

## ORIGINAL ARTICLE

# Effects of the BDNF Val66Met Polymorphism on Gray Matter Volume in Typically Developing Children and Adolescents

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## Abstract

The Val66Met polymorphism of brain-derived neurotrophic factor (BDNF) is associated with psychiatric disorders and regional gray matter volume (rGMV) in adults. However, the relationship between BDNF and rGMV in children has not been clarified. In this 3-year cross-sectional/longitudinal (2 time points) study, we investigated the effects of BDNF genotypes on rGMV in 185 healthy Japanese children aged 5.7–18.4 using magnetic resonance imaging (MRI) and voxel-based morphometry (VBM) analyses. We found that the volume of the right cuneus in Met homozygotes (Met/Met) was greater than in Val homozygotes (Val/Val) in both exams, and the left insula and left ventromedial prefrontal cortex volumes were greater in Val homozygotes versus Met homozygotes in Exam 1. In addition, Met homozygous subjects exhibited higher processing speed in intelligence indices than Val homozygotes and Val/Met heterozygotes at both time points. Longitudinal analysis showed that the left temporoparietal junction volume of Val/Met heterozygotes increased more substantially over the 3-year study period than in Val homozygotes, and age-related changes were observed for the Val/Met genotype. Our findings suggest that the presence of 2 Met alleles may have a positive effect on rGMV at the developmental stages analyzed in this study.

**Key words:** cross-sectional study, development, longitudinal study, MRI, voxel-based morphometry

## Introduction

Brain-derived neurotrophic factor (BDNF) is an essential regulator of neuronal growth, differentiation, distribution, and survival (Poo 2001; Bartkowska et al. 2010; Notaras et al. 2015). BDNF plays a central role in neuronal plasticity (Park and Poo 2013), and BDNF

knockout mice exhibit disrupted development of postnatally born hippocampal neurons (Gao et al. 2009). Changes in BDNF levels during development may have a significant impact on behavioral and neuroanatomical changes (Casey et al. 2009); however, the roles of BDNF in human development have not been well characterized.

The human *BDNF* gene is located on chromosome 11. The most common *BDNF* polymorphism is a single-nucleotide substitution of A for G at nucleotide position 196 (G196A), which results in amino acid substitution of methionine (Met) for valine (Val) that is called Val66Met (rs6265) (Hong et al. 2011; Czira et al. 2012). This single-nucleotide polymorphism (SNP) is located in the prodomain of the *BDNF* gene and leads to impaired intracellular processing, trafficking, and extracellular secretion (Egan et al. 2003) and may cause aberrant gray matter growth (Chen et al. 2006) and neural plasticity (Cheeran et al. 2008; Lamb et al. 2015). It has been suggested that Val66Met affects memory and cognition (Hariri et al. 2003; Lamb et al. 2015), and this SNP is associated with neuropsychiatric disorders including schizophrenia, depression, autism, eating disorders, and Alzheimer's disease (Notaras et al. 2015).

Reports on the relationship between the Val66Met SNP and the function and morphology of various brain structures are inconsistent. In humans, both reducing (Pezawas et al. 2004; Bueller et al. 2006; Montag et al. 2009) and increasing (Liu et al. 2014) effects of the Val66Met SNP on regional brain volume have been shown. Most studies conducted in Caucasians have merged Met homozygotes (Met/Met) and Val/Met heterozygotes into a single group of Met carriers, with the exception of a Sardinian cohort (Terracciano et al. 2010). However, in studies of Asian subjects, Met homozygotes showed different characteristics of brain morphology and cognitive functions compared with Val/Met heterozygotes (Nemoto et al. 2006; Liu et al. 2014). Therefore, further investigations comparing the 3 major genotypic groups (Val/Val, Val/Met, and Met/Met) are needed to clarify the effects of the Val66Met polymorphism on brain development, structure, and function.

Cross-sectional studies have suggested that there are age-dependent effects of Val66Met on brain morphology (Nemoto et al. 2006; Sublette et al. 2008), but these have not been well investigated in children and adolescents (Mueller et al. 2013) with the exception of a neonatal study (Knickmeyer et al. 2014). To elucidate the effects of Val66Met on neuronal and cognitive development on children, we conducted a cross-sectional and longitudinal analysis of Val/Val, Val/Met, and Met/Met individuals in a cohort of 185 healthy Japanese children by examining Met homozygotes (Met/Met) independently from Val/Met heterozygotes and then comparing these groups with Val homozygotes (Val/Val).

