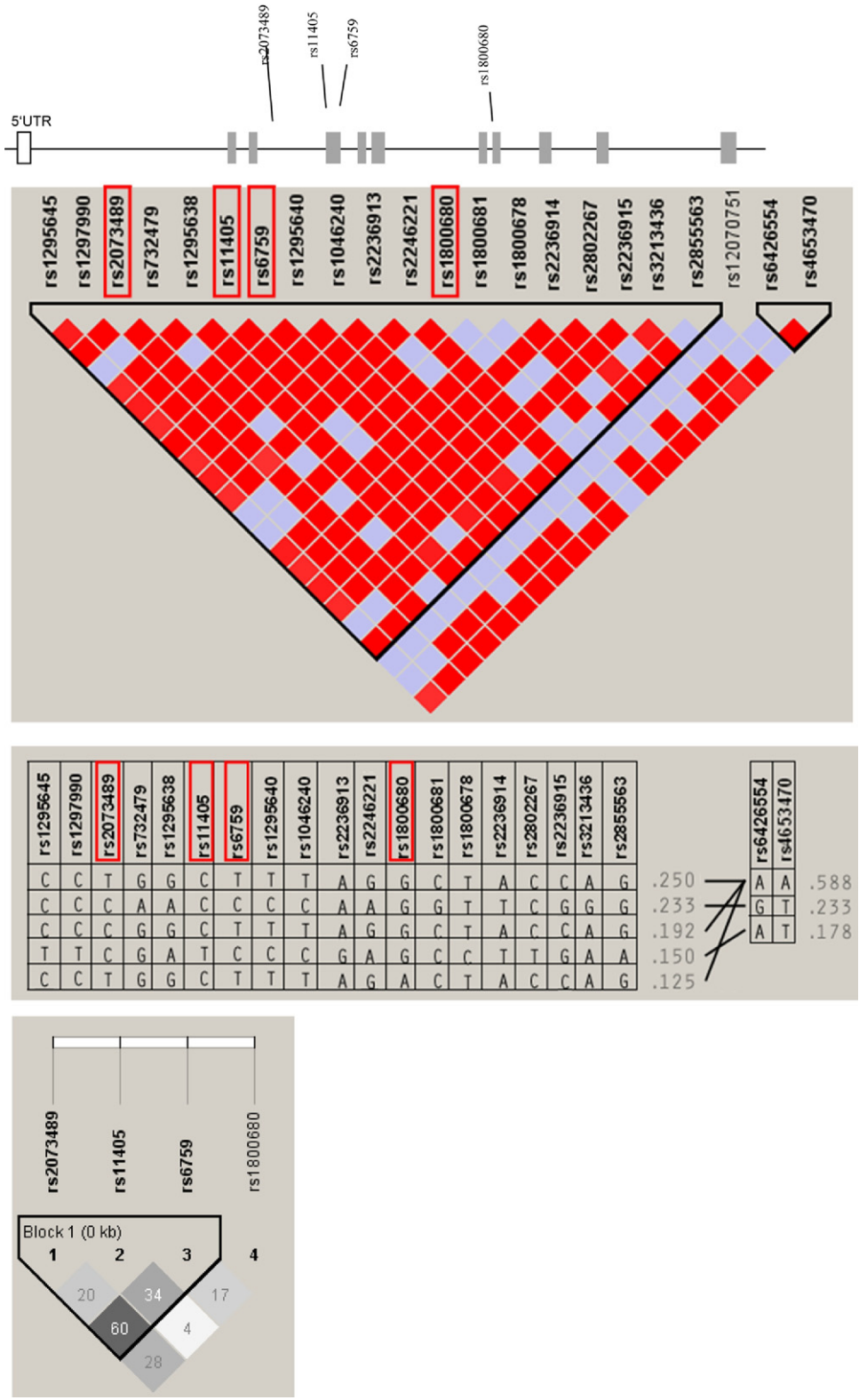


Fig. 1. Linkage disequilibrium (LD) plot of presenilin 2 (PSEN2) obtained from HapMap: tagging single nucleotide polymorphisms (SNPs) are marked in red. Each diagonal represents a definite SNP and each square indicates the pairwise magnitude of LD between 2 SNPs corresponding to the crossing diagonals. In the table below the LD plot, the 5 most frequent haplotypes as defined by the tagging SNPs are shown including the haplotype frequency. The linkage disequilibrium between the 4 tagging SNPs that were chosen is shown below using our own dataset. The numbers in the boxes representing the R2 values of the linkage between each respective SNP.

