

SHORT REPORT

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BACE1 gene variants do not influence *BACE1* activity, levels of APP or A β isoforms in CSF in Alzheimer's disease

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Abstract

The *BACE1* gene encodes the beta-site APP-cleaving enzyme 1 and has been associated with Alzheimer's disease (AD). *BACE1* is the most important β -secretase responsible for the generation of Alzheimer-associated amyloid β -proteins (A β) and may play a role in the amyloidogenic process in AD. We hypothesized that *BACE1* gene variants might influence *BACE1* activity or other markers for APP metabolism in the cerebrospinal fluid (CSF) and thereby contribute to the development of AD. We genotyped a Swedish sample of 269 AD patients for the rs638405 single nucleotide polymorphism (SNP) in the *BACE1* gene and correlated genotype data to a broad range of amyloid-related biomarkers in CSF, including *BACE1* activity, levels of A β_{40} , A β_{42} , α - and β -cleaved soluble APP (α -sAPP and β -sAPP), as well as markers for Alzheimer-type axonal degeneration, i.e., total-tau and phospho-tau₁₈₁. Gene variants of *BACE1* were neither associated with amyloid-related biomarkers, nor with markers for axonal degeneration in AD.

Findings

Cleavage of the amyloid precursor protein (APP) by β - and γ -secretase gives rise to the amyloid β -protein (A β) found in senile plaques (SP) in Alzheimer's disease (AD). The *BACE1* gene encodes the beta-site APP-cleaving enzyme 1 (OMIM 604252), which is involved in β -secretase activity [1-4]. The *BACE1* gene is associated with AD [5,6] and the *BACE1* activity is elevated both in brain tissue and in CSF in AD [7,8]. We hypothesized that *BACE1* gene variants might influence the *BACE1* activity or other amyloid-related biomarkers related to amyloid in the cerebrospinal fluid (CSF) and thereby contribute to developing AD. We tested a single nucleotide polymorphism (SNP) in the *BACE1* gene to evaluate the genetic influence on *BACE1* activity, levels of A β_{40} , A β_{42} , α - and β -cleaved soluble APP (α -sAPP and β -sAPP) in CSF from AD patients. Further we assessed the *BACE1* genetic influence on markers for Alzheimer-type axonal degeneration (total-tau and phospho-tau₁₈₁). The rs638405 SNP have a high allele frequency with a global frequency of the least common variant of 0.32.

To our knowledge this is the first study investigates *BACE1* genotype data in relation to *BACE1* activity and other amyloid-related biomarkers in CSF from AD patients.

We studied a Swedish Caucasian sample of 269 AD patients (90 men and 179 women, mean age 74.7 \pm 6.3 years) where CSF levels of total-tau, phospho-tau₁₈₁ and A β_{42} were known. We measured the *BACE1* activity, levels of α -sAPP, β -sAPP and A β_{40} in CSF samples from 84 of the patients. All participants were recruited at the Memory Clinic at the Malmö University Hospital. The patients gave informed consent to participate in the study, which was conducted according to the provisions of the Helsinki Declaration and approved by the local ethic committee. The diagnosis of "probable AD" was made according to the NINCDS-ADRDA criteria [9]. No AD patient had any of the known familial forms of autosomal dominant AD.

CSF samples were taken by lumbar puncture. *BACE1* activity was determined with a sensitive and specific solution-based assay previously described [10]. Levels of total-tau, phospho-tau₁₈₁ and A β_{42} were measured using established ELISA methods [11,12] while α -sAPP, β -sAPP and A β_{40} were quantified using MSD immunoassays (Cat#: K11120E and K111FTE), (Meso Scale Discovery, Gaithersburg, MD, USA).

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Genomic DNA was extracted from whole blood using standard methods. *BACE1* alleles were determined using the Dynamic Allele Specific Hybridization (DASH) technique as described earlier [13]. Optimal assay conditions: 1.5 mM MgCl₂, 200 μM dNTPs, 0.05 U/μl Taq polymerase, 0.15 pmol/μl forward biotinylated primer (5'-Biotin-ATCCGGCGGGAGTGGTATTATG-3'), 0.75 pmol/μl reverse primer (5'-GTCCATTGATCTC-CACCCGCAC-3') (Invitrogen, Life Technologies) and 5-20 ng DNA, 1xPCR buffer in a final volume of 25 μl. The cycling profile was: 5 min 95°C, 40 cycles: 30 sec 95°C, 45 sec 60°C, 1 min 72°C and a final step of 10 min 72°C. To identify *BACE1* alleles the probes 5'-CACAATGATCACCTCATAA-3' and 5'-CACAATGATGACCTCATAA-3' were used.

