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Original Research Article

Association of *BACE1* Gene Polymorphism with Cerebellar Volume but Not Cognitive Function in Normal Individuals

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Key Words
β-Site amyloid precursor protein-cleaving enzyme 1 · Volumetry · Cognition · Polymorphism · Cerebellum

Abstract
Aims: β-Site amyloid precursor protein (APP)-cleaving enzyme 1 *(BACE1)* is a biological and positional candidate gene for Alzheimer's disease (AD). Previous studies found that *BACE1*-null mice had impaired performance on cognition and neurodegeneration during the aging process. Additionally, a synonymous polymorphism of *BACE1* (rs638405) in exon 5 has been reported to be associated with risk for AD. We hypothesized that this *BACE1* gene variant might influence regional brain volumes and cognitive tests in normal individuals. ***Methods:*** Participants were 330 normal volunteers between 21 and 92 years of age (mean age 56.3 ± 22.0 years; 191 males, 139 females). Cognitive tests (the Mini-Mental State Examination and Digit Spans), magnetic resonance imaging, and genotyping of *BACE1* rs638405 were examined for each subject. The differences in regional gray matter (GM) volumes between G homozygotes and C-allele carriers were tested using optimized voxel-based morphometry. ***Results:*** Compared to C-allele carriers, G homozygotes exhibited significantly larger GM volumes in the left cerebellar culmen and right cerebellar lingual area, but no significant differences on cognitive function tests. ***Conclusion:*** The findings suggest that the *BACE1* rs638405 polymorphism may affect cerebellar morphology, but not cognitive function in healthy humans.

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Introduction

Amyloid plaques, composed of amyloid β (Aβ) peptides, are hallmark neuropathological lesions in brains affected by Alzheimer's disease (AD). Aβ is generated by proteolytic cleavage of the amyloid precursor protein (APP), a large type I transmembrane protein, by β- and γ-secretases [1]. β-Site APP-cleaving enzyme 1 (BACE1), also known as β-secretase, is a transmembrane aspartic proteinase [2]. BACE1 splits APP, resulting in secreted BACE1-cleaved APP ectodomain and a cell membrane-bound fragment referred to as C99. The γ-secretase then cuts the C99, releasing Aβ. The process of generating Aβ peptides is dependent on the availability of BACE1, the key rate-limiting enzyme for Aβ peptide production [1]. Hence, inhibition of the enzymatic activity of BACE1 is regarded as one of the most promising targets for treating AD patients.

BACE1 is primarily expressed within the central nervous system, and predominantly by neurons [3]. Initial studies did not reveal gross alterations in the morphology, physiology, biochemistry, and gross behavior of post-natal or adult *BACE1* knockout mice. However, recently, more precise behavioral phenotyping studies of *BACE1* knockout mice have revealed abnormalities in cognitive and emotional functions, suggesting potential mechanism-based toxicities resulting from complete *BACE1* inhibition [4].

The *BACE1* gene is located on chromosome 11q23.3, near the region with increased lod score for AD [5]. It has been speculated that variations in *BACE1* might be associated with risk for AD. In 2001, a synonymous polymorphism of *BACE1* (rs638405, C786G, Val262) in exon 5 was identified, and this polymorphism is the only single nucleotide polymorphism in the protein-coding region of *BACE1* gene with substantial frequency identified so far [6]. A significant association between the rs638405 GG genotype and AD was noted [6]. This study has been replicated by several research teams, although some found contrasting results [7].

In recent years, the intermediate phenotype concept has become a successful strategy for the biological characterization and validation of the effect of genetic variation on neuropsychiatric disease risk [8]. This approach is based on the concept that susceptibility genes for neuropsychiatric diseases increase risk by affecting risk-associated brain pathogenesis. Since the *BACE1* rs638405 polymorphism is associated with AD susceptibility, and AD is associated with significant brain atrophy and cognitive impairment [9], we tested the hypothesis that the *BACE1* rs638405 polymorphism may affect regional gray matter (GM) volume as well as cognitive function in normal individuals.