### 2.7. Comparison of results with publicly available genotype data

To assess whether the SNPs tested in our biomarker cohort also display evidence for genetic association with AD risk in publicly available genotype data (e.g., originating from candidate gene studies or genome-wide association studies [GWAS]), we consulted the AlzGene database (www.alzgene.org), a publicly available online resource of genetic association data in the field of AD (Bertram, 2007). Overall, genotype data were available for all 4 of the haplotype-defining SNPs tested here in at least 1 dataset. Whenever more than 1 dataset was available, results were combined by allele-based random-effects meta-analyses (see Bertram, 2007 for details). Note that exact haplotype reconstruction was not possible as there was no single dataset with genotype data available for all 4 haplotype-defining SNPs or their proxies.

## 3. Results

The AD group included 175 individuals (106 female and 69 male) with a mean age of 73 ranging from 60 to 87 years. MMSE score was 22.3 ± 5.4 (mean ± standard deviation). Family history was available for 152 individuals with 8% having a well-confirmed positive AD family history and 19% having relatives with cognitive decline symptoms. The clinical characteristics of the patients are summarized in Table 1.

The range of Aβ42 concentrations in the CSF in the AD patients was 176 to 598 pg/mL.

The APOE E4 allele frequency was 43.6%, 49.7% of patients were heterozygous carriers of the APOE E4 allele and 18.8% were homozygous carriers. There were no significant difference in the APOE E4 allele frequency between males and females (data not shown).

### 3.1. Haplotype analysis of PSEN2

The major PSEN2 haplotype block containing 19 SNPs covered a 23-kb region. A second minor block had 2 SNPs which were less than 1 kb apart. Both SNPs in the minor block were 100% linked to haplotypes of the major haploblock, thus, the smaller haploblock was not analyzed separately. The major 23 kb haploblock which is comprised of 98% of all possible combinations of SNPs was chosen for the selection of tagging SNPs.

Four SNPs, rs2073489, rs11405, rs6759, and rs1800680 served as tagging SNPs coding for the 5 most frequent PSEN2 haplotypes (Fig. 1).

Rs11405 and rs6759 lead to synonymous amino acid substitutions Ala23Ala and Asn43Asn in the Exon 3 of the PSEN2 gene. Rs2073489 is an intron 2 C to T exchange; rs1800680 G to A substitution is located in the intron 6.