APOE genotyping was performed using minisequencing as described before [14]. Gene designations follow the recommendations of HUGO Gene Nomenclature Committee [15].

The genotype and allele frequencies of the *BACE1* rs638405 and *APOE* ε4 are shown in table 1. The analysis of variance (ANOVA) was used to analyze the effects of *BACE1* genetic variants on MMSE, *BACE1* activity and CSF protein levels. To test the effects of known risk factors, e.g., age, sex and *APOE* ε4 we identified significantly relevant covariates for each outcome variable (MMSE and levels of AD CSF biomarkers) using forward stepwise linear regression. Hardy-Weinberg equilibrium was assessed by χ² statistics. The criterion for significance was set at p < 0.05 for all statistical tests. Statistical analyses were performed with the SYSTAT11 (SYSTAT Software GmbH, Erkrath, Germany) software.

We studied *BACE1* gene variants in relation to *BACE1* activity and levels of amyloid-related biomarkers in CSF from AD patients. In the linear regression analysis we found *APOE* ε4 to significantly interact with phospho-tau₁₈₁, Aβ₄₂ and MMSE. Subsequently, *APOE* ε4 was

Table 1 *BACE1* and *APOE* genotype and allele frequencies in AD patients

<i>BACE1</i>			
Genotype frequencies	CC	CG	GG
AD (269)	50 (0.19)	117 (0.43)	102 (0.38)
Allele frequencies	C	G	
AD (538)	217 (0.40)	321 (0.60)	
<i>APOE</i>			
Genotype frequencies	No ε4	One ε4	Two ε4
AD (269)	74 (0.28)	135 (0.50)	60 (0.22)
Allele frequencies	ε2/ε3	ε4	
AD (538)	283 (0.53)	255 (0.47)	

Abbreviations: Alzheimer's disease (AD). Number of total genotypes and alleles are given (N). Percentage of total is shown for the genotypes and alleles respectively.

Table 2 *BACE1* activity and biochemical markers in CSF and *BACE1* genotypes in AD patients

CSF protein	<i>BACE1</i> genetic variants			p-value
	CC	CG	GG	
Total-tau (pg/ml)	628.8 ± 38.7	612.7 ± 29.3	610.0 ± 33.4	0.699
Phospho-tau ₁₈₁ (pg/ml)	79.7 ± 3.9	73.8 ± 2.8	77.7 ± 3.4	0.506
Aβ ₄₂ (pg/ml)	408.7 ± 11.8	419.4 ± 9.2	408.3 ± 9.9	0.575
Aβ ₄₀ (pg/ml)	4869.9 ± 196.4	4894.8 ± 238.6	5030.7 ± 219.6	0.854
<i>BACE1</i> (pM)	29.2 ± 2.5	31.8 ± 2.0	33.6 ± 3.3	0.765
α-sAPP (ng/ml)	645.8 ± 77.0	660.5 ± 40.6	620.5 ± 41.8	0.962
β-sAPP (ng/ml)	393.3 ± 37.8	408.0 ± 24.9	399.6 ± 22.6	0.965

Abbreviations: Alzheimer's disease (AD). Mean values (± SEM) are shown. Adjusted p-values are shown for respective comparison.

included as covariate in the statistic model. We found no effect of *BACE1* gene variants on *BACE1* activity or levels of Aβ₄₀, Aβ₄₂, α-sAPP and β-sAPP (Table 2). We compared CSF levels of markers for axonal degeneration (total-tau and phospho-tau₁₈₁) between *BACE1* gene variants and found no significant differences in protein levels (Table 2). The *BACE1* SNP showed no association with MMSE (data not shown).

The *APOE* allele distribution followed the expected frequencies seen in AD populations (Table 1). Genotype frequencies conformed to Hardy-Weinberg equilibrium in AD patients.

We hypothesized that *BACE1* gene variants might influence *BACE1* activity or levels of amyloid-related biomarkers in the cerebrospinal fluid (CSF) and thereby contribute to the development of AD. A genome-wide screen of AD families has shown linkage to marker close to *BACE1* [16] and the rs638405 SNP has been tested in a number of studies [5,17]. Other studies have found association between the *BACE1* gene and AD [5,18]. Even though this SNP does not change the protein sequence it still can have functional effects based on earlier findings where *BACE1* influenced levels of Aβ in CSF [6]. We tested if the rs2069456 SNP was associated with *BACE1* activity or with levels of Aβ₄₀, Aβ₄₂, α-sAPP and β-sAPP in CSF from AD patients. Further we assessed the influence on total-tau and phospho-tau₁₈₁. Gene variants of *BACE1* were neither associated with amyloid-related biomarkers nor with markers for axonal degeneration. Our results do not support that variants of the *BACE1* gene affects *BACE1* activity or CSF levels of APP or Aβ isoforms in AD.

