













Brain volumes and Val66Met polymorphism of the BDNF gene: local or global effects?

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Abstract A common Single-Nucleotide Polymorphism brain allometry—the covariance pattern of regional brain in the Brain-Derived Neurotrophic Factor (BDNF) gene volumes—is taken into account. coding the Val66Met substitution in the pro-BDNF protein has been associated with a number of behavioural and neuroanatomical phenotypes; the latter include, for example, regional differences in volumes of the hippocampus and prefrontal grey matter. Here, we show that the observed regional differences may not stem from a local effect of this gene. Our analysis of regional brain volume in a cohort of 331 adolescents indicates that the Val66Met substitution has a global effect on brain volume, and that the observed local differences are to be expected

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Nucleotide Polymorphism (SNP) at codon 66 in the BDNF gene leads to a substitution of Valine (Val) by Methionine (Met) in the pro-BDNF protein. This Val66Met polymorphism appears to affect intracellular packaging of pro-BDNF, its axonal transport and, in turn, activity-dependent secretion of BDNF at the synapse (Chen et al. 2004). It has been associated with a number of behavioural and neuro-anatomical phenotypes.

In the case of behaviour, for example, carriers of the Met allele (vs. Val homozygotes) exhibit poorer episodic memory (Egan et al. 2003) and are more likely to suffer from major depression (Frodl et al. 2007). On the other hand, Val homozygotes appear to have higher scores of neuroticism (Sen et al. 2003) and present more often with bipolar disorder (Neves-Pereira et al. 2002; Sklar et al. 2002).

In the case of neuroanatomical phenotypes, various studies have reported that Met carriers (vs. Val/Val homozygotes) have lower hippocampal volume (Pezawas et al. 2004; Bueller et al. 2006; Frodl et al. 2007) and lower volume of grey matter in the prefrontal cortex (Pezawas et al. 2004). If these differences were due to a difference in BDNF-mediated brain plasticity throughout the life of an individual, we would expect an interaction effect of group

and age on volume, as experience should affect the two principal, and a consent form for a telephone interview genotypic groups differently. This was not the case, how were sent to the parents. Subsequently, a research nurse ever, in the age range between 18 and 60 years old conducted a structured telephone interview with interested included in the study of Pezawas et al. (2004) where no families to verify their eligibility. Additional information age-by-genotype interaction was observed. On the basis of was acquired using a medical questionnaire filled out by this finding, it was suggested that the regional difference in the child's biological parent. The main exclusion criteria volume might be established during childhood or adolescence were (1) positive history of alcohol abuse during pregnancy (Pezawas et al. 2004); (2) positive medical history for meningitis, malignancy and heart disease requiring heart surgery; (3) severe mental illness (e.g. autism, schizophrenia) or mental retardation (IQ < 70); and (4) contraindications for magnetic resonance imaging.

Here, we present the results of our analysis of magnetic resonance images (MRI) obtained in 331 adolescents, with ages ranging from 12 to 19 years old. We found a significant difference in total lobar volume, which was on average 28.34 cm³ smaller in the Met carriers than in the Val/Val carriers. The effect of the Val66Met genotype was independent of age and especially pronounced regarding the volume of white matter in the temporal and frontal lobes. These local differences, however, do not appear to result from more or less pronounced effects of BDNF in different brain compartments. Further analysis of the multivariate allometric pattern of lobar volumes suggested a global effect of the Val66Met polymorphism on brain size, which explained the observed local differences. The proportion of the total volume occupied by the different regions changed from the smallest to the largest brains, independently of Val66Met genotype. For example, volume of white matter in the frontal lobe was larger in the Val group compared with the Met group, but larger brains were found to have in general a disproportionately large volume of frontal white matter—the observed difference fell in the range of what could be expected from allometry given the difference in total volume. The same was observed regarding the small difference in hippocampal and amygdalar volumes, larger in the Val homozygotes.

Overall, our results suggest that the Val66Met polymorphism of the BDNF gene has a global effect on brain volume, independent of age and sex, and may be established earlier during development than what has been previously thought.

