

Thornton et al., 2006). Although findings to date on specific effects of these two agents on cognition have been somewhat inconsistent, beneficial effects of risperidone on several cognitive domains including memory (Bilder et al., 2002; Keefe et al., 2006; Kern et al., 1999), executive function (Keefe et al., 2006; Meltzer and McGurk, 1999; Rossi et al., 1997), and working memory (Keefe et al., 2006; Meltzer and McGurk, 1999; Mori et al., 2004), and olanzapine on memory (Keefe et al., 2006; Meltzer and McGurk, 1999; Mori et al., 2004; Sumiyoshi et al., 2005; Thornton et al., 2006) and executive function (Bender et al., 2006; Bilder et al., 2002; Keefe et al., 2006; Meltzer and McGurk, 1999) have been reported. Precedent studies have demonstrated that atypical antipsychotics had favorable effects especially on verbal memory out of memory function (Meltzer and McGurk, 1999), which was not in harmony with our results that suggested favorable effects of atypical agents on visual memory instead of verbal memory. In general, our results might be consistent with prior studies reporting superiority of atypical to conventional antipsychotics in terms of cognitive function. In the present study, however, clinical characteristics such as duration of medication, number of hospitalizations, and antiparkinsonian drug use were significantly different between the two medication groups. Moreover, CPZeq reached nearly significant difference between the two groups. In this study, therefore, causal relationship between medication type (conventional/atypical) and cognitive function is quite difficult to argue due to these confounders.

#### 4.3. Limitations

There were several limitations to the current study. The cross-sectional nature of the study did not allow drawing any definite conclusions regarding causality between antipsychotic medication and its correlates. Since control subjects in the present study performed better than the general population on the standardized WMS-R and WAIS-R, it was possible that the differences between patients and controls were exaggerated. As the patients involved in this study suffered from chronic schizophrenia, the findings can not be generalized to recent-onset schizophrenia. The sample size was not very large, which may have resulted in type II errors.

#### 4.4. Conclusion

In conclusion, this study confirms that patients with chronic schizophrenia have wide-ranging cognitive impairments and provides evidence for an association

between antipsychotic medication (both standard/non-standard and conventional/atypical) and cognitive function. The differences of medication and of cognitive function are associated but both could be due to original illness severity. Alternatively, cognitive deficits in schizophrenia could be reduced in part by specific medication pattern, especially by atypical antipsychotic monotherapy at ordinary dosage.

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## The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology

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### Abstract

We investigated the effects of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism on age-associated changes of brain morphology in 109 Japanese healthy subjects using MRI with optimized voxel-based morphometry technique. A significant age-related volume reduction was found in the dorsolateral prefrontal cortices (DLPFC), anterior cingulate cortices, and temporal and parietal cortices in all subjects. Further analysis revealed a significantly negative correlation between age and the volume of the bilateral DLPFC only in the Met-BDNF carriers, and a significant interaction between the polymorphism and age-associated volume changes in the bilateral DLPFC. Furthermore, Met-carriers showed a significant interaction ( $p < 0.0001$ ) between the gender and the genotype on the gray matter volume in the DLPFC, and female Met-carriers showed more widespread age-associated volume reduction in DLPFC than male Met-carriers. Our data suggest that the Val66Met polymorphism may impact on age-related changes of the brain, which might be associated with the functional variance of neuroprotective effects of the BDNF. Furthermore, we suggest that genotype effects of the BDNF gene on brain morphology might differ in female from in male.

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**Keywords:** Brain-derived neurotrophic factor; Val66Met polymorphism; Magnetic resonance imaging; Voxel-based morphometry; Dorsolateral prefrontal cortex; Aging

Brain-derived neurotrophic factor (BDNF), a member of neurotrophin family, has important roles in hippocampal plasticity and hippocampal-related learning and memory through long-term potentiation [15]. It also plays an important role in preventing death of neurons during development and protecting cholinergic neurons of the basal forebrain and the hippocampus from induced death in the adult brain [21].