## Materials and Methods

### Subjects

All subjects were healthy, right-handed Japanese children. We collected brain magnetic resonance imaging (MRI) scans from 290 subjects (145 males and 145 females; age range, 5.6–18.4 years) who did not have any history of malignant tumors or head trauma involving loss of consciousness. Based on self-reporting, children with a history of epilepsy, impaired color vision, diagnosis of developmental disorders, routine visits to a hospital because of illness, congenital disorders, or routine use of medications (except for over-the-counter drugs such as cold or anti-allergy medications) were excluded during the recruitment processes. We did not use specific diagnostic tools, although 1 author (Y.T.) is a radiologist who thoroughly checked the T<sub>1</sub>-weighted structural images for undiagnosed neurological diseases.

In accordance with the Declaration of Helsinki, written informed consent was obtained from each subject and his/her

parent prior to MR scanning and after a full explanation of the purpose and procedures of the study. This study was approved by the institutional review board of Tohoku University.

Exam 2 was conducted approximately 3 years after Exam 1, and 235 subjects participated. Due to issues with the quality of the imaging data (1 subject) and lack of *BDNF* genotype information (49 subjects), imaging analyses were performed in 185 subjects (95 males and 90 females). The mean interval between the 2 exams in these 185 subjects was 1108 days (623–1387 days). Effects of the interval were regressed out as a covariate of no interest in brain imaging analyses.

### Neuropsychological Testing

In both exams, trained examiners conducted intelligence tests using the Japanese version of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) or Wechsler Intelligence Scale for Children-Third Edition (WISC-III) for subjects older or younger than 16 years, respectively. The full-scale IQ (FSIQ), verbal IQ (VIQ), and performance IQ (PIQ) and 4 index scores (verbal comprehension index [VCI], perceptual organization index [POI], processing speed index [PSI], and working memory index [WMI]) were calculated as described elsewhere (Yokota et al. 2015). The FSIQs were >70 for all subjects.

### Subject Genotyping

High molecular weight DNA was isolated from saliva with Oragene containers (DNA Genotek Inc., Ottawa, Ontario, Canada), according to the manufacturer's protocol. The *BDNF* 196-G>A (rs6265) polymorphism (Val66Met) was genotyped using TaqMan analysis (assay ID: C\_11592758\_10; Applied Biosystems, Foster City, CA, USA). Genotyping was conducted in a 10- $\mu$ L volume containing 20 ng genomic DNA, 5  $\mu$ L TaqMan Mastermix (Applied Biosystems), 0.25  $\mu$ L TaqMan assay reagent, and 2.25  $\mu$ L H<sub>2</sub>O. Genotyping was performed on a CFX96™ Real-Time Polymerase Chain Reaction Detection System, and genotypes were scored using the algorithm and software supplied by the manufacturer (BioRad, Hercules, CA, USA). The genotyping assays were validated by a duplicate measurement, and blanks were used as quality controls throughout genotyping.

### Image Acquisition

All images were collected using a 3-T Philips Intera Achieva scanner (Philips, Amsterdam, the Netherlands). Three-dimensional, high-resolution, T<sub>1</sub>-weighted images (T1WI) were collected using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence. The parameters were as follows: 240 × 240 matrix, TR = 6.5 ms, TE = 3 ms, TI = 711 ms, FOV = 24 cm, 162 slices, 1.0 mm slice thickness, and scan duration of 8 min and 3 s.

### Structural Data Preprocessing and Analysis

Preprocessing of the MRI data was performed using Statistical Parametric Mapping software (SPM8; Wellcome Department of Cognitive Neurology, London, UK) following the protocol for voxel-based morphometry (VBM) analysis reported in our previous study (Hashimoto et al. 2015). Then, rGMV was calculated. T1WIs of each individual were segmented into 6 tissue sections using the new segmentation algorithm implemented in SPM8. In this protocol, the default tissue probability map (TPM) for gray matter was manipulated from maps implemented in the software such that the voxels in which the gray matter tissue probability of the default tissue gray matter TPM plus the white

matter tissue probability of the default TPM <0.25 were assigned a value of 0. This protocol makes it less likely for the dura matter to be classified as gray matter than when the default gray matter TPM is used, provided there are no other significant segmentation problems.