Materials and methods

Subjects

All participants were recruited from a relatively geographically isolated population inhabiting the Saguenay-Lac-Saint-Jean (SLSJ) region in Quebec, Canada. Details of the recruitment and testing procedures are provided in Pausova et al. (2007). Briefly, the subjects were recruited in high schools in the SLSJ region. The recruitment began with the team visiting all classrooms in a given school and presenting the study to the students. At the same time as letters containing an information brochure, a letter from the

Acquisition of MRI

Structural MRI of the brain were analysed for 331 adolescents (159 males, 172 females), 12–19 years old. High-resolution anatomical T1-weighted (T1W) images were acquired in a Phillips 1.0-T superconducting magnet using the following parameters: 3D RF-spoiled gradient echo scan with 140–160 sagittal slices, 1-mm isotropic resolution, TR = 25 ms, TE = 5 ms, flip angle = 30°.

Computational neuroanatomy

Volumes of the grey and white matter of the frontal, parietal, occipital and temporal lobes were automatically extracted using the following procedures. T1-weighted images were first corrected for non-uniformities and the intensity normalised using `nu_correct` and `inormalize` from the MINC tools (Sled et al. 1998 <http://bic.mni.mcgill.ca>).

The images were then non-linearly registered to a template brain using a 3D implementation of the rigidity-adaptable registration method of Pitiot and Guimond (2008). Next, the brain was extracted using BET from the FSL package (Smith 2002 <http://fsl.oxford.ac.uk>), and voxels were automatically classified as grey matter, white matter or cerebrospinal fluid (Zijdenbos et al. 2002; Cocosco et al. 2003). An atlas defined in template space was used to separate the grey and white matter into frontal, parietal, occipital and temporal lobes (Collins et al. 1994, 1995, 1999). The volumes were computed in native space by applying the inverse of the previous non-linear transformation. Total lobar volume was defined as the addition of the white and grey matter of the four lobes (Fig. 1). Volumes of the left and right hippocampus and amygdala were segmented automatically from the T1W images using a region-deformation method driven by anatomical landmarks and probabilistic atlases (Chupin et al. 2007). Volumes of the lobar grey matter and white matter as well as those of the hippocampus and amygdala reported here are the sum of the left and right volumes.

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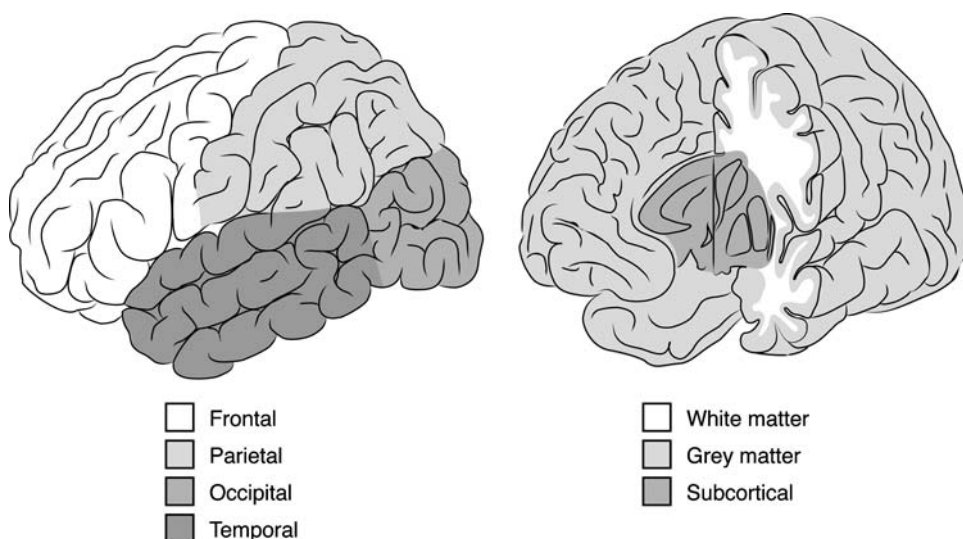
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Fig. 1 Subdivision of the hemisphere into the four lobes (left) and lobar grey and white matter (right). Note that subcortical grey and white matter and the corpus callosum are not included in the lobar volumes



