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Research report

Morphological correlates of MAO A VNTR polymorphism: New evidence from cortical thickness measurement

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ABSTRACT

A functional variant in the mono-amine oxidase A (MAO A) gene has been shown to impact neural function related to cognitive and affective processing and increase risk for conduct disorders. However, whether MAO A could be a candidate gene for structural variation in the human brain remains to be clarified. This study is the first to investigate the effect of this genotype on brain morphology by measuring cortical thickness. We genotyped 59 healthy male subjects (36 carrying the MAO A High-activity allele and 23 the MAO A Low-activity allele) who underwent structural MRI at 3 T. Models of the grey-white and pial surfaces were generated for each individual's cortices, and the distance between these two surfaces was used to compute cortical thickness within *a priori* regions of interest of the orbitofrontal and cingulate cortices. Surface-based analysis of the cortical mantle showed that the MAO A genotype was associated with structural differences in the orbitofrontal cortex bilaterally, where the MAO A High-activity group showed the highest cortical thickness value and the MAO A Low-activity group the lowest. Otherwise, no significant difference was detected within the cingulate cortex. Thus, we confirm the hypothesis that the MAO A genotype has a specific impact on human brain morphology. In particular, thickness measurement of the orbitofrontal cortex provides new evidence about the biological impact of the MAO A genotype on neural systems relevant to the pathophysiology of behavioural disorders.

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1. Introduction

Mono-amine oxidase (MAO) is a mitochondrial enzyme that degrades the neurotransmitters serotonin (5-HT) and (to a lesser extent) noradrenaline and dopamine [50]. There are two distinct forms of the enzyme: A and B. MAO A provides the major enzymatic clearing step for serotonin and norepinephrine during brain development [50]. The MAO A coding gene (Xp11.4–Xp11.3) presents a well-characterized variable number tandem repeat (VNTR) functional polymorphism in the promoter region, which has two common alleles that selectively influence protein transcription and, hence, enzymatic activity. Enzyme expression is relatively high for carriers of 3.5 or 4 repeats (MAO A High) and lower for carriers of 2, 3 or 5 repeats (MAO A Low) [48].

The presence of this functional polymorphism has stimulated several studies on its association at an intermediate phenotypic level (gene-brain function or gene-brain structure relationships) or at phenotypic level (gene-cognitive function or gene-behavioural disorder relationships). Unfortunately, major parts of this research were characterized by conflicting findings. Whereas the association of this genotype with antisocial behaviour in human cross-sectional studies underlined the role of the MAO A High-activity allele in males as a risk factor [36], population studies investigating the gene-by-environment interaction defined a clear and pronounced effect of the MAO A Low variant to predict conduct disorders in males with adverse early experiences [6,23]. Similarly, several imaging genetic studies investigating the neurofunctional correlate of the MAO A VNTR polymorphism presented different interpretations as to whether the High- or Low-activity allelic variant is the risk factor. One study highlighted the under-activation of the

Abbreviations: MAO A, mono-amine oxidase A; MRI, magnetic resonance imaging; VBM, voxel-based morphometry; 5-HT, serotonin; VNTR, variable number tandem repeat; ROIs, regions of interest; PUs, parcellation units; GLM, general linear model; DOSS, different offsets same slopes; DODS, different offsets different slopes; ICV, intracranial volume.

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orbitofrontal cortex or the anterior cingulate cortex in individuals carrying the MAO A Low variant during cognitive control paradigm [38], whereas others underscored the hyperactivity of the same areas in the High-activity carriers during functional magnetic resonance imaging (fMRI) tasks involving equivalent cognitive process [7,17,41,42]. Even the employment of structural MRI to investigate the neuroanatomic effects of this genotype did not help to discern this doubt. In fact, in two different studies using an optimized version of voxel-based morphometry (VBM), Meyer-Lindenberg et al. [38] and Cerasa et al. [8] both found similar findings but had divergent interpretations about the impact of this genotype on brain morphology.

Thus, the aim of this study is to provide, for the first time, evidence of physical characteristics associated with the MAO-A High and MAO-A Low activity variants by using an *in vivo* cortical thickness measurement. Given the aforementioned abundant evidences showing how this genotype affects both brain function and structure of the orbitofrontal and cingulate cortices, we computed the average cortical thickness in these specific regions in individual subjects.

