The Cumulative Effect of Genetic Polymorphisms on Depression and Brain Structural Integrity

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Abstract: In major depressive disorder (MDD), the need to study multiple-gene effect on brain structure is emerging. Our aim was to assess the effect of accumulation of specific *SERT*, *BDNF* and *COMT* gene functional polymorphisms on brain structure in MDD patients. Seventy-seven MDD patients and 66 controls underwent a clinical assessment, genetic testing and MRI scan. Compared with controls, patients were more *BDNF*-Val homozygotes, *COMT*-Met carriers and *SERT*-L' carriers. Thus, subjects were split into three groups: 1. High-frequency susceptibility polymorphism group (hfSP, subjects with all three SPs); 2. Intermediate-frequency SP group (ifSP, two SPs); and 3. Low-frequency SP group (lfSP, one/none SP). Cortical thickness, volumetry of hippocampus, amygdala and subcortical structures, and white matter (WM) tract integrity were assessed. Compared to controls, hfSP patients showed thinning of the middle frontal cortex bilaterally, left frontal pole, and right lateral occipital cortex, and smaller hippocampal volume bilaterally; and both hfSP and lfSP patient groups showed thinning of the left inferior parietal cortex and reduced WM integrity of the corpus callosum. Compared to patients, hfSP controls showed greater integrity of the fronto-occipital cortices and corpus callosum. We showed that cortical prefrontal and occipital damage of MDD patients is modulated by the SP accumulation, while damage to the parietal cortex and corpus callosum seem to be independent of genetic accumulation. HfSP controls may experience protective mechanisms leading to a preserved integrity of critical cortical and WM regions. Investigating the effect of multiple genes is promising to understand the pathological mechanisms underlying MDD. *Hum Brain Mapp* 37:2173–2184, 2016.

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Additional Supporting Information may be found in the online version of this article.

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Key words: major depression disorder; functional polymorphisms; cortical thickness; tractography; accumulation model

INTRODUCTION

One of the main theories for the aetiology of complex human diseases, including major depressive disorder (MDD), is the “common disease-common variant hypothesis” that suggests a role of numerous common genetic variants that individually exert small biological effects, but combined may mediate the risk for a particular disease, due to cumulative or synergistic effects [Singleton and Hardy, 2011]. So far, several genetic polymorphisms have been associated with susceptibility for depression, but results can explain only a small portion of the phenotypic expression of this condition. In MDD, three of the “prime suspects” are: the serotonin transporter gene (*SERT*) 5-HTTLPR polymorphism, which has been shown to be associated with neuroticism and reaction to stress [Caspi et al., 2003]; catechol-O-methyltransferase gene (*COMT*) Val158Met, which exerts an effect on emotional processing and predisposes to an increased vulnerability to depression [Antypa et al., 2013]; and the brain-derived neurotrophic factor gene (*BDNF*) Val66Met known to modulate neuroplasticity [Hosang et al., 2014]. However, the association between these genetic polymorphisms and depressive features are often inconsistent and even sometimes contradictory, suggesting that the simple “protective/vulnerable” dichotomy is not applicable

for these polymorphisms and that their effects are likely to be much more complex. Furthermore, there is no consensus on which polymorphism makes individuals most vulnerable to depression. For instance, although the S’ allele was suspected to be a susceptibility polymorphism for the *SERT* 5-HTTLPR genotype [Caspi et al., 2003], recent evidences suggested that both the L’ and the S’ allele carry the risk for MDD, but each exhibits the effect in different environmental conditions [Belsky et al., 2009]. Similarly, meta-analyses on *BDNF* Val66Met [Verhagen et al., 2010] and studies assessing *COMT* Val158Met [Domschke et al., 2012] questioned whether Val or Met is the susceptibility polymorphism in these genes.

Imaging studies reported structural and functional alterations of several brain regions of depressed patients (mostly fronto-temporal regions, hippocampus and amygdala) in association of each of the three polymorphisms. However, findings did not provide a clear and widely accepted explanation for the pathophysiological mechanisms of MDD. One possible approach to study depression is to analyze the interaction or accumulation effects of functional polymorphisms on cerebral tissue integrity. In line with this approach, a synergistic effect on the pathophysiology of MDD was suggested for *SERT* 5-HTTLPR and *BDNF* Val66Met, *SERT* 5-HTTLPR and *COMT* Val158Met, and *BDNF* Val66Met and *COMT* Val158Met polymorphisms.

The aim of this study was to assess whether there was an effect of accumulation of specific *BDNF*, *COMT* and *SERT* functional polymorphisms on clinical symptoms, cortical thickness, volumetry of hippocampus, amygdala and subcortical structures, and white matter (WM) tract architecture in patients affected by MDD compared to healthy controls. Susceptible gene polymorphisms were selected according to their distribution among our patients and controls.