A common missense polymorphism of the BDNF gene producing a valine to methionine amino acid substitution (Val66Met) affects the activity dependent secretion of BDNF in neurons and affects memory function [6,8]. Neuroimaging studies revealed that this polymorphism affected memory-related

neuronal activities measured by functional magnetic resonance imaging (MRI) and macroscopic morphology of the hippocampus [8,12,23,28]. Regarding the brain morphology in normal individuals, Pezawas et al. [23] reported that Met-BDNF carriers had smaller volumes of the hippocampi and the prefrontal cortices as compared to individuals with homozygous Val-BDNF. This result was recently replicated in another mixed study of healthy and schizophrenic subjects [28]. Although several neuroimaging studies have indicated that environmental factors considerably impact on human brain structures even in normal adult brains [18], these data suggest that genetic factors such as polymorphism of BDNF might also strongly affect human brain morphology, and contribute to individual differences of brain morphology.

Aging is another factor which strongly affects brain morphology in human. There are several studies that demon-

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strated morphological changes associated with normal aging in vivo [10,24]. A general trend in the in vivo volumetric studies of healthy volunteers points to the prefrontal cortex as the cortical region in which the largest age-related volume reduction is observed. Considering the previous findings that BDNF is expressed abundantly in the prefrontal cortex [25] and that BDNF has a neuroprotective effect, Val66Met polymorphism might have some impacts on age-related morphological changes. However, there is no datum whether this polymorphism is associated with age-related morphological changes.

To clarify whether the BDNF polymorphism impacts on morphological changes associated with aging, we analyzed structural MR images in 109 normal individuals using optimized voxel-based morphometry (VBM) technique.

One hundred and thirty healthy subjects participated in the study. Written informed consent was obtained from all subjects in accord with ethical guidelines in place at local ethical committee. All of the subjects were recruited from local advertisements and underwent a Japanese version of National Adult Reading Test (JART) that is essentially the same as National Adult Reading Test [22] and MRI scanning. We employed JART as a convenient tool to measure IQ for each participant because previous study reported that it showed high correlation with IQ in healthy subjects [20]. All subjects were screened by a questionnaire regarding medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the central nervous system, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, asymptomatic or symptomatic cerebral infarction detected by T2 weighted MRI, hypertension, chronic lung disease, kidney disease, chronic hepatic disease, cancer, or diabetes mellitus. Template creation for the optimized VBM was based on a sample of the 120 subjects, aged  $36.2 \pm 12.1$  years (range 20–72). All subjects were Japanese. Since single nucleotide polymorphism (SNP) genotyping, described in the next section, was done successfully in 109 subjects, the MR images of these 109 subjects were used for subsequent analyses. According to the polymorphism, subjects were categorized into the following three groups: a homozygous Val-BDNF group ( $n=41$ ), a Val/Met-BDNF group ( $n=51$ ), or a homozygous Met-BDNF group ( $n=17$ ). The genotype distribution of this SNP was not deviated with Hardy–Weinberg equilibrium ( $\chi^2=0.03$ ,  $p=0.86$ ). Because of the small number of subjects with homozygous Met-BDNF, the Val/Met-BDNF group and homozygous Met-BDNF group were treated as one group, the Met-BDNF carriers ( $n=68$ ). The demographic data of these groups are the following; the homozygous Val-BDNF comprised 26 females and 15 males, two were left-handed, aged  $36.9 \pm 13.0$  years (range 21–68), and the mean education period and JART score were  $16.2 \pm 2.8$  years (range 12–24) and  $75.5 \pm 13.3$  (equivalent to  $108.8 \pm 9.55$  for full scale IQ (range 50–96; equivalent to 90.5–123.6 for full scale IQ), respectively. The Met-BDNF carriers comprised 45 females and 23 males, three were left-handed, aged  $35.8 \pm 11.6$  years (range 20–72), and their mean education period and JART score were  $16.9 \pm 3.0$  years (range 12–28) and  $78.0 \pm 11.6$  (equivalent to  $110.7 \pm 8.3$ ) for full scale IQ (range

45–99; equivalent to 86.9–125.8 for full scale IQ), respectively. The mean age, gender ratio, handedness, education period, or JART score did not differ between the two groups (two sample *t*-test, data not shown).