2. Methods

2.1. Participants

One hundred fifty-five healthy individuals (Caucasian, age-range: 18–70) were recruited by local advertisements. Inclusion criteria were: (1) male; (2) right handedness, according to the Edinburgh Handedness Inventory [39]; (3) vision and hearing sufficient for compliance with testing procedures; (4) neuropsychological scores above the cutoff scores, corrected for age and educational level, identifying normal cognitive level in the Italian population (see Section 2.2). We included only men for two reasons: (a) MAO A polymorphism maps in a region of the X chromosome is suspected to escape the normal inactivation [5] which makes it very difficult to compare homozygous males (carrying either one MAO A High-activity allele or one MAO A Low-activity allele) to homozygous females (carrying either two MAO A High-activity alleles or two MAO A Low-activity alleles) or heterozygous females (carrying both one MAO A High-activity allele and one MAO A Low-activity allele) in terms of enzymatic activity; (b) there is evidence that the effects of MAO A alleles on the serotonergic function *in vivo* vary as a function of both ethnicity and gender [38,55]. Exclusion criteria were: (1) major medical illnesses, known or suspected history of alcoholism or drug dependence and abuse during lifetime; (2) mental disorders (i.e., schizophrenia, mood disorders, anxiety disorders, personality disorders, and any other significant mental disorder), according to DSM-IV criteria assessed by the Structured Clinical Interviews for DSM-IV Axis I (SCID-I) [18] and Axis II (SCID-II) [19], and/or neurological disorders diagnosed by an accurate clinical neurological examination; (3) dementia, according to DSM-IV criteria or mild cognitive impairment according to Petersen criteria [44] and confirmed by the administration of the Mental Deterioration Battery (MDB) [4]; (4) Mini Mental State Examination (MMSE, [25]) score < 27; (5) presence of vascular brain lesions, brain tumour and/or marked cortical and subcortical atrophy on MRI scan. From the initial sample of 155 subjects, 61 subjects (39.4%) were excluded from the sample for being of female gender, 11 (7.1%) were excluded because of substance abuse including cannabis, 8 (5.2%) because of a dementia diagnosis or MMSE score lower than 27, 13 (8.4%) because of medical illness or neuropsychiatric disorder, and 3 (1.9%) because of previous traumatic brain injury. After the initial screening, 59 subjects were considered eligible. All subjects signed written, informed consent. The study procedures were undertaken in accordance with the guidance of Santa Lucia Foundation Ethics Committee.

All male subjects were genotyped based on the High-activity (no. 36; 3.5 or 4 repeats) and the Low-activity (no. 23; 2, 3 and 5 repeats) allelic variants of the MAO A VNTR polymorphism. To check for known potentially confounding variables, since differences in brain anatomy have been previously associated with a functional polymorphism in the targeting region of the BDNF gene (Val^{66}Met)[45] as well as with the 5-HTT variants of the serotonin transporter gene (5-HTTLPR) [46], we genotyped our group according to these polymorphisms to account for potential confounds in interpreting MAO A effects on brain morphology (Table 1).

2.2. Neuropsychological assessment

Two trained neuropsychologists, who were blind to the aim of the study, conducted the cognitive assessment, which was performed within 15 days of MRI. We selected the following tests from the MDB in order to provide information about the functionality of different cognitive domains such as: verbal memory (Rey's 15-word Immediate Recall (RIR) and Delayed Recall (RDR)), short-term visual memory (Immediate Visual Memory (IVM)), logical reasoning (Raven's Progressive Matrices' 47 (PM47)) and language (Phonological Verbal Fluency (PVF) and Sentence Construction (SC)).

As "executive functioning" denotes a set of different cognitive abilities that are involved in complex, goal-directed thought and behaviour, the following executive dimensions were assessed: (a) attention control, (b) set-shifting, and (c) working memory [51,52]. (a) In order to assess abilities of attention control and inhibition, we administered the Stroop test (ST) [53]. Time of performance was chosen as a measurement. (b) Set-shifting or cognitive flexibility was assessed using the Modified Wisconsin Card Sorting test (MWCST) [30]. The number of perseverative/no-perseverative errors was chosen as a measurement. (c) In order to measure verbal, spatial and visual working memory we administered the n-back test. In this test, participants were required to continuously monitor a sequence of verbal/spatial/visual stimuli (a total of 22 items for each task, visually presented on a screen) and to select items that appeared as n-back items in any sequence. The number of correct responses was generally considered as index of working memory performance. In this study we only considered highly cognitive demanding $n-2$ level performance.