METHODS AND MATERIALS

Subjects

Eighty-five patients who fulfilled the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-R) criteria for a current major depressive episode without psychotic features were consecutively recruited at the Institute of Mental Health, Belgrade, Serbia, from June 2011 to April 2013. Seventy-three age- and sex-matched healthy subjects were also enrolled. Participants were excluded if they: (a) were < 18 and > 65 years old; (b) had any (other) central nervous system disease or other causes of focal or diffuse brain damage, including lacunar infarction(s) and/or WM hyperintensities at routine MRI; (c) had any psychiatric

Abbreviations

ANOVA	Analysis of variance
BDI	Beck Depression Inventory
<i>BDNF</i>	Brain-derived neurotrophic factor gene
CC	Corpus callosum
<i>COMT</i>	Catechol-O-methyltransferase gene
CSF	Cerebrospinal fluid
CT	Cortical thickness
DE	Dual-echo
FDR	False discovery rate
GABA	Gamma-aminobutyric acid
GM	Gray matter
HDRS	Hamilton Depression Rating Scale
ILF	Inferior longitudinal fasciculi
MD	Mean diffusivity
MDD	Major depressive disorder
MNI	Montreal Neurological Institute
SCID-I	Structured Clinical Interview for Axis-I
<i>SERT</i>	serotonin transporter gene
SLF	Superior longitudinal fasciculi
TFE	Turbo field echo
TR	Repetition time
TSE	Turbo spin echo
WM	White matter
WMH	WM hyperintensity

TABLE I. Demographic, clinical and genetic features of patients and controls

	MDD patients	Healthy controls	P
Number of subjects	77	66	–
Age [years]	44.6 ± 10.6 (22–63)	45.4 ± 10.8 (23–63)	0.63
Age at onset [years]	36.1 ± 12.7 (11–59)	–	–
Women	46 (60%)	35 (53%)	0.73
Education	3.2 ± 1.0 (1–5)	4.4 ± 1.2 (2–6)	<0.001
WMH load [ml]	0.2 ± 0.6 (0–3.8)	0.1 ± 0.2 (0–0.8)	0.22
FH	28 (36.4%)	7 (10.6%)	<0.001
HDRS	22.9 ± 4.8 (11–36)	–	–
BDI	30.4 ± 12.2 (5–59)	2.2 ± 3.4 (0–21)	<0.001
<i>BDNF</i>			
Val66Val; Val66Met/Met66Met	53 (69%); 24 (31%)	32 (48%); 34 (52%)	0.01
Val66Val/Val66Met; Met66Met	71 (92%); 6 (8%)	65 (98%); 1 (2%)	0.12
<i>COMT</i>			
Val158Val; Val158Met/Met158Met	14 (18%); 63 (82%)	21 (32%); 45 (68%)	0.04
Val158Val/Val158Met; Met158Met	59 (77%); 18 (23%)	51 (77%); 15 (23%)	1.00
<i>SERT</i>			
5-HTTLPR S'S'; L'S'/L'L'	12 (16%); 65 (84%)	19 (29%); 47 (71%)	0.04
5-HTTLPR S'S'/L'S'; L'L'	64 (83%); 13 (17%)	52 (79%); 14 (21%)	0.53
Cumulative model: <i>BDNF</i> : Val-Val (SP)/ Met-carriers (noSP); <i>COMT</i> :Val-Val (noSP)/ Met-carriers (SP); <i>SERT</i> : S'S'(noSP)/L'-carriers (SP)			
VVS* (1 SP; Low-frequency SP)	3 (4%)	3 (5%)	0.85
VVL (2 SPs; Intermediate-frequency SP)	6 (8%)	7 (11%)	0.56
VMS (2 SPs; Intermediate-frequency SP)	4 (5%)	5 (8%)	0.56
VML (3 SPs; High-frequency SP)	40 (52%)	17 (26%)	0.001
MVS (0 SP; Low-frequency SP)	0 (0%)	2 (3%)	0.12
MVL (1 SP; Low-frequency SP)	5 (6%)	9 (13%)	0.16
MMS (1 SPs; Low-frequency SP)	5 (6%)	9 (13%)	0.16
MML (2 SPs; Intermediate-frequency SP)	14 (19%)	14 (21%)	0.65

Values denote means ± standard deviations (range or frequency). Education scale: 1= no school; 2= primary school; 3= high school; 4= college; 5= university degree; 6= master degree or doctoral degree. In the cumulative model, patients and controls were defined V, M or L, depending on the two allelic polymorphisms of each of the three genes: Val/Val (V) vs. Met carriers (M) for the *BDNF* and *COMT* genes, and S'/S' (S) vs. L' carriers (L) for the *SERT* gene. *The order is the following: *BDNF* (V or M), *COMT* (V or M), *SERT* (S' or L').