In this novel segmentation process, default parameters were used, except in the case of affine regularization, which was performed using the International Consortium for Brain Mapping (ICBM) template for East Asian brains. We then proceeded into the diffeomorphic anatomical registration through exponentiated lie (DARTEL) algebraic registration process implemented in SPM8. In this process, we used DARTEL-imported images of the 6 TPMs created using the above-mentioned segmentation process. First, the template for the DARTEL procedures was created using the T1WI data from the Exam 1 scans of all the subjects. Next, using this existing template, DARTEL procedures were performed using both T1WI scans of all of the subjects included in the study and the default parameter settings. The resulting images were then spatially normalized to the Montreal Neurological Institute (MNI) space to obtain images with  $1.5 \times 1.5 \times 1.5 \text{ mm}^3$  voxels. In addition, we performed a volume change correction (modulation) by modulating each voxel with the Jacobian determinants derived from the spatial normalization, allowing for the determination of regional differences in the absolute amount of brain tissue. Subsequently, all images were smoothed by convolving them with an isotropic Gaussian kernel of 8-mm full width at half maximum (FWHM), which is the standard version of the smoothing for DARTEL. Smoothing kernel of 8–10 mm is appropriate for DARTEL-VBM (Shen and Sterr 2013), although a 12-mm FWHM has been recommended for cluster-forming thresholds (Silver et al. 2011). The resulting rGMV calculations were used for cross-sectional imaging analyses.

Finally, the signal change in rGMV between Exam 1 and 2 images was computed at each voxel for each participant. In this computation, we included only voxels that showed GMV values >0.10 in both exams to effectively limit the images to areas likely to be GM. The resulting maps representing the rGMV change

between the MRI scans (rGMVexam – rGMVexam1) were then used for longitudinal imaging analyses.

### Structural Data Statistical Analysis

Statistical analyses of imaging data were performed with SPM8. To assess the rGMV differences among the 3 genotypic groups, analysis of covariance (ANCOVA) was employed using age, sex, and the interval between Exam 1 and Exam 2, and changes of total intracranial volume were considered covariates of no interest. F-contrasts of SPM were not applicable because of the statistical (cluster level) threshold described below.

In addition, the effects of PSI on rGMV were investigated with ANCOVA using PSI scores as a covariate of interest, because there were significant differences in PSI between BDNF genotypic groups in both exams. The statistical significance level was set as  $P < 0.05$  (family-wise error corrected at the cluster level) with an uncorrected  $P < 0.001$  at the voxel level. In addition, correlations (Pearson's  $r$ ) between PSI scores and rGMV of the region detected by the above-mentioned analysis were calculated. Finally, the effects of age on rGMV changes over 3 years in 3 genotypes were analyzed by dividing subjects into younger and older age groups using 1-way analysis of variance (ANOVA), because age-related rGMV changes could be observed during development (Brain Development Cooperative 2012) in longitudinal analyses.

## Results

### Genotypic Distribution

The BDNF genotypic distributions of the 185 sampled subjects were as follows: Val/Val ( $n = 68$ , 36.8%), Val/Met ( $n = 84$ , 45.4%), and Met/Met ( $n = 33$ , 17.8%) (Tables 1 and 2). Note that the Met allele is widely prevalent in the Japanese population (Kunugi et al. 2004). Tests for the Hardy–Weinberg equilibrium exhibited no deviations from the expected genotype distribution ( $\chi^2 = 0.63$ ,  $P > 0.05$ ). There were no significant differences in age ( $F = 0.04$ ,  $P = 0.39$ ) or sex ( $\chi^2 = 0.09$ ,  $P = 0.96$ ).

### Differences in IQ Scores

A 3-way ANOVA (3 BDNF genotypes  $\times$  2 exams  $\times$  7 IQ scores) was used to reveal IQ score differences between BDNF genotypes using Statistical Package for Social Science software (SPSS ver. 22). There were significant main effects for exams ( $F = 16.98$ ,  $P < 0.001$ ) and IQ scores ( $F = 8.68$ ,  $P < 0.001$ ) (Table 3). In addition, there were significant interactions for Exams  $\times$  IQ scores ( $F = 8.06$ ,  $P < 0.001$ ) and Genotypes  $\times$  IQ scores ( $F = 3.60$ ,  $P < 0.001$ ) (Table 3). Post hoc analysis (Ryan's method) of the genotypes  $\times$  IQ scores

Table 1 Demographic information

	BDNF genotype		
	Val/Val	Val/Met	Met/Met
N (%)	68 (36.8)	84 (45.4)	33 (17.8)
Male/Female	34/34	44/40	17/16
Exam 1 Age (mean $\pm$ SD)	11.5 $\pm$ 3.2	11.3 $\pm$ 3.1	10.6 $\pm$ 3.0
Exam 2 Age (mean $\pm$ SD)	14.5 $\pm$ 3.2	14.4 $\pm$ 3.1	13.6 $\pm$ 3.0

Table 2 Mean IQ and index scores  $\pm$  SD of each genotypic group in Exams 1 and 2