Although none of the participants met the criteria for major depressive episodes or other psychiatric disorders, we further investigated the presence of depressive and anxiety symptoms using the Hamilton Depression rating scale (HDRS) and the Hamilton Rating Scale Anxiety (HAM-A), respectively [28,29].

2.3. Genotyping

DNA was extracted from blood samples obtained from all subjects according to standard procedures. Genotyping for the MAO A VNTR, 5-HTTLPR, and BDNF Val^{66}Met polymorphisms was performed as described previously (see Supplementary material) [7,8,41,42].

2.4. Magnetic resonance imaging

Each of the 59 participants underwent the same imaging protocol with a whole-brain T1-weighted scan using a 3 T Allegra MR imager (Siemens, Erlangen, Germany) with a standard quadrature head coil. Whole-brain T1-weighted images were obtained in the sagittal plane using a modified driven equilibrium Fourier transform (MDEFT) [14] sequence (TE/TR = 2.4/7.92 ms, flip angle 15°, voxel-size 1 mm × 1 mm × 1 mm).

2.5. Cortical thickness

MRI-based quantification of cortical thickness was performed using *Freesurfer* (v. 4.05) software package (http://surfer.nmr.mgh.harvard.edu). This method has been previously described in detail [13,20,21]. The procedure involves segmentation of white matter, tessellation of the grey/white matter junction, inflation of the folded surface, tessellation patterns and automatic correction of topological defects in the resulting mainfold. Cortical thickness measurements were obtained by reconstructing representations of the grey/white matter boundary and the cortical surface. The distance between these two surfaces was calculated individually at each point across the cortical mantle. This method uses both intensity and continuity information from the entire 3D MRI volume in segmentation and deformation procedures to construct representations of cortical thickness. The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The entire cortex in each individual subject was then visually inspected, and any inaccuracies in Talairach-transformation, skull stripping and segmentation were manually corrected and re-inspected. The anatomic accuracy of the grey and white matter surfaces was reviewed with particular attention to the temporal pole where non-brain tissue often needs to be excluded. Thickness measurements can be mapped onto the "inflated" surface of each participant's reconstructed brain, thus allowing visualization without interference from cortical folding. Maps were smoothed using a circularly symmetrical Gaussian kernel across the surface with a standard deviation of 12.6 mm and averaged across participants using a non-rigid high-dimensional spherical averaging method to align cortical folding patterns [20]. This procedure provides accurate matching of morphologically homologous cortical locations among participants on the basis of each individual's anatomy while minimizing metric distortions, resulting in a mean measure of cortical thickness for each group at each point on the reconstructed surface. This spherical morphing procedure was used to construct the cortical thickness difference brain maps.

2.6. Computation of average cortical thickness within ROIs

Given the substantial evidence highlighting the influence of the MAO A genotype on the function and structure of specific brain regions, the primary aim of this study was to focus on group differences within two regions of interest (ROIs) or parcellation units (PUs): (a) the orbitofrontal cortex (including the sub-regions pars triangularis, pars orbitalis, medial and lateral orbitofrontal cortices) and (b) the cingulate cortex (including the sub-regions isthmus, posterior, rostral- and caudal-anterior cortices). Cortical ROIs or PUs were drawn on maps of average folding patterns on the cortical surface, with reference to an anatomical atlas (Fig. 1). For each of these structures the right- and left-hemisphere measurements are esti-

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Table 1
Group demographics for cortical thickness analysis.