Abbreviations: BDI= Beck Depression Inventory; FH= positive Family History for psychiatric disorders; HDRS= Hamilton Depression Rating Scale; MDD= Major Depression Disorder; SP= susceptibility polymorphism; WMH= White Matter Hyperintensities.

comorbidity within the Axis I, except for anxiety disorders (specifically panic disorder, phobic and generalized anxiety disorders).

Eight patients (9%) and 7 healthy controls (9.5%) were not included in the final analyses due to failure to complete the clinical and/or the MRI protocol. Therefore, the final sample included 77 patients and 66 healthy controls (Table I). All patients were treated with antidepressants (selective serotonin reuptake inhibitors, being the most prevalent class, followed by tetracyclics, tricyclics and serotonin and norepinephrine reuptake inhibitors), in association with at least one benzodiazepine (mostly clonazepam) (Supporting Information Table I). Since our sample consisted only of inpatients, some of them experiencing a treatment-resistant depression and a chronic course of the disease also received adjunct treatment such as antipsychotics (first or second generation) or stabilizers (Supporting Information Table I).

The Ethics committees of the Institute of Mental Health and the School of Medicine, University of Belgrade approved the study. Patients and controls were included after signing an informed written consent.

Clinical Assessment

All participants underwent a comprehensive evaluation including detailed psychiatric history and examination, blood sampling and MRI. Socio-demographic and clinical data and family history (according to patient knowledge, information given by family members or existing medical charts) were obtained during a detailed interview with the psychiatrist. Positive family history was identified by questions regarding knowledge of any family member (up to and including third degree relatives) that suffered from any mental disorder, underwent treatment for depression

or any other psychiatric disorder, had suicide attempts, suicides, alcoholism and/or drug addiction.

The Structured Clinical Interview for Axis-I for DSM-IV (SCID-I) [First et al., 2002] and the Hamilton Depression Rating Scale (HDRS) [Hamilton, 1960] were administered to patients. The Structured Clinical Interview for Axis II for DSM-IV (SCID II) was administered to 61 out of 77 patients. The self-reporting Beck Depression Inventory (BDI) [Beck et al., 1961] was administered to both patients and controls.

Genetic Testing

DNA was extracted from blood using standard protocols. Genotypes of selected single-nucleotide polymorphisms in *BDNF* (rs6265) and *COMT* (rs4680) genes were determined using predesigned TaqMan assays (Applied Biosystems, Foster City, CA). The (*SERT*) 5-HTTLPR deletion (L/S) polymorphism was assayed using gel electrophoresis of PCR products as previously reported [Alexander et al., 2009]. Additionally, all samples containing the L allele were genotyped for SNP rs25531 (A/G) by restriction fragment length polymorphism analysis with HpaII restriction enzyme.

MRI Acquisition

Brain MRI scans were obtained using a 1.5 T scanner (Achieva, Philips Medical Systems, Best, the Netherlands) with a SENSE-head 8 coil. The following sequences were acquired from all subjects: dual-echo (DE) turbo spin echo (TSE) [repetition time (TR) = 3,124 ms; echo time (TE) = 20/100 ms; flip angle = 90°; 88 contiguous, 3-mm-thick, axial slices; matrix size = 256 × 246; FOV = 240 × 240 mm²]; 3D T1-weighted turbo field echo (TFE) (TR = 7.09 ms; TE = 3.23 ms; flip angle = 8°; 180 contiguous axial slices with voxel size = 1 × 1 × 1 mm³; matrix size = 256 × 256; FOV = 256 × 256 mm²); and pulsed-gradient SE echo planar with sensitivity encoding (TR = 6,714 ms; TE = 86 ms; 3,300 contiguous, 2.6-mm-thick, axial slices; number of acquisitions = 1; after SENSE reconstruction, the matrix dimension of each slice was 112 × 112, with an in-plane pixel size = 2 × 2 × 2.6 mm and a FOV = 224 × 224 mm²) and diffusion gradients applied in 65 noncollinear directions using a gradient scheme which is standard on this system (gradient over-plus) and optimized to reduce echo time as much as possible. The *b* factor used was 1,000 s/mm². Fat saturation was performed to avoid chemical shift artefacts. All slices were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum (CC).

MRI Analysis

WM hyperintensity (WMH) load was measured by a semi-automatic threshold-based approach using the Jim

software package (Version 6.0, Xinapse Systems, Northants, UK, <http://www.xinapse.com>).