	Exam 1			Exam 2		
	Val/Val	Val/Met	Met/Met	Val/Val	Val/Met	Met/Met
FSIQ	100.8 $\pm$ 11.5	103.5 $\pm$ 10.8	101.9 $\pm$ 13.7	103.9 $\pm$ 13.4	105.0 $\pm$ 11.4	103.9 $\pm$ 12.5
PIQ	99.4 $\pm$ 12.9	100.7 $\pm$ 11.7	101.1 $\pm$ 13.9	103.4 $\pm$ 13.5	102.8 $\pm$ 11.7	103.8 $\pm$ 14.0
VIQ	101.6 $\pm$ 12.7	105.4 $\pm$ 11.7	102.2 $\pm$ 13.6	103.7 $\pm$ 14.9	106.1 $\pm$ 12.6	103.4 $\pm$ 11.2
VCI	101.3 $\pm$ 14.1	105.5 $\pm$ 13.0	101.6 $\pm$ 13.7	104.5 $\pm$ 15.8	107.1 $\pm$ 12.3	103.2 $\pm$ 12.1
POI	100.8 $\pm$ 13.9	101.6 $\pm$ 12.6	99.5 $\pm$ 15.6	102.1 $\pm$ 14.1	102.4 $\pm$ 13.0	100.2 $\pm$ 13.9
PSI	99.1 $\pm$ 12.0	99.9 $\pm$ 11.9	107.4 $\pm$ 14.1	104.9 $\pm$ 12.4	105.7 $\pm$ 11.9	113.0 $\pm$ 12.8
WMI	99.0 $\pm$ 12.8	99.7 $\pm$ 11.4	100.8 $\pm$ 13.4	98.7 $\pm$ 10.6	99.1 $\pm$ 12.9	100.2 $\pm$ 14.8

Note: FSIQ, full-scale IQ; PIQ, performance IQ; VIQ, verbal IQ; VCI, verbal comprehension index; POI, perceptual reasoning index; PSI, processing speed index; WMI, working memory index.

interaction identified by 3-way ANOVA demonstrated that Met/Met homozygotes exhibited a higher processing speed index (PSI) than Val/Val homozygotes ( $P = 0.001$ ) and Val/Met heterozygotes ( $P = 0.002$ ) (Fig. 1). There were no significant differences in the other IQ metrics (FSIQ, PIQ, VIQ, VCI, POI, or WMI) between genotypic groups.

### Cross-Sectional Differences in rGMV

VBM analyses showed significant differences between groups (Table 4). In Exam 1, Met/Met homozygotes showed a larger rGMV in the right cuneus than Val/Val homozygotes (Fig. 2B). In contrast, Val/Val homozygotes showed a larger rGMV in the left insula and the left ventromedial prefrontal cortex (VMPFC) than Met/Met homozygotes (Fig. 2A). In Exam 2, Met/Met homozygotes likewise displayed a larger rGMV in the right cuneus than Val/Val homozygotes (Fig. 3).

### Effects of PSI on rGMV

In Exam 1, greater effects of PSI on rGMV in the cerebellum ( $x, y, z$  coordinates:  $[-3, -39, -15]$ , 2187 voxels,  $Z$  score = 4.44) were

Table 3 3-way ANOVA results

	df	F	P
Main effects			
BDNF genotypes	2	0.43	0.64
Exam 1/2	1	16.98	<0.001
IQ scores	6	8.67	<0.001
Interactions			
Genotypes $\times$ Exam1/2	2	0.383	0.68
Genotypes $\times$ IQ scores	12	3.59	<0.001
Exam 1/2 $\times$ IQ scores	6	8.06	<0.001
Genotypes $\times$ Exam 1/2 $\times$ IQ scores	12	0.11	1.00

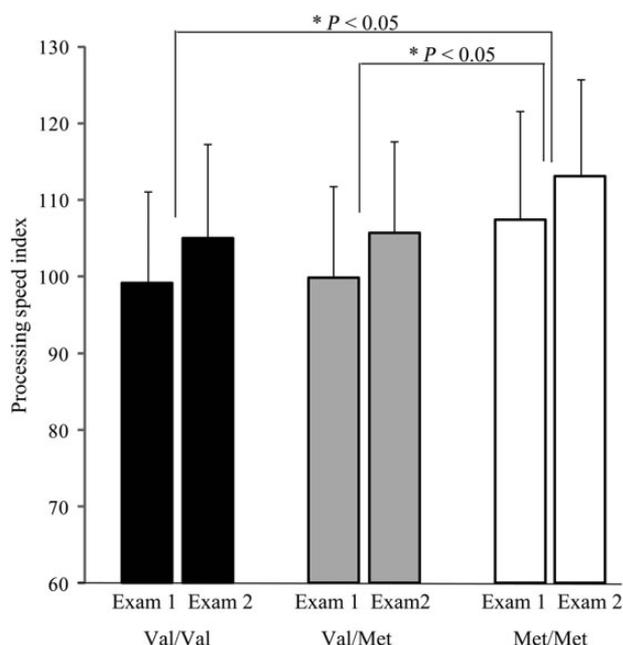


Figure 1. Processing speed index differences among the Met/Met, Val/Met, and Met/Met genotypic groups. Post hoc analysis (Ryan's method) of the 3-way ANOVA revealed significant differences in both Exams 1 and 2 between Met/Met and Val/Val individuals, and between Met/Met and Val/Met individuals.