Genotype

The Val66Met SNP of the BDNF gene (rs6265, A/G) with the minor G allele was genotyped using KASP. KASP is a competitive allele-specific PCR-based SNP fluorescent genotyping system that uses FRET quencher cassette oligos (KBioscience, Herts, UK). The call rate was 98% and the SNP was in Hardy–Weinberg equilibrium. Based on this SNP, subjects were classified as either Val homozygotes ($n = 217$) or Met carriers ($n = 114$, 98 Val/Met heterozygotes, 16 Met/Met homozygotes).

corresponding to the volume of region i , $i = 1, \dots, 8$, with $R_i = \{Fw, Pw, Ow, Tw, Fg, Pg, Og, Tg\}$, then, following Jolicoeur (1963), the multivariate allometric relationship between the regions is

$$\left(\frac{R_1}{M_1}\right)^{1/PC1_1} = \left(\frac{R_2}{M_2}\right)^{1/PC1_2} = \dots = \left(\frac{R_8}{M_8}\right)^{1/PC1_8}$$

$$M_i = \frac{1}{331} \exp\left(\sum_j \log(R_{ij})\right),$$

with $j = 1, \dots, 331$, the 331 subjects.

Statistics and multivariate allometry

The univariate allometric equation $y = bx^a$ has been widely used to study the relationship in the size of different structures of the vertebrate brain (Jerison 1973; Finlay et al. 2001). Depending on the value of a , this equation can describe two structures whose volume is directly proportional ($a = 1$), or a structure whose volume is non-linearly related to the volume of the other ($a > 1$ for a disproportionate increase, $a < 1$ for a decrease). The allometric coefficient a can be easily estimated by linear regression in a log–log plot or, more appropriately, from the first component of the covariance matrix of $\log(\text{structure volume})$.

The principal components method can be easily extended to the analysis of allometric relationships between multiple structures, as first proposed by Jolicoeur (1963). In our case, let A be a matrix with 8 columns representing the frontal, parietal, occipital and temporal (respectively, P , O and T) grey and white matter (respectively, w and v), and 331 rows representing each of the subjects. The loadings of the first principal component of the covariance matrix of A provide the linear combination of regional volumes that explains most of the variance. If we call P_1 the loading

If the proportion of the eight volumes were to be conserved, i.e. if all brains were geometrically equivalent, then, as the norm of the principal component vectors is always 1, the coefficients $PC1_i$ should be all equal to $1/\sqrt{8}$. If the volume of two regions n and m were proportional, their corresponding loadings should be equal, $PC1_n = PC1_m$, otherwise, their volumes will be non-linearly related.

Lobar volumes The total lobar volume was on average 28.34^3 cm^3 larger in the Val homozygotes group (mean: $1,007.40 \pm 8.70 \text{ cm}^3$) compared with the Met carriers (mean: $979.05 \pm 90.79 \text{ cm}^3$). The difference was statistically significant, with $P = 0.0112$ ($F = 6.51$). Analysis of variance showed a significant three-way interaction between tissue, lobe and genotype $P = 0.0012$; tissues: grey and white matter; lobes: frontal, parietal, occipital and temporal; genotypes: Val homozygotes and Met carriers; tissue and lobe were within-subject variables, genotype was a between-subjects

Table 1 Observed differences in lobar volumes between Val homozygotes and Met carriers for the Val66Met SNP of the BDNF gene (cm

Region	Mean volume	Met	Val	Difference	CI	<i>t</i>	<i>P</i>
Fw	174.29	172.76	180.95	8.19	2.81, 13.56	3.0	0.0030*
Pw	93.20	92.11	96.66	4.56	1.49, 7.63	2.9	0.0038*
Ow	35.25	35.27	37.56	2.30	0.70, 3.89	2.8	0.0050*
Tw	83.96	83.61	87.14	3.53	0.76, 6.30	2.5	0.0127*
Fg	247.48	245.23	248.03	2.80	-2.58, 8.18	1.0	0.8468
Pg	112.73	113.62	114.27	0.65	-1.98, 3.27	0.5	0.6250
Og	65.08	64.43	66.57	2.14	0.56, 3.71	2.7	0.0081*
Tg	171.74	172.04	176.21	4.18	0.23, 8.13	2.1	0.0384

False discovery rate corrected threshold was 0.0081 for $P = 0.05$

* Statistically significant differences

average). Principal components analysis showed that the first two components account for more than 90% of the variance (71.83 and 20.76%, respectively).