Materials and Methods

This study included 330 healthy Chinese subjects recruited from the community in northern Taiwan (191 males and 139 females) with a mean age of 56.3 ± 22.0 years (range 21–92). Each subject was evaluated by a trained research assistant using a diagnostic structured Mini-International Neuropsychiatric Interview (MINI) [10]. The exclusion criteria included the following: (1) presence of any diagnosis on axis I of the DSM-IV, such as mood disorders or psychotic disorders; (2) neurobiological disorder, such as dementia, head injury, stroke, or Parkinson's disease; (3) illiteracy, and (4) subjects with Clinical Dementia Rating Scale score of >0.5. All participants had sufficient visual and auditory acuity to undergo cognitive testing. They were administered the Mini-Mental State Examination (MMSE) and the Wechsler Digit Span test (forward and backward). The research was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Taipei Veterans General Hospital. Written informed consent was

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obtained from all subjects after they had been given an adequate understanding of the study.

For genotyping, genomic DNA was extracted from peripheral blood with a commercial kit (Qiagen, Gentra Puregene Blood Kit). rs638405 genotyping was performed using high-throughput MALDI-TOF mass spectrometry. Briefly, polymerase chain reaction and single-base extension primers were designed using Sequenom (San Diego, Calif., USA). The genotyping analysis was performed by using iPLEX Gold Reagent Kit (Sequenom) according to the manufacturer's instructions. The purified extension products were spotted onto a 384 SpectroCHIP II array using a MassARRAY Nanodispenser RS1000 following by analyzing on a MassARRAY Compact Analyzer (Sequenom). The resulting spectra were processed and alleles called with SpectroTYPER (Sequenom).

All magnetic resonance (MR) scanning was performed at National Yang-Ming University, Taiwan, using a 3.0-Tesla Siemens MR imaging scanner with 12 channel head coil (Siemens Magnetom Tim Trio, Erlangen, Germany). High-resolution structural MR images were acquired with 3D magnetization-prepared rapid gradient echo sequence (3D-MPRAGE; TR = 2,530 ms, TE = 3.5 ms, TI = 1,100 ms, FOV = 256 mm, flip angle = 7°, matrix size = 256 × 256, 192 sagittal slices, voxel size = 1.0 × 1.0 × 1.0 mm, no gap). All images were acquired parallel to the anterior commissure-posterior commissure line. To minimize motion artifact generated during image acquisition, each subject's head was immobilized with cushions inside the coil. A Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL)-based T1 VBM approach was used for preprocessing and subsequent analysis of whole-brain T1-weighted volumetric images [11]. Details of the procedure were described in our previous report [12].

Statistical analyses were performed using the SPSS 13.0 program (SPSS Inc., Chicago, Ill., USA). Student's t test and χ^2 test were applied to compare the continuous and categorical variables between the two groups (C-carriers, and G/G), respectively. Smoothed modulated GM segments were analyzed with SPM8 utilizing the framework of General Linear Model (GLM). Analysis of covariance (ANCOVA) was employed by covarying age, education, and total intracranial volume (TIV) to investigate the regional GM volume differences between the two genotypic groups. To avoid possible partial volume effects around the margin between GM and white matter, all voxels with a GM probability value <0.2 (range 0–1) were eliminated. The differences were deemed to be significant at the individual voxel level when the uncorrected p value was <0.001 and the extended cluster size was >338 voxels which was calculated from the expected number of voxels per cluster according to the theory of Gaussian random fields. We used the icbm2tal function from the GingerALE toolbox (The BrainMap Development Team; http://brainmap.org/ale/index.html) to transform Montreal Neurological Institute (MNI) coordinates into Talairach coordinates, and to minimize coordinate transformation discrepancy between MNI and Talairach space. Anatomical structures of the coordinates representing significant clusters were identified on the basis of the Talairach and Tournoux atlas (Talairach and Tournoux 1988). To evaluate the neuroanatomical correlates of individual differences between genotype groups, partial correlation analysis using age, education level, and TIV as confounding covariates was performed to correlate the clinical scores (only the scores showing group differences) with the regional GM volume in all participants. The regional GM volumes were extracted and summed up from the peak coordinates showing significant differences. The threshold for statistical significance was set at $p < 0.05$ with Bonferroni correction for multiple comparisons.