observed for Met/Met than Val/Val, and significant correlations between PSI and the cerebellum volume in Met/Met ( $r = 0.36$ ,  $P < 0.05$ ) were detected (Fig. 4). No significant differences in the effects of PSI scores on rGMV were found between BDNF genotypes in Exam 2.

### Longitudinal Changes in rGMV

As shown in Table 4, Val/Met heterozygotes showed a significant rGMV increase in the left temporoparietal junction (TPJ) over the 3-year study period in comparison to the Val/Val homozygotes (Fig. 5). At younger ages, children with a Val/Met SNP displayed greater increases in rGMV, which decreased substantially at older ages; in contrast, Val (and also Met) homozygotes showed moderate rGMV changes with age (Fig. 5, bottom). Three (genotypes)  $\times$  2 (younger and older age groups) ANOVA revealed significant effects of age on rGMV ( $F_{1,179} = 12.74$ ,  $P = 0.0005$ ) and a significant genotype  $\times$  age interaction ( $F_{2,179} = 3.15$ ,  $P = 0.0455$ ). A significant simple main effect for Val/Met  $\times$  age ( $F_{1,179} = 16.86$ ,  $P < 0.0001$ ) was detected.

### Discussion

Effects of 3 BDNF Val66Met genotypes (Met/Met, Val/Met, and Val/Val) on cognitive and neural development in children were examined in the 3-year follow-up study. In cross-sectional analyses, we found that Met homozygotes had higher PSI scores than Val homozygotes and Val/Met heterozygotes in both exams. Compared with Val homozygotes, the right cuneus volume was greater in Met homozygotes in both exams. Longitudinal analysis revealed greater volume increases in the left TPJ of Val/Met heterozygotes than in Val/Val homozygotes. Thus, this is the first study to suggest that Met alleles of BDNF may convey cognitive and neural development advantages in children.

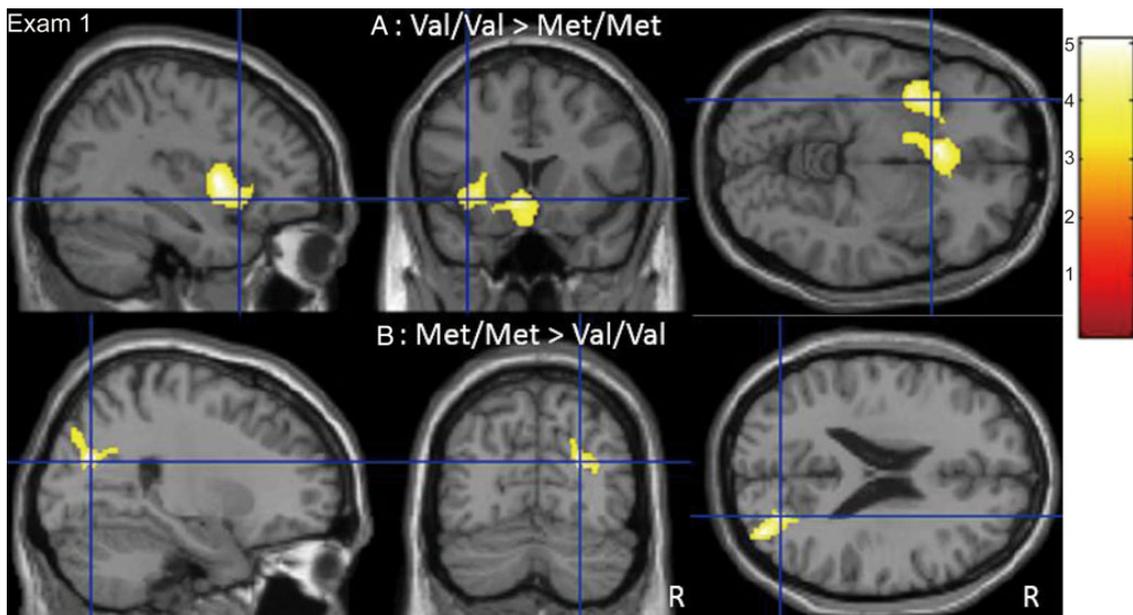
We were surprised to observe enhancement of cognitive and neural development in Met homozygous children since negative effects of the Met allele on brain development and function have been suggested. However, because of the lower frequency of the Met allele in Caucasians (20%) compared with Asians (40–50%) (Petryshen et al. 2010), effects of Met homozygosity have not been adequately demonstrated in previous studies with Caucasian cohorts. Recent studies have indicated that the Met allele is associated with positive effects on rGMV in healthy adults (Liu et al. 2014) and in patients with multiple sclerosis (Ramasaamy et al. 2011), systemic lupus erythematosus (Oroszi et al. 2006), and major depression (Gonul et al. 2011). In addition, the Met allele was correlated with positive effects on IQ scores (Tsai et al. 2004; Vyas and Puri 2012). Although the underlying mechanism remains unknown, it has been hypothesized that reduced BDNF secretion caused by Val66Met substitution may lead to a compensatory increase in the extracellular release of pro-BDNF (Liu et al. 2014). Pro-BDNF would in turn be cleaved by extracellular serine protease plasmin and matrix metalloproteinases to form mature BDNF (Lee et al. 2001), which might ultimately have protective effects on rGMV. We speculate that this may be the mechanism for the positive effects of Met homozygosity on childhood brain development reported in this study.