We used the first principal component to estimate the allometric relationship between lobar volumes. This relationship is described by the formula:

$$\begin{aligned} \left(\frac{Fw}{12.08}\right)^{1/0.42} &= \left(\frac{Pw}{11.45}\right)^{1/0.45} = \left(\frac{Ow}{10.50}\right)^{1/0.58} \\ &= \left(\frac{Tw}{11.35}\right)^{1/0.42} = \left(\frac{Fg}{12.41}\right)^{1/0.16} \\ &= \left(\frac{Pg}{11.64}\right)^{1/0.11} = \left(\frac{Og}{11.09}\right)^{1/0.16} \\ &= \left(\frac{Tg}{12.06}\right)^{1/0.22} \end{aligned}$$

The composition of the first principal component was not significantly different in the Val homozygotes and compared with the carriers of the Met allele. The loadings of the first principal components of the two genotype groups can be considered as the coordinates of two 8-dimensional vectors (Krzanowski 1979). The angle between these two vectors, 2.58° was very small when

compared with 10,000 random permutations of the group memberships ($r = 0.8103$, see Fig. 4). We also compared the composition of the first principal component between young and old subjects in our group (median split at 15.2 years) and between females and males (Fig. 5). In both cases, the differences were not significant (10,000 permutations of the group memberships; young vs. old: $t = 7.37$, $P = 0.1718$; females vs. males: $t = 6.87$, $P = 0.2092$).

The expected variation of individual lobar volumes with total lobar volume is illustrated in Fig. 6. The volume

of frontal, parietal, occipital and temporal white matter increased in general faster than that of the grey matter as brain volume moved from the smallest to the largest brains. If we examined the proportion of the frontal, parietal, occipital and temporal grey and white matter at different brain sizes (bottom plot in Fig. 6), we observed a significant effect of the Val66Met SNP on brain volume, which was larger in the Val homozygotes compared with the carriers of the Met allele. The total difference was relatively small, namely, 28.34 cm³ (2.8%) of the total lobar volume on average. When different

Fig. 6), we can observe that larger brains have relatively more white matter, especially in the frontal lobe.

Based on the allometric pattern of brain growth, and given the difference observed in total lobar volume in the Val and Met groups, we computed the expected regional differences (Table 2). All expected differences fall within the 95% confidence interval of the observed differences (Table 1).

Allometry of the hippocampal and amygdalar volumes

The same was observed for the difference in hippocampal and amygdalar volumes between the Val and Met groups, which were also explained by their scaling with total lobar volume. The allometric relationships were

$$\log(\text{Hipp}) = 3.19 + 0.39 \log(\text{TLV}),$$

$$\log(\text{Amy}) = 1.34 + 0.47 \log(\text{TLV}),$$

where Hipp and Amy are, respectively, the expected hippocampal and amygdalar volumes for a total lobar volume TLV.

Given the observed difference in total lobar volume between the Val and Met groups, the expected difference in hippocampal volume was 62.39 mm³ and the expected difference in amygdalar volume was 35.81 mm³. Both values fall within the 95% confidence interval of the observed differences.

Discussion

We observed a significant effect of the Val66Met SNP on brain volume, which was larger in the Val homozygotes compared with the carriers of the Met allele. The total difference was relatively small, namely, 28.34 cm³ (2.8%) of the total lobar volume on average. When different