The detail process of genotyping of BDNF Val66Met SNP (dbSNP accession: rs6265) was described previously [13]. Primers and probes for detection of the SNP (TaqMan SNP Genotyping assays on demand) were purchased from Applied Biosystems (ABI, Foster City, CA, USA). PCR cycling conditions were: at 95 °C for 10 min, 50 cycles of 92 °C for 15 s and 60 °C for 1 min.

All MR studies were performed on a 1.5 T Siemens Magnetom Vision plus system. A three dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of 144 sagittal sections using an MPRage sequence (TE/TR, 4.4/11.4 ms; flip angle, 15°; acquisition matrix, 256 × 256; INEX, field of view, 31.5 cm; slice thickness, 1.23 mm).

Data were analyzed with Statistical Parametric Mapping 2 (SPM2) (<http://www.fil.ion.ucl.ac.uk/spm/>; Wellcome Department of Imaging Neuroscience, London, UK) running on MATLAB 6.5 R1 (MathWorks, Natick, MA). Before analyses, each image was confirmed by a neuroradiologist to eliminate images with artifacts, and then anterior commissure–posterior commissure line was adjusted. First, we made a customized anatomical T1 template and prior probability images from the sample of 120 brains [10]. Then, images were processed using an optimized VBM script ([dbm.neuro.uni-jena.de/vbm.html](http://dbm.neuro.uni-jena.de/vbm.html)). The detail of this process is described elsewhere [2,10]. The normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization function to encode the deformation field for each subject as tissue density changes in the normal space. Finally, images were smoothed using a 12 mm full width half maximum of isotropic Gaussian kernel. Statistical analyses were performed with SPM2, which implemented a General Linear Model. Proportional scaling was used to achieve global normalization of voxel values between images. First, we used a two-sample *t*-test to test regional population effect on gray matter volume. For this analysis, we set  $p < 0.005$  without a correction for multiple comparisons, followed by applying small volume correction to each cluster with a false discovery rate (FDR) < 0.05. For the small volume correction, spheres with radius 10 mm around the peak were set as regions of interest (ROIs). The resulting sets of *t*-values constituted the statistical parametric maps {SPM (*t*)}. Anatomic localization was according to both MNI coordinates and Talairach coordinates, obtained from M. Brett's transformations (<http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml>) and presented as Talairach coordinates. Since a previous study with Caucasians demonstrated a significant reduction of volumes in the hippocampi and the frontal cortices in Met-BDNF carriers, we applied an additional hypothesis-driven ROI method to test regional population effects in these regions by using the Wake Forest University PickAtlas [19].

The genotype effects on age-related morphological changes were tested using a single subject condition and covariate model. Since several studies reported gender different age-related mor-

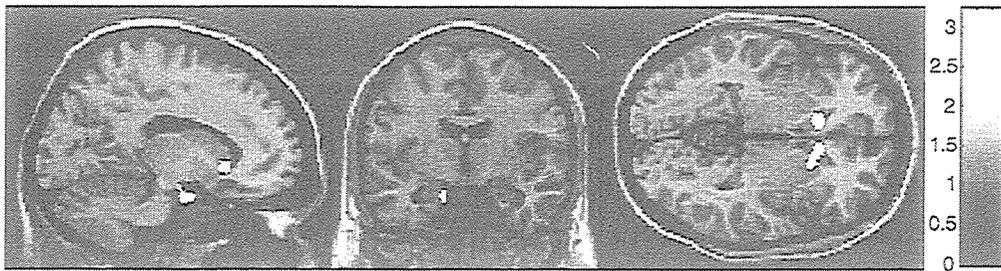


Fig. 1. The volume reduction of Met-BDNF carriers compared to that of individuals with homozygous Val-BDNF ( $p < 0.05$ , small volume correction with FDR). A significant reduction of volumes of the left parahippocampal gyrus ( $t$ -value: 2.92, Talairach coordinates (TAL):  $-12, -3, -19$ ) and the bilateral heads of caudate nucleus (left:  $t$ -value: 3.23, TAL:  $-9, 22, -3$ , right:  $t$ -value: 3.02, TAL:  $10, 21, -4$ ) in the Met-BDNF carriers was noted.

phological changes in the brain [7], we additionally examined genotype effects on age-related morphological changes in each gender, separately. Orthogonalized first order polynomial expansion of age was treated as a covariate of interest to determine the linear effects of age [5]. Since second- and third-order polynomial expansions did not contribute to the age effect model of our sample, we removed them from a design matrix. Considering the possible association between IQ and brain morphology, we treated JART score as a nuisance variable. For this analysis, we applied  $p < 0.001$ , corrected for multiple comparisons with FDR  $< 0.05$  as a statistical threshold [9]. MarsBar program (marsbar.sourceforge.net/) was also used to extract data from the regions of interest.