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Table 1. Total MMSE scores and Wechsler Digit Span task scores among the *BACE1* rs638405 genotypic groups in normal subjects

Genotype	MMSE	p	Digit forward	p	Digit backward	p
C-carriers	27.7 (2.3)	0.276	13.5 (2.6)	0.595	7.2 (4.1)	0.281
G/G	28.1 (2.3)		13.6 (2.7)		7.8 (4.0)	

Data are presented as mean (standard deviation). p represents the p value adjusted for age and education years.

Results

The *BACE1* rs638405 genotype distribution for the 330 subjects was: C/C = 43, C/G = 143, and G/G = 144. The distributions of the genotype did not differ significantly from Hardy-Weinberg equilibrium (p = 0.427). No significant differences were observed with respect to age, years of education, or gender among the three *BACE1* genotypic groups. Using age and total years of education as covariates, no significant differences in MMSE, the Digit forward score, or the Digit backward score were demonstrated for the *BACE1* rs638405 genotypic groups (table 1).

By using SPSS software, the ANOVA test did not yield any significant results for differences in TIV and total GM volume between the *BACE1* genotype groups. DARTEL-based T1 VBM analyses revealed that G homozygote carriers had larger GM volumes in the left cerebellar culmen and right cerebellar lingual areas than the C-allele carriers (fig. 1).

Discussion

To the best of our knowledge, this is the first study to examine the effect of the *BACE1* rs638405 polymorphism on cognitive function and brain structural changes in normal individuals. The major findings of this study showed that *BACE1* rs638405 G homozygotes exhibited larger GM volumes in the cerebellum than C-allele carriers (fig. 1). Animal studies have demonstrated that *BACE1* is expressed in specific areas in the cortex, hippocampus, cerebellum, pons, and spinal cord [3]. The study's results indicate that the *BACE1* rs638405 polymorphism or other *BACE1* functional polymorphisms that are in linkage disequilibrium with this polymorphism may affect cerebellum volume in normal individuals. Even though this *BACE1* rs638405 polymorphism does not change the protein sequence, it can still have functional effects, based on earlier findings where *BACE1* rs638405 genotypes influenced levels of Aβ in the cerebrospinal fluid [13].

The mechanisms underlying the *BACE1* genetic effect on cerebellum volume are unclear. In a recent study, Ewers et al. [14] demonstrated that an increase in cerebrospinal fluid-BACE1 activity is associated with decreased left and right hippocampus volume in AD patients. In an animal study, loss of neurons was found in the dentate gyrus and CA3 regions in *BACE1*-null mice examined at 2 years of age [15]. It is suggested that sustained asynchronous stimulation arising from abnormally higher neuronal activity may have triggered neurodegeneration during the aging process [15]. Recently, in a cellular model of spinocerebellar ataxia 17, it was shown that upregulation of *BACE1* by decreased miR-29a/b levels enhanced neuronal apoptosis [16]. Taken together, these studies and our findings suggest that *BACE1* may modulate brain morphology and neurodegeneration.