Demographic data	MAO A High activity	MAO A Low activity	*p* values
No.	36	23	
Age (years)	37.8 ± 14	41.9 ± 16.8	0.31[b]
Educational level (years)	13 (8–19)	13 (11–21)	0.43[c]
Verbal memory			
RIR	43.3 ± 7.9	42.9 ± 7.4	0.84[b]
RDR	9.52 ± 2.5	9.3 ± 2.2	0.8[b]
Short-term visual memory			
IVM	19.3 ± 1.3	19.6 ± 1.2	0.41[b]
Logical reasoning			
PM 47	29.6 ± 3.1	29.1 ± 2.1	0.45[b]
Language			
PVF	31.95 ± 11.4	34.5 ± 11.1	0.41[b]
SC	19.6 ± 3.2	18 ± 4.3	0.12[b]
Executive function			
ST read (time, s)	13.5 ± 2.6	13.7 ± 3.3	0.79[b]
ST color (time, s)	16.9 ± 3.1	18.1 ± 4	0.25[b]
ST color-word (time, s)	28.5 ± 6.6	29.6 ± 7.6	0.57[b]
MWCST PE	0.42 ± 1.12	0.43 ± 1	0.98[b]
MWCST No PE	0.42 ± 0.75	0.9 ± 0.9	0.04[b]
Verbal N-back 2-back	17.5 ± 2.6	14.9 ± 5.4	0.02[b]
Spatial N-back 2-back	17.5 ± 3	14.6 ± 5.4	0.01[b]
Visuo-spatial N-back 2-back	18.2 ± 2.4	16.1 ± 5.1	0.052[b]
Psychological variables			
HAM-A	3.94 ± 4.6	5.14 ± 4.2	0.34[b]
HDRS	2.3 ± 2.4	3.1 ± 2.8	0.23[b]
Genetic background			
BDNF Val^{66}Met (%) val group val/val	69.5%	69.6%	0.89[a]
BDNF Val^{66}Met (%) met group grcarriers	30.5%	30.4%	
5-HTTLPR (%) short variant	80.6%	72.8%	0.19[a]
5-HTTLPR (%) long variant	19.4%	27.2%	

Data are given as mean values (SD) or median values (range) when appropriate. RIR and RDR, Rey's 15-word Immediate and Differite Recall; IVM, Immediate Visual Memory; PM 47, Raven's Progressive Matrices'47; PVF, Phonological Verbal Fluency; SC, Sentence Construction; ST, Stroop Task. MWCST PE and No PE, Modified Wisconsin Card Sorting test, perseverative and no-perseverative errors. HAM-A, Hamilton Rating Scale Anxiety. HDRS, Hamilton Depression Rating Scale. BDNF, Brain Derived Neurotrophic Factor; 5-HTTLPR, Serotonin Transporter gene polymorphism.

[a] Chi-square test.
[b] One-way ANOVA.
[c] Mann–Whitney test.

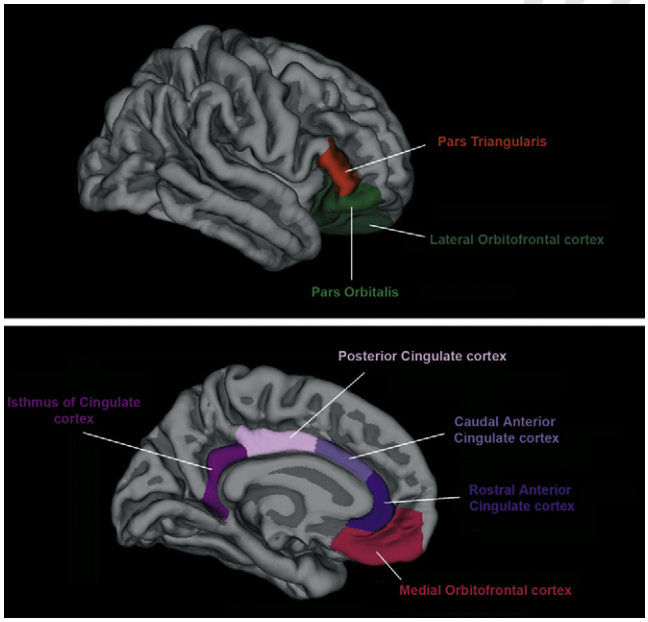

Fig. 1. Cortical parcellation units (PUs) involved with *a priori* hypothesis. The orbitofrontal cortex was composed by: pars triangularis, pars orbitalis, medial, and lateral orbitofrontal cortex. The cingulate cortex included the sub-regions isthmus, posterior and rostral- and caudal-anterior cortices. Only one hemisphere is shown.