Fig. 1 shows a significant reduction of gray matter volumes of the left parahippocampal gyrus (Brodmann area (BA) 34), and bilateral heads of the caudate nucleus in Met-BDNF carriers when compared to homozygous Val-BDNF individuals. Even in hypothesis-driven ROI approach with a lenient statistical threshold (uncorrected  $p = 0.05$ ), we could not find any significant differences of hippocampal nor prefrontal cortical volumes between the two groups. The results were essentially unchanged

even when the restricted samples of subjects (female group, male group, or young group aged under 40 years old) were analyzed (data not shown).

Fig. 2 shows morphological changes related to normal aging. A significant negative correlation between age and the gray matter volumes was noted in the bilateral dorsolateral prefrontal cortices (DLPFC; BA9, 46), right superior temporal gyrus (STG; BA22), bilateral insulae (BA13), bilateral caudate nuclei, left anterior cingulate gyrus (BA24), bilateral inferior parietal lobules (BA40), bilateral precuneus (BA7), and bilateral fusiform gyri (BA37) in all subjects. In homozygous Val-BDNF individuals, a significant age-related volume reduction was found in the bilateral insulae (BA13) and right STG (BA22). On the other hand, Met-BDNF carrier showed an additional negative correlation of the gray matter volumes in the bilateral DLPFC (BA9, 46) and right dorsal premotor area (BA6) with age. Additional analyses in each gender revealed a significant interaction ( $p < 0.0001$ ) in Met-carriers between the gender and the genotype on the gray matter volume in the DLPFC, and female Met-carriers showed more widespread age-associated volume reduction in DLPFC than male Met-carriers. Male Met-carrier also showed volume