Studies of *BACE1* knockout mice suggest that *BACE1* is essential for cognitive, emotional, and synaptic functions [4]. The results of our study indicate that, in our sample of normal

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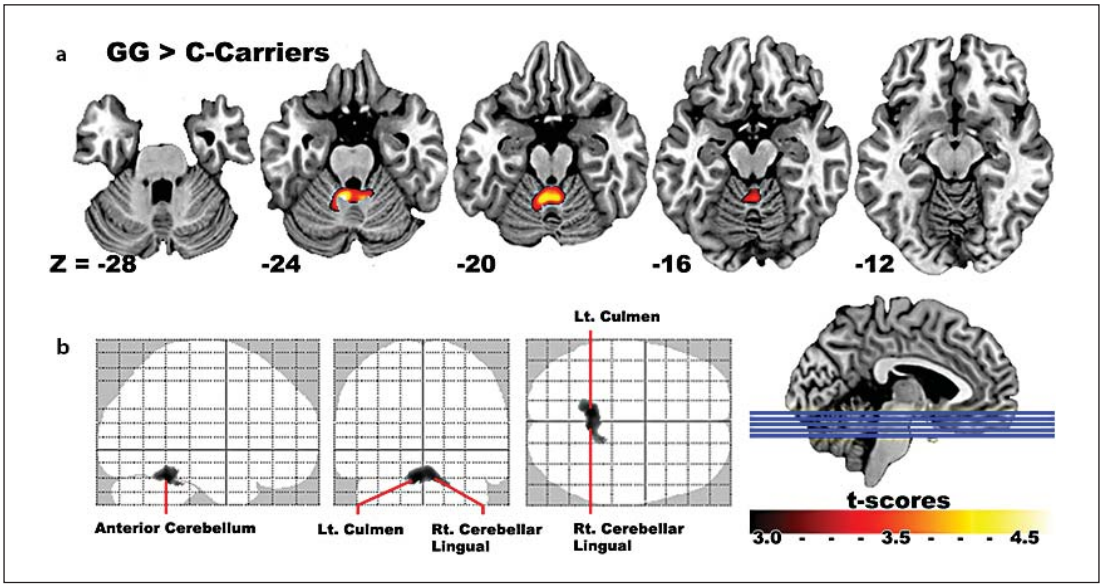

Fig. 1. Regions showing significant GM volume reduction in C-carriers of *BACE1* genotype compared with GG homozygotes. **a** Regions showing larger regional GM volume in GG homozygotes than C-carriers in the left cerebellar culmen and right cerebellar lingual area. **b** The glass brain map showing the spatial distribution of GM reduction in subjects with C allele of *BACE1* (statistical criteria: uncorrected $p < 0.001$ with cluster size = 503 voxels). No area of regional GM volume reduction was found in GG homozygotes. Rt = Right; Lt = left.

subjects, the *BACE1* rs638405 polymorphism has no effects on cognitive function. There are several explanations for our negative findings. First, the *BACE1* gene may not play a role in cognitive function. Second, our negative results could arise from a lack of statistical power due to a small sample size. If the *BACE1* rs638405 polymorphism had a minimal effect on cognition, the sample size in this study may not have been able to detect the difference. Finally, this study merely analyzed a single polymorphism, *BACE1* rs638405, overlooking other potentially essential *BACE1* polymorphisms. For instance, a recent report demonstrated that two polymorphisms (918G/A, rs4938369; 2014T/C, rs3017608) in the *BACE1* promoter are associated with AD susceptibility [17]. Thus, before arbitrarily excluding participation of *BACE1* in cognitive function, further association studies might benefit from investigating these polymorphisms, or even additional tag-SNP markers selected from public SNP databases.

Our study has strengths and limitations. We were able to employ genetic analysis in a relatively large sample that met the requirements recommended by previous researchers [8]. However, our study was limited by several factors. The first is the nature of its cross-sectional design. It is difficult to determine whether the *BACE1* rs638405 polymorphism influences age-related or, alternatively, aging-related brain GM changes in normal subjects. Second, we only included normal subjects in this study. Future studies including normal controls and AD patients might add knowledge as to the *BACE1* genetic effect on AD pathogenesis.

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Disclosure Statement

No competing financial interests exist.

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