mated separately. This method has been validated against manual tracings in healthy controls and is part of the publicly available *Freesurfer* package (derived using the surface-based morphing procedure as described by Fischl et al. [22]). Each ROI was mapped back onto each individual subject's unfolded surface by applying the same algorithm that morphed each subject's unfolded surface to the average spherical surface representation in reverse. Mean thickness for each ROI was calculated by averaging the mean cortical thickness measurements at each vertex within a given ROI. Statistical analysis was performed within each ROI by using AnCOVA with age and gender as covariates of no-interest. The level of statistical significance for each ROI was set at $p \leq 0.01$ after correction for the number of multiple comparisons: $p < 0.05/4 = 0.0125$. As a measure of the effect sizes, the Cohen's d [12] was calculated, which indicates the magnitude of mean differences (using the estimated marginal means) in SD units. We also tested for correlations between the cortical thickness measurement in the ROIs and all neuropsychological measures (Pearson' r). To reduce type I errors, the level of statistical significance for correlation analysis was set at $p \leq 0.01$.

2.7. Computation of statistical cortical thickness difference maps in the whole-brain

To further characterize the morphological correlates of the MAO A genotype we adopted a voxel-wise brain mapping approach to the entire cortical mantle (results are presented in the Supplementary Material). For each hemisphere, estimation of statistical effects was generated by computing a general linear model (GLM) of the effects of the MAO A genotype on cortical thickness at each vertex. Two types of designs were used in these analyses [16]. A different offset, same slope (DOSS) design was used to test whether a main effect of group on thickness could be found. A different offset, different slope (DODS) design was used to test whether cortical thickness was more related to age in one genotype group than in the other. This is conceptually similar to an interaction between age and genotype. First, to explore the effects of MAO A polymorphism on regional cortical thickness independent of participant age, we conducted a GLM with the MAO A genotype (High activity, Low activity) as a classification variable assuming identical age-related slopes between groups (main effect). To test whether or not the MAO A genotype was associated

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Table 2
Mean cortical thickness in genotype groups within individual Parcellation Units (PUs). Q5

Left hemisphere	Cortical thickness, mean ± SD (mm)		*p*-level
	MAO A High activity	MAO A Low activity	
Orbitofrontal cortex			
Pars triangularis	2.406 ± 0.18	2.347 ± 0.19	0.24
Pars orbitalis	2.691 ± 0.21	2.666 ± 0.19	0.65
Lateral orbitofrontal cortex	2.68 ± 0.18	2.561 ± 0.15	0.01*
Medial orbitofrontal cortex	2.511 ± 0.21	2.454 ± 0.26	0.35
			Cingulatecortex
Rostral-anterior cingulate cortex	2.53 ± 0.24	2.52 ± 0.23	0.82
Caudal-anterior cingulate cortex	2.38 ± 0,37	2.362 ± 0.2	0.83
Posterior cingulate cortex	2.581 ± 0.18	2.559 ± 0.19	0.66
Isthmus	2.704 ± 0.23	2.61 ± 0.21	0.12
Right hemisphere	Cortical thickness, mean ± SD (mm)		*p*-level
	MAO A High activity	MAO A Low activity	
Orbitofrontal cortex			
Pars triangularis	2.523 ± 0.16	2.411 ± 0.12	0.006*
Pars orbitalis	2.812 ± 0.2	2.75 ± 0.26	0.83
Lateral orbitofrontal cortex	2.715 ± 0.18	2.581 ± 0.22	0.01*
Medial orbitofrontal cortex	2.428 ± 0.22	2.329 ± 0.22	0.1
			Cingulatecortex
Rostral-anterior cingulate cortex	2.585 ± 0.33	2.428 ± 0.21	0.06
Caudal-anterior cingulate cortex	2.394 ± 0,28	2.411 ± 0.26	0.81
Posterior cingulate cortex	2.571 ± 0.18	2.501 ± 0.21	0.17
Isthmus	2.611 ± 0.21	2.556 ± 0.23	0.35

AnCOVA analysis corrected for age.