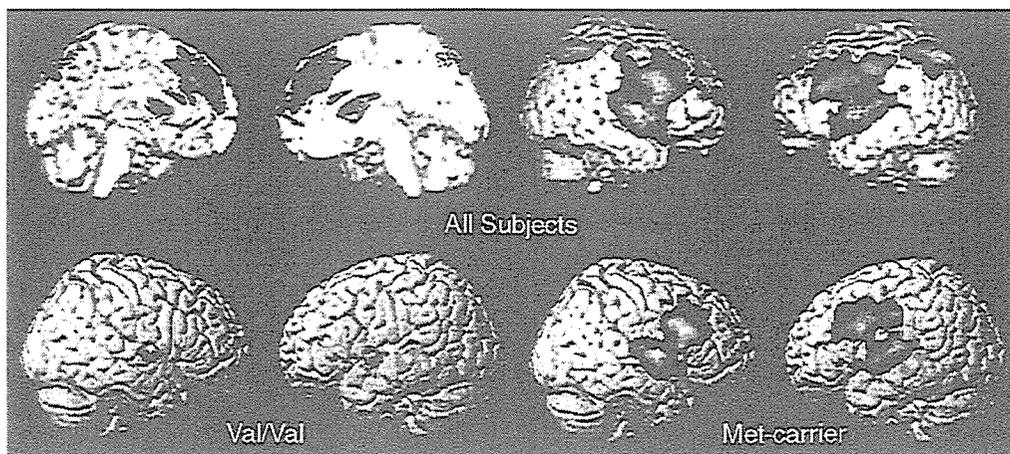


Fig. 2. (Top) The volume reduction associated with normal aging in all subjects ( $p < 0.05$ , FDR corrected). All subjects showed negative correlation with age in the bilateral DLPFC, right STG, bilateral insulae, bilateral caudate nuclei, left anterior cingulate gyrus, bilateral inferior parietal lobules, bilateral precuneus, and bilateral fusiform gyri. (Bottom) The volume reduction associated with normal aging in each genotypic group ( $p < 0.05$ , FDR corrected). (Left) Results of individuals with homozygous Val-BDNF. Individuals with homozygous Val-BDNF showed negative correlation with age in the bilateral insulae (right:  $t$ -value: 4.36, TAL:  $42, -2, 4$ ; left:  $t$ -value: 4.52, TAL:  $-43, -2, 4$ ) and the right superior temporal gyrus ( $t$ -value: 4.57, TAL:  $47, 9, -4$ ). (Right) Results of Met-BDNF carriers. The Met-BDNF carriers showed negative correlation with age in the bilateral dorsolateral prefrontal cortices (right:  $t$ -value: 6.5, TAL:  $52, 21, 26$ ; left:  $t$ -value: 6.12, TAL:  $-48, 19, 32$ ) as well as the bilateral insulae and the superior temporal gyri.

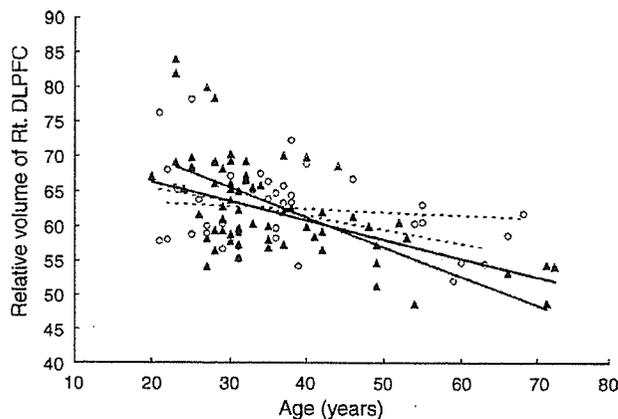


Fig. 3. Scatter plot of relative gray matter volume of the right DLPFC against age in each genomic group. The Met-BDNF carriers showed more significant volume reduction with normal aging compared to homozygous Val-BDNF subjects in the bilateral DLPFC in each gender (right: male Met-BDNF carriers:  $y = -0.27x + 71.8$ ,  $r = -0.71$ ,  $p < 0.0001$ , male homozygous Val-BDNF subjects:  $y = -0.046x + 64.2$ ,  $r = -0.12$ ,  $p = 0.67$ , female Met-BDNF carriers:  $y = -0.43x + 78.4$ ,  $r = -0.56$ ,  $p < 0.001$ , female homozygous Val-BDNF subjects:  $y = -0.20x + 69.5$ ,  $r = -0.41$ ,  $p = 0.03$ ; left: male Met-BDNF carriers:  $y = -0.20x + 67.2$ ,  $r = -0.53$ ,  $p = 0.01$ , male homozygous Val-BDNF:  $y = -0.11x + 65.3$ ,  $r = -0.25$ ,  $p = 0.367$ , female Met-BDNF carriers:  $y = -0.48x + 77.0$ ,  $r = -0.71$ ,  $p < 0.0001$ , female homozygous Val-BDNF:  $y = -0.14x + 65.3$ ,  $r = -0.27$ ,  $p = 0.18$ ). Due to limitations of space, only the plot at the right DLPFC in each gender is shown. Blue stands for male subjects and red stands for female subjects. Open circle: homozygous Val-BDNF; closed triangle: Met-BDNF carrier. Dotted lines are the regression line of homozygous Val-BDNF, whereas solid lines are those of Met-BDNF carrier.

reduction in the right inferior parietal lobules (BA40,  $t$ -value: 3.86, Talairach coordinates: 40, -43, 53). We found a significant interaction effect (male:  $p = 0.003$ , female:  $p < 0.0001$ ) between the aging effect and the genotype on the gray matter volume in the DLPFC in each gender. (right: male Met-BDNF carriers:  $r = -0.71$ ,  $p < 0.001$ , male homozygous Val-BDNF subjects:  $r = -0.12$ ,  $p = 0.67$ ; female Met-BDNF carriers:  $r = -0.56$ ,  $p < 0.001$ , female homozygous Val-BDNF subjects:  $r = -0.41$ ,  $p = 0.03$ ; left: male Met-BDNF carriers:  $r = -0.53$ ,  $p = 0.01$ , male homozygous Val-BDNF subjects:  $r = -0.25$ ,  $p = 0.367$ , female Met-BDNF carriers:  $r = -0.71$ ,  $p < 0.0001$ , female homozygous Val-BDNF subjects:  $r = -0.27$ ,  $p = 0.18$ ) (Fig. 3).