Met homozygotes had higher PSI scores and displayed greater right cuneus volumes compared with Val homozygotes and Val/Met heterozygotes. Furthermore, positive correlations between PSI and the cerebellum volume in Met homozygotes were found in Exam 1. These results suggest that the presence of 2 Met alleles (i.e., Met/Met, rather than Val/Met) might be required to induce these positive effects. Given the Met-enhancing mechanism reported by Liu et al. (2014), 2 Met alleles (Met/Met) might

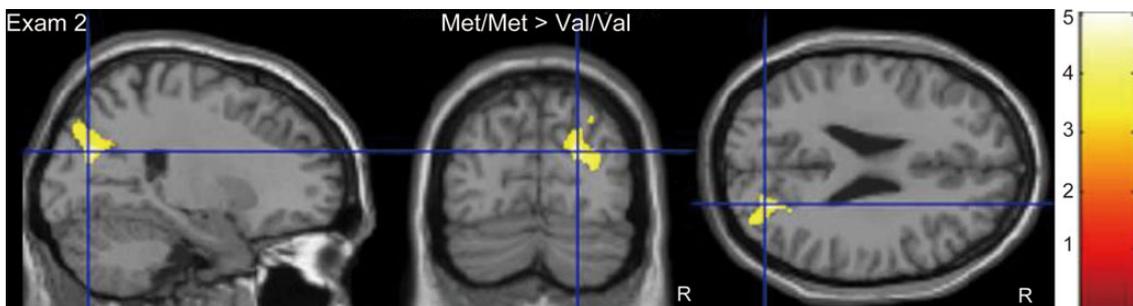
**Table 4** Regional gray matter differences in genotypic groups in cross-sectional and longitudinal analyses

Brain area	MNI coordinates			Number of voxels	Cluster P value	Peak level Z score
	x	y	x			
<b>Exam 1</b>						
Val/Val > Met/Met						
Left insula	-38	5	3	1874	0.001	4.83
Left VMPFC	-6	20	-8	2164	0.000	4.64
Met/Met > Val/Val						
Right cuneus	26	-72	22	1029	0.02	4.72
<b>Exam 2</b>						
Met/Met > Val/Val						
Right cuneus	24	-75	25	1240	0.009	4.81
Longitudinal change (Exam 2-1)						
Val/Met > Val/Val						
Left TPJ	-51	-42	21	2483	0.000	4.48

Note: MNI, Montreal Neurological Institute; VMPFC, ventromedial prefrontal cortex; TPJ, temporoparietal junction.

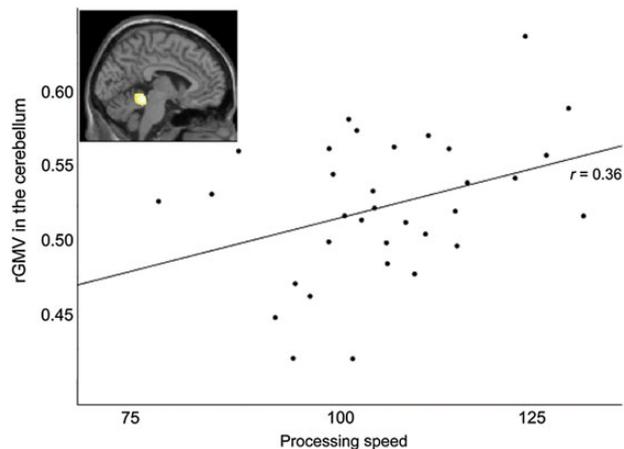


**Figure 2.** Brain regions showing significant volume differences between groups in Exam 1. (A) The left insula and ventromedial prefrontal cortex (VMPFC) showed significantly greater volume in Val/Val homozygotes compared with Met/Met homozygotes. (B) The right cuneus showed significantly greater volume in Met/Met homozygotes compared with Val/Val homozygotes. The color bar indicates the *t* value and R denotes right.