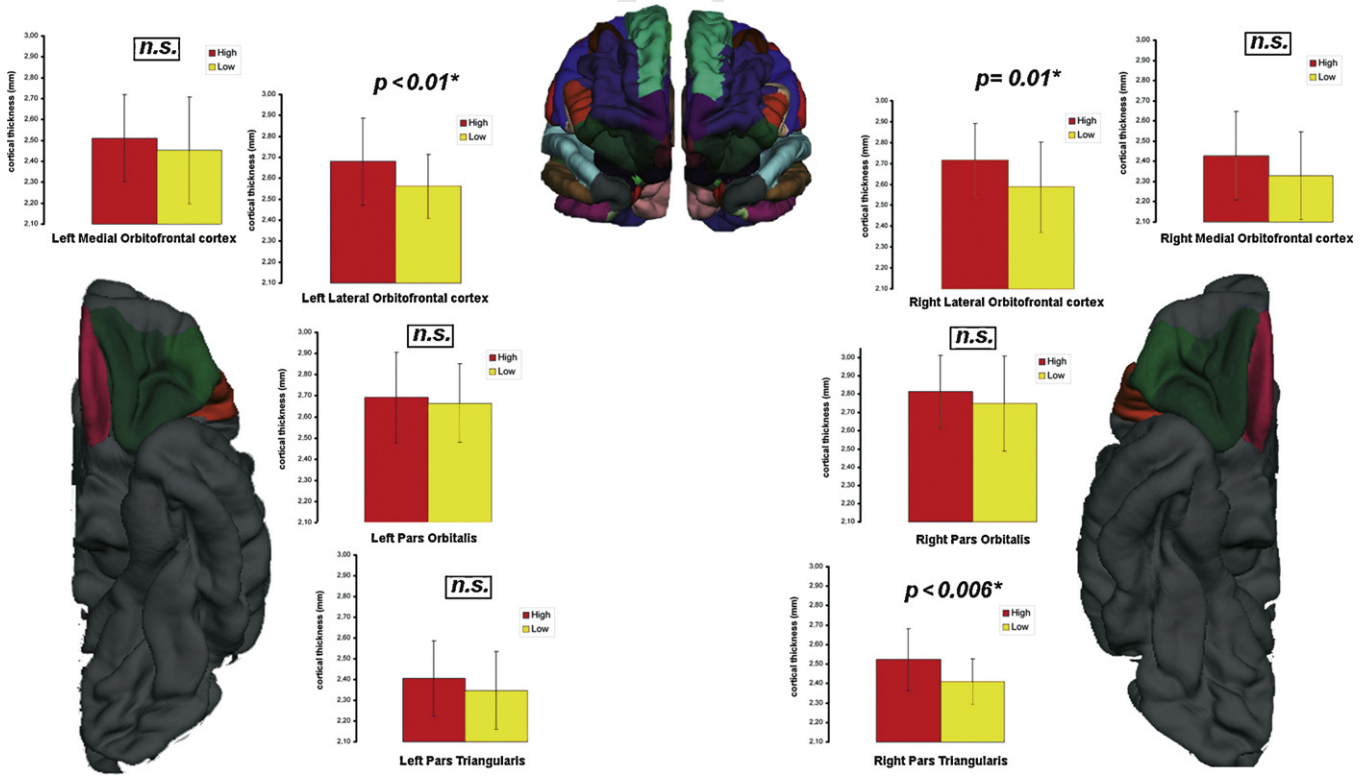

Fig. 2. Mean cortical thickness for the genotype groups within the orbitofrontal cortex as automatically parcellated by Freesurfer. The MAO A High activity represented in red and MAO A Low activity in yellow. A significant difference was detected in the lateral orbitofrontal cortex bilaterally and in the right pars triangularis sub-regions where the individuals carrying the MAO A High-activity variant showed an increased thickness with respect to carriers of MAO A Low variant. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.) Q4

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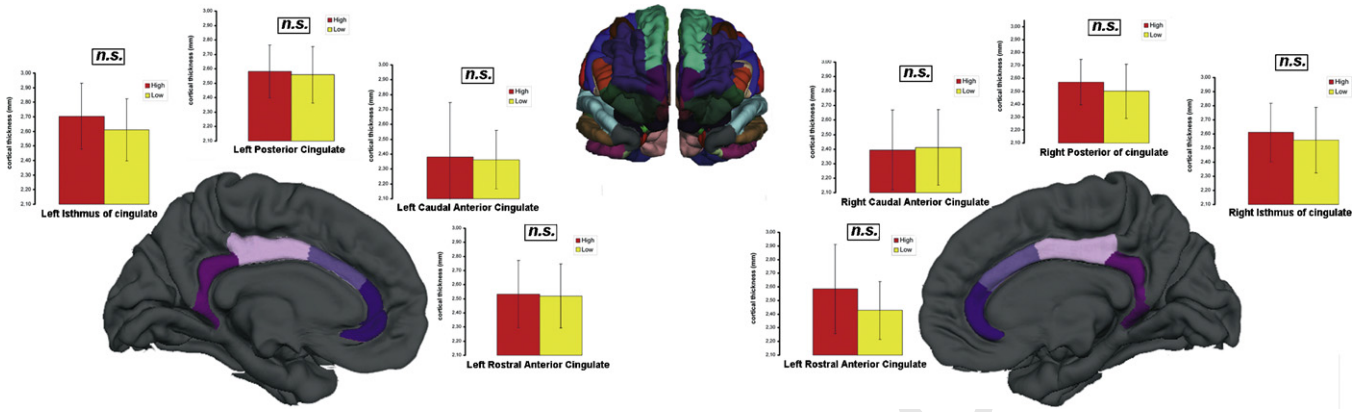