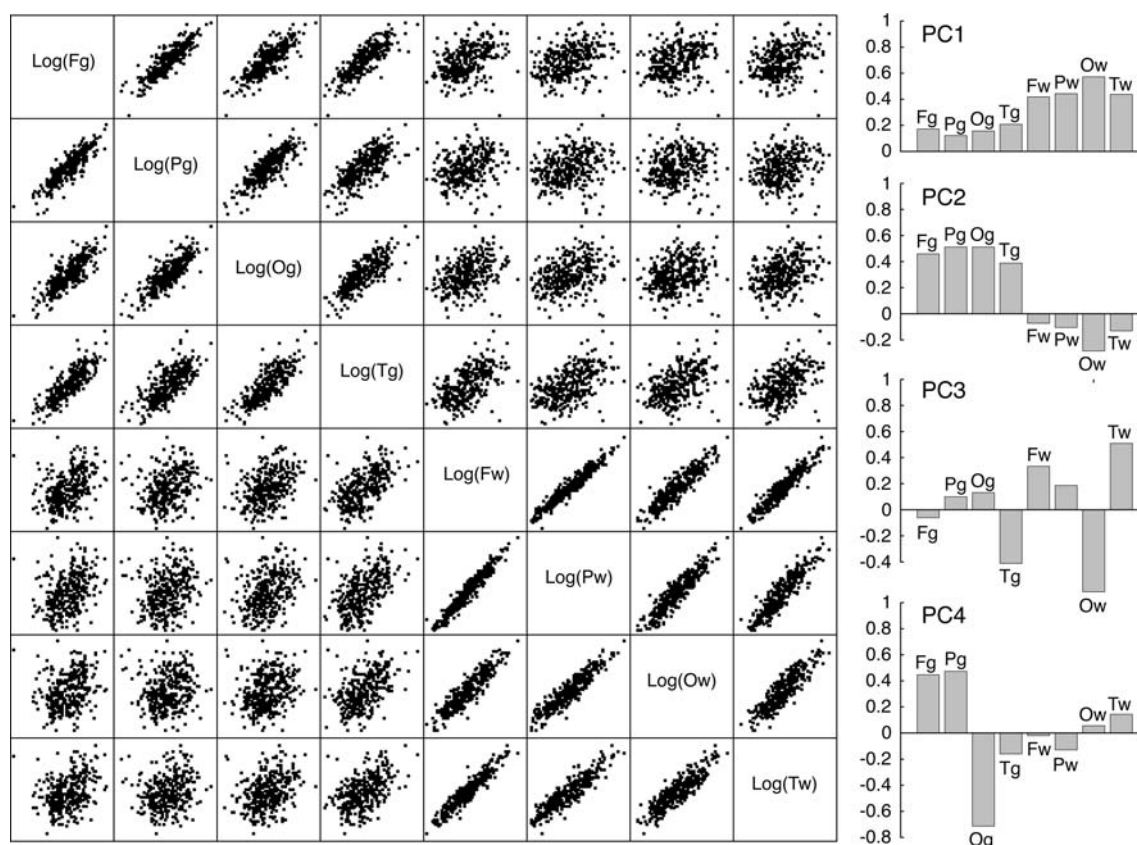


Fig. 3 Pattern of covariation of lobar volumes. We observed a very strong $r = 0.36$ on average). The first two principal components (strong correlation of the white matter across the four lobes 0.92 of the covariance matrix account for more than 90% of the variance on average), and of the grey matter across the lobes 0.82 on (71.83 and 20.76%, respectively) average). White and grey matter volumes also show a correlation, but

regions were studied separately, however, we observed a stronger effect in the white matter volume, 4.8% larger in the volume of white matter may be a geometric consequence of the increase in cortical surface area. All lobar volumes as well as hippocampal and amygdala area. In a previous study, we have shown that the degree of cortical folding is also disproportionately higher in the Val/Val group. The maximum relative difference was in the occipital white matter volume, 6.3% larger. The maximum absolute difference was in the white frontal volume, 8.19 cm³ larger. As Zhang and Sejnowski suggested, this exposure of the BDNF gene under study may have an effect on early

cortical development, leading to a slight but significant increase in the volume of white matter. One to think of a local effect of the Val66Met SNP of the difference in cortical surface area and associated increase in BDNF gene on the development of the white matter or in white matter volume.