This is the first study which investigated the impacts of BDNF Val66Met polymorphism on age-associated brain morphological changes in normal individuals. We found an exaggerated age-related volume reduction of the DLPFC in the Met-BDNF carriers.

Several studies demonstrated morphological changes associated with normal aging in the STG, insula, inferior parietal lobules, motor cortex, ACC, and DLPFC [10,24]. In consistent with previous studies, our data also showed age-related volume reduction in similar regions in all subjects' analysis of each gender. Further analysis revealed that the Met-BDNF carriers showed a stronger negative correlation between age and gray matter volume in the DLPFC and right precentral gyrus when compared to individuals with homozygous Val-BDNF. Though the mechanisms underlying the predilection of the prefrontal

cortex for age-related volume reduction are still unclear, the prefrontal cortex exhibits the greatest age-related alteration of GABA and glutamate [11], and glucose metabolism and age-related declines in regional cerebral blood flow [4]. Though there has been no study investigating the relationship between Val66Met SNP and vulnerability to age-related changes, BDNF protein itself is reported to be associated with aging. Amounts of BDNF protein in hippocampal pyramidal neurons and dentate granule cells are decreased during aging in monkeys [14]. Further, several studies demonstrated neuroprotective effects of BDNF [3,29]. Our data suggest that the Met-BDNF carriers, particularly females carrying Met-BDNF allele, may be more vulnerable to aging than individuals with homozygous Val-BDNF. Considering the fact that prefrontal cortex is one of the regions in which BDNF is expressed abundantly [25], we suggest that the Val66Met polymorphism may be associated with functional variances of neuroprotective and stress resistant effects of BDNF, which results in different effects on age-related morphological changes. Furthermore, we found a reduction of the striatal volumes in met-BDNF carriers as compared to individuals with homozygous Val-BDNF. It has been postulated that enhancement of BDNF in the cortex may be involved in protection of striatal neurons against damage via anterograde transport because BDNF exerts neuroprotective effects against excitotoxicity in the striatum [1,16]. The result, reduced volumes in the striatum in met-BDNF carriers, may again suggest the reduced neuroprotective effects of met-BDNF. Since there has been no direct evidence of differential regulation of vulnerability to neurodegenerative process by BDNF Val66Met polymorphism, further study such as investigating how Val66Met SNP affects cell survival in a cellular model is required to clarify our speculation.

Although we could not replicate results of the previous studies, the smaller hippocampus in the Met-BDNF carriers [23,28], our data also suggest that BDNF polymorphism should have impacts on brain morphology associated with episodic memory. The discrepancy between our results and those of the previous studies could be partially explained by the racial difference. Binding its receptor TrkB, BDNF activates several pathways including the PI3-kinase/Akt, the mitogen-activated protein kinase, and PLC-gamma1 pathway [15]. These signals are known to be critical for survival of neuron, suggesting that not only Val66Met polymorphism of BDNF, but also interaction of polymorphism of each signal or molecule has effects on brain morphology. Racial differences might be related to such interactions, resulting in the different findings. This may partially contribute to the discrepancy in associations between BDNF polymorphism and the prevalence of neuropsychiatric diseases in Asian and Caucasian populations [17,27].

Finally, we mention a limitation of this study. To explore the association between aging effects on the brain morphology and the Val66Met polymorphism, we performed a cross-sectional study. There is a secular bias, which can be resolved by a longitudinal study. In this context, our data may be considered preliminary rather than conclusive. However, a recent longitudinal MR study of normal aging demonstrated that cross-sectional and longitudinal estimates of atrophy rates were similar [26].

In conclusion, we found that Val66Met polymorphism of BDNF had impacts on age-associated morphological changes in Japanese subjects. Our data suggest that Val66Met polymorphism of BDNF may play important roles for vulnerability to age-related morphological changes as well as the efficiency of plasticity, especially in DLPFC. Furthermore, we suggest that genotype effects of the BDNF gene on brain morphology might differ in female from in male.

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