Fig. 3. Mean cortical thickness within the cingulate cortex plotted as a function of participants' MAO A genotype (MAO A High-activity individual represented in red and MAO A Low activity in yellow). No significant difference was detected between the two groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

with different age-related slopes, we submitted the data to another GLM where different age-related slopes were allowed to emerge (interaction effect).

2.8. Statistical analysis

Statistical analyses for demographic data (Table 1) were performed with Statistical Package for Social Sciences software-SPSS (version 12.0, Chicago IL, USA). Assumptions for normality were tested for all continuous variables. Normality was tested using the Kolmogorov–Smirnov test. All variables were normally distributed, except for the number of years of formal education (K–$S = 0.2$, $p < 0.05$). ANOVAs, Mann–Whitney U-test (educational level) and χ^2 (genotype distributions) were used to assess potential differences between the genotype groups for all demographic variables. All statistical analyses had a two-tailed α level of <0.05 for defining significance.

3. Results

3.1. Demographical data

The allelic distribution of BDNF and 5-HTTLPR genotypes were in Hardy–Weinberg equilibrium in both MAO A genotype groups. Demographic and cognitive variables were well matched between groups, although individuals carrying the Low-activity allele had higher no-perseverative errors in Wisconsin card sorting test and lower working memory performance with respect to individuals carrying the High-activity variant (Table 1).

3.2. Cortical thickness differences in ROIs

Table 2 presents the mean differences in cortical thickness between the genotype groups in the two ROIs for each hemisphere. A significant effect of MAO A polymorphism was detected on the lateral orbitofrontal cortex bilaterally ($F = 6.57$; $p < 0.01$; $F = 6.24$; $p = 0.01$; respectively for the left and right hemispheres) and in the right pars triangularis ($F = 8.19$; $p < 0.006$) (Fig. 2) where the MAO A High-activity group showed the highest value and the MAO A Low-activity group the lowest. Effect sizes for significant findings, as reflected in Cohen's d, were as follows: left lateral orbitofrontal cortex $d = 0.72$, right lateral orbitofrontal cortex $d = 0.67$, right pars triangularis $d = 0.79$.There were no significant effects on the other sub-regions within the orbitofrontal and cingulate ROIs (Figs. 2 and 3). Finally, Pearson correlations between cortical measurements and neuropsychological scores did not reveal significant relationships.

4. Discussion

The present study provides compelling new evidence that genetic variation in the MAO A gene is associated with different values of cortical thickness in the orbitofrontal cortex. In particular, the individuals carrying the High-activity variant showed the highest mean cortical thickness (~2.7 mm), while the Low-activity carriers had the lowest (~2.57 mm). Several lines of evidence have highlighted the role of MAO A in modulating serotonergic function [36,37], in particular in the orbitofrontal cortex that presents a high expression of the MAO A protein [25] and a dense serotonergic innervation [11]. In agreement with this evidence, recent fMRI studies demonstrated the abnormal activation of this prefrontal area during the execution of inhibitory control and working memory tasks in association with this genotype [76,38,41,42]. Our structural data are consistent with the reported influence of the MAO A genotype on function of the orbitofrontal cortex.