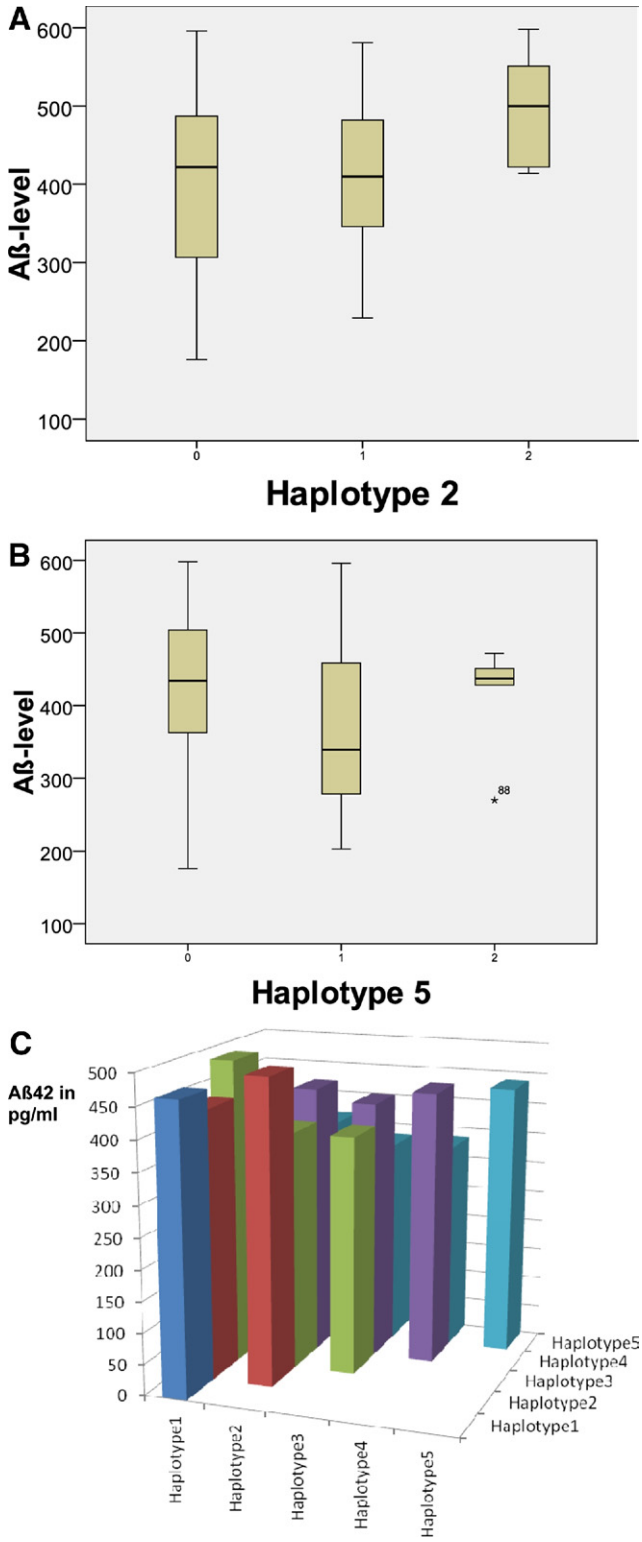


Fig. 2. A box plot graph showing the median (black), the 25th and 75th percentile (boxes) and the data range (antennas) of the beta-amyloid 42 (Aβ42) values in cerebrospinal fluid (CSF). (a) Aβ42 in heterozygous and homozygous carriers of haplotype 2; (b) depicts the same for haplotype 5; and (c) for all haplotype combinations.

Table 2
PSEN2 haplotypes and correlation with Aβ42 CSF level in (*p*-values are from 1-way ANOVA test)

| Haplotype | Number of haplotype copies | AD | | |
|---|---|---|---|---|
| | | Number of cases | Aβ42, pg/mL (mean ± SD) | ANOVA *p*-value |
| Haplotype 1 | | | | |
| TCTG | 0 | 98 | 400.95 ± 104.14 | 0.092 |
| | 1 | 64 | 404.52 ± 108.205 | |
| | 2 | 13 | 467.85 ± 73.99 | |
| Haplotype 2 | | | | |
| CCCG | 0 | 107 | 398.80 ± 110.25 | 0.021 |
| | 1 | 58 | 407.76 ± 93.43 | |
| | 2 | 10 | 494.30 ± 64.97 | |
| Haplotype 3 | | | | |
| CCTG | 0 | 137 | 403.24 ± 103.80 | 0.472 |
| | 1 | 32 | 427.28 ± 103.06 | |
| | 2 | 6 | 391.33 ± 138.80 | |
| Haplotype 4 (rs11405) | | | | |
| CTCG | 0 | 104 | 420.53 ± 103.08 | 0.097 |
| | 1 | 69 | 386.20 ± 105.39 | |
| | 2 | 2 | 440.50 ± 79.90 | |
| Haplotype 5 (rs1800680) | | | | |
| TCTA | 0 | 115 | 427.50 ± 98.35 | 0.001 |
| | 1 | 55 | 364.44 ± 108.05 | |
| | 2 | 5 | 411.64 ± 80.89 | |
| Rs2073489 | | | | |
| CC | 0 | 55 | 427.62 ± 97.35 | 0.142 |
| CT | 1 | 86 | 392.31 ± 107.41 | |
| TT | 2 | 34 | 411.95 ± 106.21 | |
| Rs6759 | | | | |
| TT | 0 | 57 | 419.90 ± 113.97 | 0.01 |
| TC | 1 | 84 | 383.95 ± 96.00 | |
| CC | 2 | 34 | 443.47 ± 97.81 | |

Key: Aβ42, beta-amyloid 42; AD, Alzheimer's disease; ANOVA, analysis of variance; APOE, apolipoprotein E; CSF, cerebrospinal fluid; PSEN2, presenilin 2.