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Authors' contributions

AS participated in the design of the study, performed the genetic analyses, analyzed the data statistically and drafted the manuscript. HZ participated in the design of the study, helped in analyzing the data and helped in drafting the manuscript. UA performed the activity analyses and the immunoassays and revised the manuscript critically. LM collected the clinical material and revised the manuscript critically. KB participated in the design of the study, helped in analyzing the data and revised the manuscript critically. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, *et al*: Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Mol Cell Neurosci* 1999, **14**:419-427.
2. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, *et al*: Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 1999, **402**:537-540.
3. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, *et al*: Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 1999, **286**:735-741.
4. Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, *et al*: Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* 1999, **402**:533-537.
5. Nowotny P, Kwon JM, Chakraverty S, Nowotny V, Morris JC, Goate AM: Association studies using novel polymorphisms in BACE1 and BACE2. *Neuroreport* 2001, **12**:1799-1802.
6. Kirschling CM, Kolsch H, Frahnert C, Rao ML, Maier W, Heun R: Polymorphism in the BACE gene influences the risk for Alzheimer's disease. *Neuroreport* 2003, **14**:1243-1246.
7. Zetterberg H, Andreasson U, Hansson O, Wu G, Sankaranarayanan S, Andersson ME, Buchhave P, Londos E, Umek RM, Minthon L, *et al*: Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease. *Arch Neurol* 2008, **65**:1102-1107.
8. Zhong Z, Ewers M, Teipel S, Burger K, Wallin A, Blennow K, He P, McAllister C, Hampel H, Shen Y: Levels of beta-secretase (BACE1) in cerebrospinal fluid as a predictor of risk in mild cognitive impairment. *Arch Gen Psychiatry* 2007, **64**:718-726.
9. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984, **34**:939-944.
10. Wu G, Sankaranarayanan S, Tugusheva K, Kahana J, Seabrook G, Shi XP, King E, Devanarayan V, Cook JJ, Simon AJ: Decrease in age-adjusted cerebrospinal fluid beta-secretase activity in Alzheimer's subjects. *Clin Biochem* 2008, **41**:986-996.
11. Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E: Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? *Mol Chem Neurobiol* 1995, **26**:231-245.
12. Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, Minthon L, Wallin A, Blennow K, Vanmechelen E: Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma. *Amyloid* 2000, **7**:245-258.
13. Prince JA, Feuk L, Howell WM, Jobs M, Emahazion T, Blennow K, Brookes AJ: Robust and accurate single nucleotide polymorphism genotyping by dynamic allele-specific hybridization (DASH): design criteria and assay validation. *Genome Res* 2001, **11**:152-162.
14. Blennow K, Ricksten A, Prince JA, Brookes AJ, Emahazion T, Wasslavik C, Bogdanovic N, Andreasen N, Batsman S, Marcusson J, *et al*: No association between the alpha2-macroglobulin (A2M) deletion and Alzheimer's disease, and no change in A2M mRNA, protein, or protein expression. *J Neural Transm* 2000, **107**:1065-1079.
15. Eyre TA, Ducluzeau F, Sneddon TP, Povey S, Bruford EA, Lush MJ: The HUGO Gene Nomenclature Database, 2006 updates. *Nucleic Acids Res* 2006, **34**:D319-321.
16. Blacker D, Bertram L, Saunders AJ, Moscarillo TJ, Albert MS, Wiener H, Perry RT, Collins JS, Harrell LE, Go RC, *et al*: Results of a high-resolution genome screen of 437 Alzheimer's disease families. *Hum Mol Genet* 2003, **12**:23-32.
17. Murphy T, Yip A, Brayne C, Easton D, Evans JG, Xuereb J, Cairns N, Esiri MM, Rubinsztein DC: The BACE gene: genomic structure and candidate gene study in late-onset Alzheimer's disease. *Neuroreport* 2001, **12**:631-634.
18. Clarimon J, Bertranpetit J, Calafell F, Boada M, Tarraga L, Comas D: Association study between Alzheimer's disease and genes involved in Abeta biosynthesis, aggregation and degradation: suggestive results with BACE1. *J Neurol* 2003, **250**:956-961.

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