Other specific brain regions, such as the hippocampus. Our previous reports on the effect of the Val66Met SNP on allometric analysis suggests, however, that at least in brain anatomy have overlooked brain allometry. Given the present case these differences do not reflect a local effect of non-linear nature of brain allometry, simply dividing the BDNF but are the product of the tight relationship between regional volumes by total volume may be insufficient and a misleading way of correcting for inter-individual differences in brain size. In Table 4 we illustrate the problem by comparing this type of relative volumes of white matter but that the larger brains, compared with the smaller brains, have in general a disproportionately larger volume of white matter. It is interesting to note that the values represent the proportion of the total lobar volume occupied by the individual regions. The comparison and Sejnowski (2000) in their study of the brains of different species of mammals, ranging in size from shrew to elephant, still shows significant regional differences, which could be interpreted as a regional effect of the Val66Met SNP,

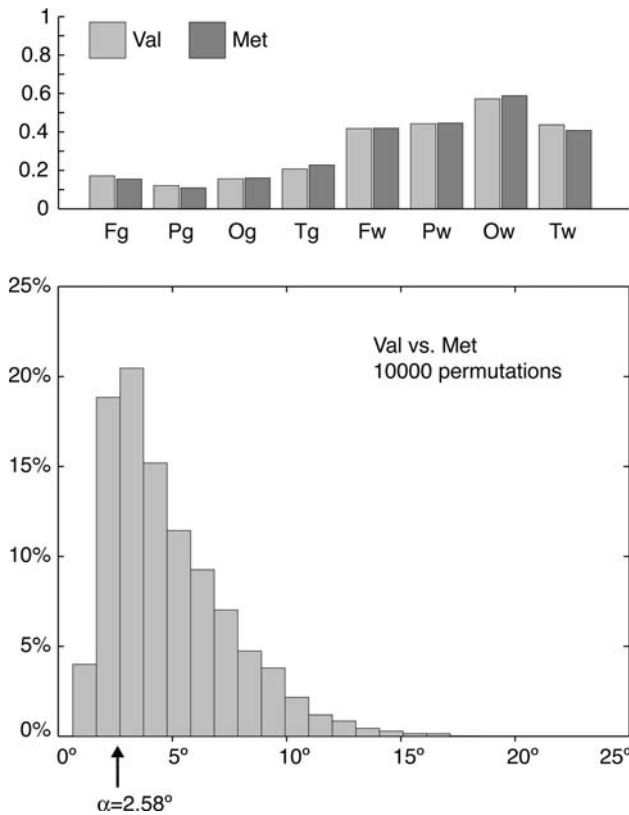
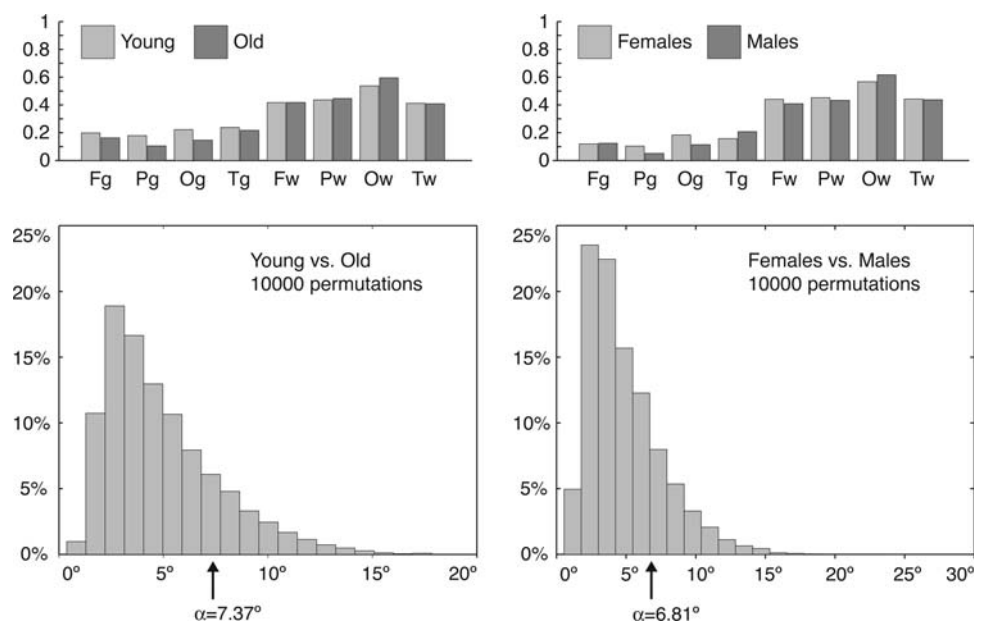