### 3.2. Analysis of influence of PSEN2 haplotypes on Aβ42 concentrations in CSF

Table 1 shows the clinical characteristics of the patients, and the distribution of the APOE genotypes.

We analyzed the Aβ42 CSF concentrations in homozygous, heterozygous, and noncarriers of each PSEN2 haplotype (Fig. 2, Table 2). Higher Aβ42 concentrations in CSF were detected with increasing copy numbers of haplotype 2 ($p = 0.021$, ANOVA) with mean levels of 399, 408, and 494 pg/mL in carriers of none, 1, or two haplotypes 2 (Fig. 2a and c). Under the additive model for haplotype 2, including APOE genotype, this association was confirmed ($p = 0.037$). Only analyzing rs6759, a similar association with higher Aβ42 in homozygous carriers but not in heterozygous carriers was visible in the patients, ($p = 0.01$). No difference in MMSE or age of onset of disease was observed in the haplotype 2 carriers compared with noncarriers.

Further, an association between haplotype 5 (rs1800680) and lower Aβ42 concentrations in heterozygous carriers was observed. However this effect was less in the 5 individuals who were homozygous haplotype 5 carriers (means of 428 vs. 364, and 412 pg/mL in homozygous carriers, $p = 0.001$, Fig. 2b and c). An analysis taking into account APOE genotype yielded comparable results where $p = 0.0003$.

There was a significantly lower Aβ42 concentrations in CSF in homozygous carriers of the APOE E4 allele than in the heterozygous or non-E4 carriers (361 pg/mL in homozygous allele 4 carriers vs. 430 pg/mL in heterozygous and 398 pg/mL in non-E4 carriers $p = 0.009$, ANOVA) (Fig. 3a and b).

Looking at clinical correlations with Aβ42, we observed significant positive correlation between Aβ42 CSF concentrations and MMSE value in the whole AD group ($r = 0.173$, $p = 0.032$). Although there was a slightly higher percentage of females in the AD group (60%) there was no association between sex and Aβ42 levels in CSF. There was