A new finding of our imaging genetic study is the presence of the increased cortical thickness in carriers of the High-activity allele with respect to the Low-activity individuals. There are few studies investigating the morphological correlates of this genotype in healthy individuals [8,38]. These studies found a significant decrease of the main parameter obtained from VBM analyses within the orbitofrontal cortex in MAO A High-activity individuals. Differences between our cortical thickness study and previous VBM results could be attributed both to biology [54] and/or methodology [33]. Indeed, VBM provides a mixed measure of cortical grey matter including cortical surface area and/or cortical folding, as well as cortical thickness, and it has been demonstrated that thickness and surface area are biologically independent and differently influenced by genetic factors [40,56]. Consequently, VBM has a limited benefit because this method could not discriminate between these two neuroanatomical traits [56]. Early evidence regarding the distinct sensitivity of cortical thickness and VBM measurements to detect the influence of genetic factors was observed in recent morphological studies investigating the impact of the $Val^{108}Met$ polymorphism in the catechol-O-methyltransferase (COMT) gene [9,10,31,49]. Although, different populations were investigated (adults [9,10,31] versus adolescents [49]) the main finding arising from these studies was the inverse correlation between the main parameter obtained from VBM analyses and the cortical thickness measurement of the prefrontal brain morphology as a function of the number of *Met* alleles.

We did not detect any significant association between the morphology of the cingulate cortex and MAO A genotype. The reason for this lack of significant association might be dependent upon some

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factors. First, as previously discussed VBM and thickness measurements are independent and may not be equally sensitive methods for detecting morphological variations caused by genetic factors [56]. Therefore, the apparent discrepancies between our data and a previous VBM finding [38] could only be caused by the different neuroanatomical traits investigated (i.e., thickness, surface or volume). Second, as demonstrated by a previous morphological study [38], gender might modulate the effects of the MAO A genotype on brain anatomy. Indeed, given the poorly established cellular mechanisms underlying MAO A dosage differences between females and males and the well-known interactive influence of sex hormone expression on orbitofrontal and cingulate cortices and regulation of mono-amine metabolism [2,27,34], we decided to only include male individuals. The sample selection that we adopted eliminates potential confounders and helps with interpretation of the results, though it might question the generalization of our findings.

At a phenotypic level, our individuals with the Low-activity allele had reduced working memory performances. This finding seems to be in agreement with another independent fMRI study coming from our research group in which we detected the positive association between the presence of this genotype and altered function of the orbitofrontal cortex during the *n*-back task [7]. However, the small sample size employed and the lack of significant correlation with the intermediate phenotype (cortical thickness) prevents us from making a general conclusion about this finding. Studies with larger cohorts of subjects are needed in order to confirm whether alteration in the serotonergic system as determined by MAO A VNTR polymorphism may affect working memory performance.

The aim of this study was to provide a new objective intermediate phenotypic marker of the MAO A VNTR polymorphism on brain anatomy by using cortical thickness measurements. Variation of anatomy in the adult human brain is primarily genetically determined [43,476]. Determining the extent to which focal brain morphology is influenced by genes is important for improving our knowledge of individual variation in brain functioning, and it facilitates the interpretation of the morphological changes found in psychiatric disorders [32]. Given the recent evidence about the lack of correspondence between MAO A VNTR and MAO A activity in a cohort of healthy adults by using *in vivo* measurement (C^{11}clorgyline positron emission tomography (PET)) [26] we can hypothesize that our structural findings may not necessarily be related to serotonergic neurotransmission. More complex and long-acting molecular mechanisms could be involved, as 5-HT has been highly implicated for being involved in development and differentiation of neurons [15,35]. Other factors, such as environmental risk factors, need to be considered as well. In fact, as recently stated by Belsky et al. [1] the MAO A genotype could be more appropriately conceptualized as a "plasticity gene", rather than putative "vulnerability genes" or "risk alleles", because they seem to make individuals more susceptible to environmental influences. This new neurobiological model of gene–environment interactions resembles that reported previously by Buckholtz and Meyer-Lindenberg [2], namely that the MAO A Low-activity variant, by altering 5-HT and noradrenaline levels during a critical window for the development of corticolimbic circuitry, labilizes the neural network involved in social decision making and affect regulation, rendering risk allele carriers more vulnerable to the influence of adverse early life experience. Thinning of the orbitofrontal cortex in individuals carrying the MAO A Low-activity variants would seem to support this hypothesis.

In conclusion, our data provides further validation of the biological impact of MAO A genetic variation on a neural system, which is relevant to the pathophysiology of behavioural disorders. In particular, thickness measurement of the orbitofrontal cortex may represent a new promising morphometric endophenotype for future studies.

Uncited references

[3,24,47].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbr.2010.03.021.

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