Fig. 4 Difference in the composition of the first principal component in the Val/Val group compared with the Met-carrier group. The loadings of the first principal components of the two groups can be considered as the coordinates of two 8-dimensional vectors. The angle between these two vectors, 2.58°, was very small compared with 10,000 random permutations of the group memberships. Then, the first principal component was not significantly different between Val/Val and Met-carrier groups ($P = 0.8103$)

independent of brain size (the same is true if the normalisation uses total brain volume instead of total lobar volume, the two measurements correlate with 0.94). On the contrary, if we correct for brain size by subtracting from the lobar volumes the volumes expected from allometry, the residuals do not show any regional effect (Table 4). Beyond our present analysis, brain allometry should be always considered when comparing groups of subjects exhibiting a significant difference in brain size. For example, autistic children present with a larger brain volume compared with typically developing children, and have been also found to have a higher volume of frontal white matter. What has been interpreted as the effect of an “abnormally profuse” frontal connectivity (Carper et al. 2002; Herbert et al. 2004) may be, at least in part, the normal consequence of a difference in total brain volume.

The analysis of brain anatomy during adolescence poses two major challenges. First, there are still significant regional age-related changes in cortical grey matter (decrease) and white matter (increase) even though the overall size of the brain approximates the adult values by the age of 10 years. Second, there exist significant sex-related differences in brain maturation (Giedd 2008; Perrin et al. 2008). For example, in our cohort the rate of change of the white matter was three times larger in males compared with females. But the fact that brain allometry is the same within the Val/Val and Met-carrier groups should not be affected by age- or sex-related differences. On the one hand, the interaction between age and genotype was not significant, nor was the mean age between the two genotype groups ($F = 1.50, P = 0.2211$), or the allometry (the composition of the first principal component) of the

Fig. 5 Difference in the composition of the first principal component between young and old individuals, and between females and males. The composition of the first principal component was not significantly different between young (age less than 15.2 years) and old (age greater than or equal to 15.2 years) individuals (angle 7.37°, $P = 0.1718$, 10,000 permutations), nor between females and males (angle 6.81°, $P = 0.2092$, 10,000 permutations)