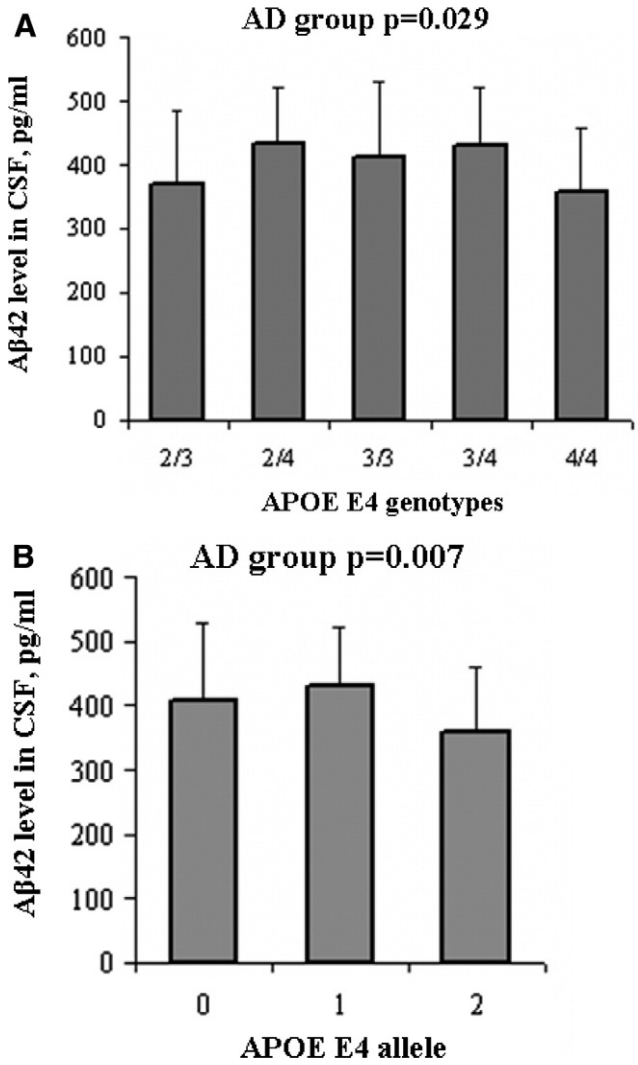


Fig. 3. (a) Beta-amyloid 42 (Aβ42) for apolipoprotein E (APOE) different genotypes; (b) depicts Aβ42 for APOE E4 allele non-carriers (0), heterozygous (1), and homozygous (2) carriers.

no correlation between age of onset of Alzheimer's disease and Aβ42 concentrations in CSF.

For explorative analysis of single effects of genotypes or clinical variables on Aβ variability, univariate ANOVAs were performed. Additionally, additive models were applied where the per-allele effects at single SNPs were investigated. After this, the variables found to be significant were included in the multifactorial model, in addition to the significant covariates.

After exploratory analysis of the single factors in a univariate model, we analyzed the number of haplotypes together with all significant covariates (which were significant in the univariate model), based on an additive regression model. In this model, we chose haplotype 1 as the reference haplotype and included the numbers of the other haplotypes (haplotype 2–5) per individual as factors in the model. The covariates included were gender, APOE genotype, MMSE, and age of onset (AAO). In this model, significant effects on Aβ42 were observed for APOE genotype ($p = 0.024$), haplotype 4 ($p = 0.064$), and haplotype 5 ($p = 0.04$), whereas gender, age of onset, and MMSE remained insignificant.

In a simultaneous analysis of all haplotypes in relation to reference haplotype 1 in one additive statistical model under consideration of the APOE genotype, significant effects on Aβ42 were observed for APOE genotype ($p = 0.007$), haplotype 4 ($p = 0.063$), and haplotype 5 ($p = 0.0009$). Gender, age of onset, and MMSE had been excluded from the model beforehand due to insignificant association with Aβ42.

Finally, we investigated whether any of the 4 tested SNPs showed evidence for association with AD risk in publicly available genotype data extracted from the AlzGene database (Supplementary Table 1). With the possible exception of rs11405 (which defines haplotype 4; $p$-value 0.15) there was no evidence supporting a correlation of these SNPs with disease risk. It should be noted, however, that quantitative trait analyses on CSF Aβ42 levels cannot be directly compared with results obtained from investigating binary endpoints such as AD vs. no AD. Furthermore, and probably more importantly, only haplotypes 4 and 5 could be assessed in this way due to the lack of sufficient genotype data in the available datasets. Therefore, more extensive de novo genotyping will be necessary to independently replicate our findings.

## 4. Discussion

While genetic variations in presenilin genes are known to influence familial Alzheimer's disease, the genetic underpinnings for sporadic cases are less characterized. Because Aβ42 concentrations in CSF are used as a biological marker for severity of AD, our intention was to analyze associations between genetic variants in presenilin genes and the Aβ42 concentrations in AD patients.

We studied the correlation between PSEN2 haplotypes and Aβ42 concentrations in CSF because low levels of Aβ42, and high level of tau-protein are used as a biomarker for AD (Otto et al., 2008; Skoog et al., 2003), and presenilin-dependent γ-cleavage is the last step in Aβ-production. A correlation between a lower Aβ42 concentration in CSF and greater cognitive decline as well as a higher abundance of amyloid plaques in the brain has been shown (Fagan et al., 2007; Gustafson et al., 2007; Stomrud et al., 2007). It is known that PSEN1 and 2 mutation carriers show decreased Abeta levels (Nikisch et al., 2008; Portelius et al., 2010; Uttner et al., 2010). However little is known about reasons for and significance of variations of Aβ42 in sporadic AD patients.