**Figure 3.** Brain regions showing significant volume differences between groups in Exam 2. The right cuneus showed significantly greater volume in Met/Met homozygotes compared with Val/Val homozygotes in Exam 2. The color bar indicates the *t* value and R denotes right.

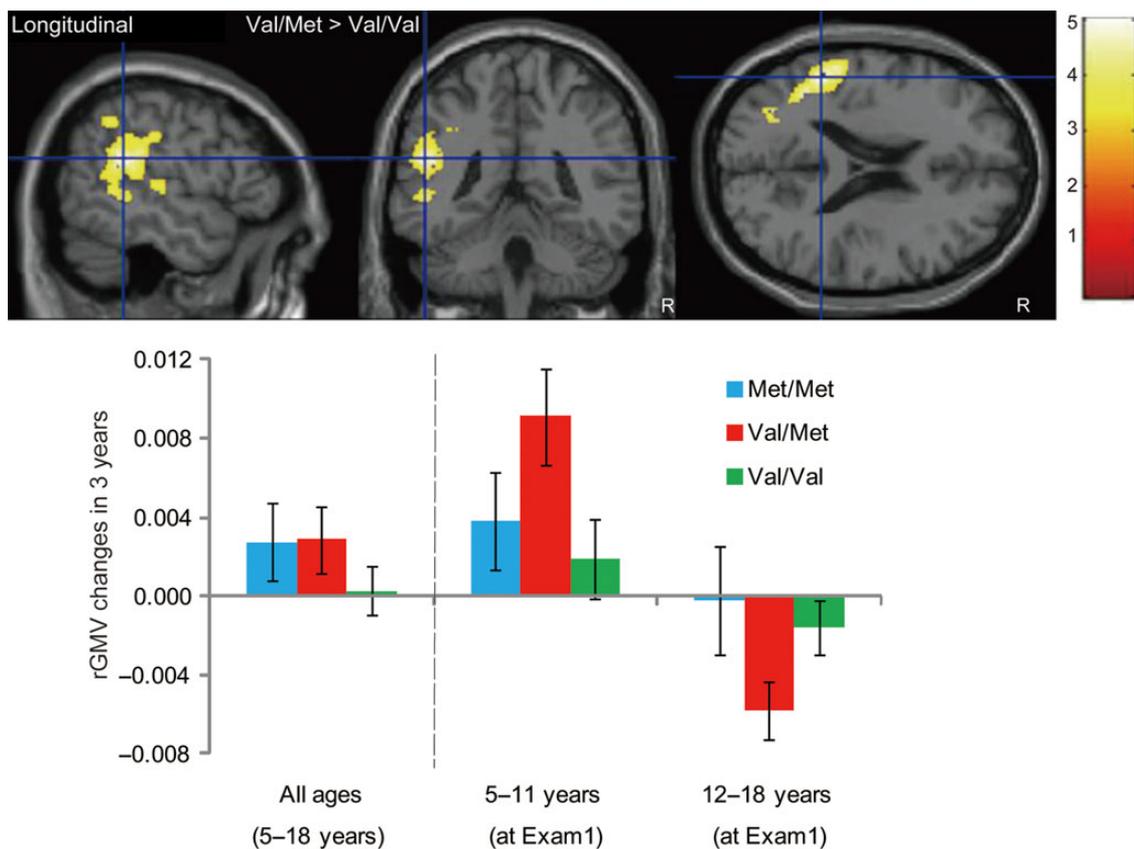
significantly augment the release of pro-BDNF, whereas only one Met allele (Val/Met) might have only a minor effect. Although no previous studies have demonstrated a relationship between PSI and BDNF genotypes in children, adult Val homozygotes demonstrated faster processing speed than Met carriers (Miyajima et al.



**Figure 4.** Effects of PSI on rGMV in Met/Met homozygotes in Exam 1. Positive correlations between PSI and the cerebellum (peak coordinates:  $[-3, -39, -15]$ , mean of 2187 voxels, shown on the top left) volume in Exam 1 are shown. A regression line and a correlation coefficient ( $r$ ) are shown.