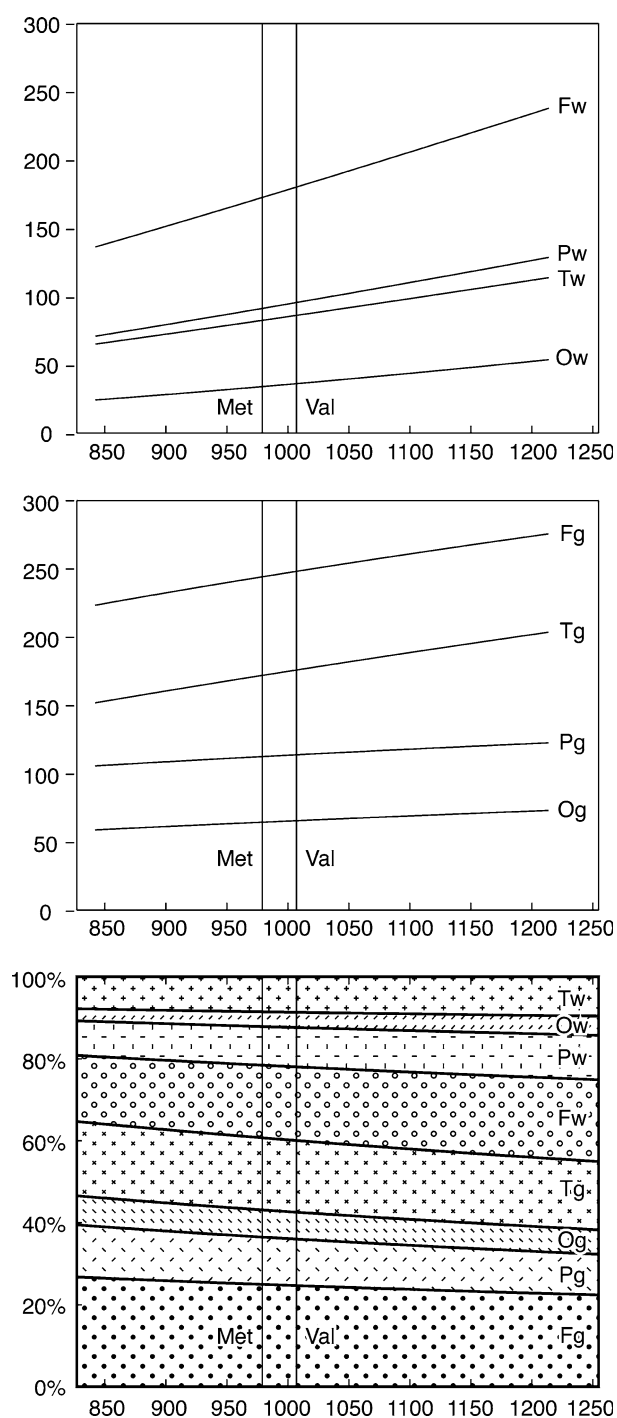


Fig. 6 Expected variation of individual lobar volumes with total lobar volume. The volume of frontal, parietal, occipital and temporal white matter (*top plot*) increases more steeply than that of the grey matter (*middle plot*) as we move from the smallest to the largest brains (the scale of both plots is the same). In the *bottom plot*, the previous curves have been stacked to show the proportion of white and grey matter in the four lobes for brain volumes varying from 850 to 1,250 cm³. If small and large brains were geometrically similar, all lines in this plot should be horizontal but we see, for example, that the proportion of grey matter is larger in a small brain than in a large brain. The vertical lines labelled Met and Val indicate the average brain volume of the subjects in those groups

Table 2 Expected differences in lobar volumes between Val homozygotes and Met carriers based on multivariate allometry and total lobar volume difference (mean total lobar volume for the Val and Met group, 1,007.40 and 979.05 cm³, respectively)

Region	Met	Val	Difference
Fw	173.41	181.09	7.68
Pw	92.35	96.70	4.35
Ow	35.20	37.39	2.19
Tw	83.61	87.29	3.68
Fg	244.27	248.31	4.04
Pg	112.96	66.11	1.31
Og	65.01	66.11	1.10
Tg	172.41	176.22	3.99

All expected differences fall within the 95% confidence interval of the observed differences (Table 1)

Table 3 Val66Met-related differences in lobar volumes: relative volumes

Region	Mean volume	Met	Val	Difference	<i>P</i>
Fw	0.17791	0.1761	0.1793	0.0031	2.13 0.0340*
Pw	0.09946	0.0939	0.0957	0.0018	1.99 0.0479*
Ow	0.03643	0.0359	0.0371	0.0012	2.15 0.0328*
Tw	0.08565	0.0853	0.0864	0.0011	1.27 0.2038
Fg	0.25827	0.2508	0.2466	-0.0041	-2.56 0.0109*
Pg	0.11495	0.1163	0.1138	-0.0025	-2.43 0.0161*
Og	0.06665	0.0659	0.0662	0.0003	0.55 0.5830
Tg	0.17514	0.1759	0.1750	-0.0009	-0.93 0.3558

* Statistically significant differences

Table 4 Val66Met-related differences in lobar volumes: residuals of allometry (cm³)

Region	Met	Val	Difference	<i>t</i>	<i>P</i>
Fw	-0.056	0.164	0.220	0.34	0.7370
Pw	0.041	0.077	0.036	0.09	0.9314
Ow	0.765	0.721	-0.004	-0.02	0.9868
Tw	0.289	0.001	-0.287	-0.57	0.5669
Fg	1.724	0.394	-1.330	-0.58	0.5647
Pg	0.935	0.253	-0.683	-0.55	0.5842
Og	-0.369	0.640	1.009	1.42	0.1559
Tg	0.453	0.549	0.095	0.06	0.9491

- of the dynamics of changes in regional volumes, a description of the variation of allometry during development, and a more accurate understanding of the effect of the Val66Met polymorphism on brain anatomy.
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