A certain stability over time of this marker has been shown with no consistent change of the CSF Aβ42 level of patients during month 10 and month 20 indicating that measuring Aβ42 might serve as a kind of biological marker for Alzheimer disease (Andreasen et al., 1999).

We were able to replicate prior findings of APOE E4 genotype association with decreased Aβ42 levels in CSF in sporadic AD patients (Prince et al., 2004; Sunderland et al., 2004).

PSEN2 haplotypes were analyzed by 4 tagging SNPs in their relation to CSF Aβ42 concentrations in AD patients. In addition to APOE, a significant influence of the rs1800680 defining haplotype 5 of PSEN2 on Aβ42 was detected with lower Aβ42 in carriers than in non-carriers. Recently, PSEN2 was also studied in relation to schizophrenia because it lies in a schizophrenia susceptibility locus (Zhang et al., 2009) and an influence of rs6759 and rs11405 on mRNA (messenger ribonucleic acid) expression in blood lymphocytes from schizophrenic patients but not in healthy controls, and an association with the rs1295645 and schizophrenic symptoms (Zhang et al., 2009). In our analysis, only the rs1800680 which is in no LD with any of the SNPs studied in this analysis on schizophrenia showed a significant effect on Aβ42.

The reason to choose PSEN2 and not PSEN1 was the finding that in the PSEN1 gene, there are no distinct regions of high LD resulting in few distinct haplotype blocks but in a large number of possible combinations of variants.

A recent study investigating the association between PSEN1 variants and the risk for AD development, revealed a marginally significant protective effect of the intronic rs165932 G allele ($p = 0.03$) (Belbin et al., 2008). Even though the approach to choose the most frequent variants in a gene for genotyping may cover the most frequent genetic constellations (as combinations of the respective variants), the lack of distinct haplotype blocks with numerous variants causes significant multiple testing problems.

Our study suggests that the AD association with PSEN2 haplotypes is functionally related to APP/Aβ metabolism, however we cannot rule out that this is

actually the result of another, correlated effect, or that it represents a false positive finding altogether. There are a number of limitations of the present analysis. First, due to the cross-sectional design, no follow-up of the A$\beta$42 levels exists. Thus, we could not account for the changes in A$\beta$42 over the time. Second, we do not have longitudinal clinical data. As we found no association with gender, APOE genotype, MMSE score, and AAO, analysis of associations with longitudinal parameters like disease progress or parameters like efficacy of medication will be a topic in future studies. However, it is known from the familial AD cases carrying PSEN mutations that AAO exhibits a wide range and other genes modifying AAO are discussed in this context (Pastor et al., 2003). MMSE itself decreases over time in contrast to the genetic factors analyzed here. Therefore an association with MMSE would be surprising. As now first trials with amyloid modifying drugs are conducted analyses of responders regarding their PSEN2 haplotype might be of particular interest. Third, we do not have imaging data, because it has been shown that PSEN2 SNPs are associated with alterations is gray matter density and hippocampal volume in magnetic resonance imaging (MRI) (Sloan et al., 2010).

Finally, despite the strong statistical support for the associations between CSF Abeta levels and PSEN2, these findings have to be confirmed in other CSF based studies and the relevance on a population-wide level remains to be determined.

This study was indeed a replication for the previous studies on the relation between APOE genotype and A$\beta$42, and, similar, an independent replication of this finding on haplotype 5 would be desired.

Taken together, the associations described will likely have no major role serving as a diagnostic or predictive tool for AD in a clinical setting, unless other (e.g., rarer) variants of higher penetrance are linked to the observed effects. However, and depending on their exact functional role in AD pathogenesis, the identified haplotypes might still be essential in advancing our understanding of the biochemical processes leading to AD. A better understanding of genetic influences on biomarkers like CSF A$\beta$42 might help to develop appropriate and effective means to target these processes therapeutically.

## Disclosure statement

All authors disclose no conflicts of interest.

Appropriate approval concerning human subjects was used: the study was approved by the university ethical review board (No. 2001/67).

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## Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2010.07.017.

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