2008; Raz et al. 2009); however, there were very few Met homozygotes in this cohort. In contrast, Met homozygosity in elderly individuals has been associated with higher non-verbal reasoning (Harris et al. 2006), while better response inhibition has been reported in young adults with Met alleles (Beste et al. 2010). Enhanced processing speed across development is related to a shorter visual component of event-related potentials (Couperus 2011). The cuneus lies in the dorsal visual pathway and is adjacent to the parieto-occipital sulcus that is involved in visual motion processing (Pitzalis et al. 2010). The cuneus shows functional connectivity with both dorsal and ventral visual regions including the intraparietal sulcus and fusiform gyrus (Bray et al. 2015). Taken together, these results suggest that greater rGMV in the cuneus may have facilitated visual information processing in the Met homozygous children in our study. Moreover, cerebellar volumes are related to information processing and gait speed in older adults (Nadkarni et al. 2014). Improved processing speed has been associated with greater rGMV in the precentral gyrus and has no effects on other cognitive domains including working memory and creativity in young adults (Takeuchi et al. 2011). The greater cerebellar and cuneus volumes in our Met homozygous children might be associated with their higher processing speed.

Val homozygotes demonstrated greater volumes in the left insula and left VMPFC compared with Met homozygotes in Exam 1 but not in Exam 2. These findings suggest that rGMV in the left insula and VMPFC increased more rapidly over the 3 years of the study in Met homozygotes versus Val homozygotes. The



**Figure 5.** Effects of Val66Met on brain development identified in longitudinal analyses. The left TPJ showed significantly increased volume over the 3-year study period in Val/Met heterozygotes compared with Val/Val homozygotes (top). The color bar indicates  $t$  value and R denotes right. Mean rGMV changes in the left TPJ (peak coordinates:  $[-51, -42, 21]$ , mean of 2483 voxels) of the 3 genotypes are shown (bottom left). Blue, red, and green graphs indicate Met/Met, Val/Met, and Val/Val, respectively. Error bars display standard error. Younger ( $N = 109$ , Met/Met: 24, Val/Met: 49, Val/Val: 36) and older ( $N = 76$ , Met/Met: 9, Val/Met: 35, Val/Val: 32) subjects are shown separately (bottom center and right).

genotypic differences in the left insula and VMPFC might be transiently associated with this developmental stage. Compared with Met carriers, Val homozygotes have been shown to exhibit greater volumes in several brain regions including the hippocampus, dorsolateral prefrontal cortex, and amygdala (Pezawas et al. 2004; Bueller et al. 2006; Montag et al. 2009). However, we did not observe differences in these regions in this study. This discrepancy might be explained by differences between children and adults. Although smaller hippocampal volumes in healthy adult Met carriers were reported in a meta-analysis (Molendijk et al. 2012), many previous studies might suffer from small effect sizes (Harrisberger et al. 2015) with few Met carriers.

In the longitudinal analysis, Val/Met heterozygotes showed greater rGMV increases of the left TPJ than Val homozygotes. In all 3 genotypic groups, the left TPJ volume increased at earlier ages and declined in older subjects. Reduced rGMV in the TPJ over childhood development may reflect functional and morphological reorganization during development (Paus et al. 2008). The observation of greater volume increases in childhood compared with adolescence in Val/Met heterozygotes suggests age-related effects on brain volume in this region. However, significant differences between the 3 genotypes were not detected in the TPJ in either exam. The presence of longitudinal but not cross-sectional effects on gray matter volume suggests that age-dependent effects on regional neural plasticity might be more prominent in Val/Met heterozygotes.

Some limitations of our study should be noted. The positive effects of Met homozygosity in children found in this study might be derived from ethnicity or population bias (Notaras et al. 2015) rather than age. We could not detect an effect of PSI, which was higher in Met homozygotes, on rGMV in Exam 2, possibly due to age-dependent effects and/or the small number of Met homozygous subjects. Interpretation of developmental changes in brain morphology should be done cautiously, because age-related decreases in rGMV and increases in rWMV have been observed in adolescents (Brain Development Cooperative 2012), whereas both region-specific rGMV increases and decreases have been reported in adults (Tamnes et al. 2010). Furthermore, a smaller rGMV in Asian adult Met carriers than Val homozygotes (Kim et al. 2013) also suggests that careful interpretation is required for our results. White matter structures rather than rGMV may be associated with PSI (Penke et al. 2012), and this variable was not assessed in this study. Therefore, future research conducted with a larger sample and other imaging methods (e.g., diffusion tensor imaging) that also accounts for ethnicity or population bias is desirable.

In conclusion, this study revealed that Met homozygosity had positive effects on rGMV and processing speed in healthy Japanese children. Our results may challenge the conventional view that the Val66Met BDNF polymorphism has negative effects on brain development and behavior in children.

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