Gray matter: cortical thickness

Cortical reconstruction and estimation of cortical thickness (CT) were performed on the 3D T1-weighted FFE images using the FreeSurfer image analysis suite, version 5.0 (<http://surfer.nmr.mgh.harvard.edu/>) [Fischl and Dale, 2000]. After registration to Talairach space and intensity normalization, the process involved an automatic skull stripping, which removes extra-cerebral structures, cerebellum and brainstem, by using a hybrid method combining watershed algorithms and deformable surface models. Images were then carefully checked for skull stripping errors. After this step, images were segmented into gray matter (GM), WM, and cerebrospinal fluid (CSF); cerebral hemispheres were separated, and subcortical structures were divided from cortical components. The WM/GM boundary was tessellated and the surface was deformed following intensity gradients to optimally place WM/GM and GM/CSF borders, thus obtaining the WM and the pial surfaces [Dale et al., 1999]. The results of this segmentation procedure were inspected visually, and if necessary, edited manually by adding control points. Afterwards, surface inflation and registration to a spherical atlas were performed [Dale et al., 1999] and the cerebral cortex parcellated into 34 regions per hemisphere, based on gyral and sulcal structures, as described by Desikan et al. [2006]. CT was then estimated as the average shortest distance between the WM boundary and the pial surface. Surface maps were generated following registration of all subjects' cortical reconstructions to a common average surface and then smoothed using a surface-based Gaussian kernel of 10 mm full width half-maximum. The mean CT of the 34 regions of interest per hemisphere was calculated.

Gray matter: volumetry

Using the FreeSurfer automatic subcortical segmentation toolkit (<http://freesurfer.net/fswiki/SubcorticalSegmentation>), we obtained the volumetric values from the hippocampus, amygdala and several subcortical structures (thalamus, caudate, putamen, pallidum, and accumbens nuclei) bilaterally.

White matter: DT MRI preprocessing and tractography

DT MRI analysis was performed using the FMRIB software library (FSL) tools (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT>) and the JIM6 software (Version 6.0, Xinapse Systems, Northants, UK, <http://www.xinapse.com>). Diffusion-weighted images (DWI) were corrected for distortions caused by eddy currents and movements, using an implementation of the algorithm described in Rohde et al. [2004] (<http://white.stanford.edu/newlm/index>).

php/DTI_Preprocessing#dti_Raw_Preprocessing_Pipeline). This eddy-current/motion correction step combines a rigid-body 3D motion correction (six parameters) with a constrained non-linear warping (eight parameters) based on a model of the expected eddy-current distortions. Previous transformations were concatenated to a further affine transformation calculated to register DWI onto the Montreal Neurological Institute (MNI) space and applied to DW data. The DT was estimated on a voxel-by-voxel basis using the DTIfit toolbox, part of the FMRIB Diffusion Toolbox within FSLv4.1.7 (<http://www.fmrib.ox.ac.uk/fsl/>) in order to obtain maps of mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity (axD) and radial diffusivity (radD).

Seeds for tractography of the cingulate bundle (split in the anterior cingulum and parahippocampal tract), CC (whole tract, as well as genu, body and splenium), superior longitudinal (SLF), inferior longitudinal (ILF) and uncinate fasciculi were defined in the MNI space on the FA template provided by FSL, as previously described [Canu et al., 2015]. Fiber tracking was performed in native DT MRI space using a probabilistic tractography algorithm implemented in FSL (probtrackx), which is based on Bayesian estimation of diffusion parameters (Bedpostx) [Behrens et al., 2007]. Fiber tracking was initiated from all voxels within the seed masks in the diffusion space to generate 5,000 streamline samples with a step length of 0.5 mm and a curvature threshold of 0.2. Tract maps were then normalized taking into consideration the number of voxels in the seed masks. To do so, the number of streamline samples present in the voxels of the tract maps was divided by the way-total, which corresponds to the total number of streamline samples that were not rejected by the exclusion masks. The tract maps obtained were thresholded at a value equal to 40% of the 95th percentile of the distribution of the intensity values of the voxels included in the tract. This normalization procedure allowed to correct for possible differences between tracts due to the different sizes of the starting seeds. In this way, we also excluded the background noise and avoided a too restrictive thresholding when the maximum intensity value was an outlier. Using a “single-seed” approach, the reconstructions of the entire CC, genu, body and splenium of the CC, and bilateral anterior cingulum, parahippocampal tract, SLF, ILF and uncinate fasciculus were obtained. Group probability maps of each tract were produced to visually check their anatomical accuracy across study subjects. For each tract, the average MD, FA, axD, and radD were calculated in the native space.

Statistical Analysis

For each gene, we tested the frequency of the homozygous status of one allele *vs.* the homozygous plus the heterozygous status of the other, and compared patients and controls in any of these combinations in order to assess

those polymorphisms that were more frequent in MDD patients, i.e., susceptibility polymorphisms (SPs). Based on their distribution among patients and controls (Table I), *BDNF* Val66Val, *COMT* Val158Met and Met158Met, and 5-HTTLPR L'S' and L/L' were considered SPs for MDD. Patients and controls were classified into three groups using a cumulative SP approach as follows: (1) high-frequency SP (hfSP) group, i.e., subjects who had all the three SPs; (2) intermediate-frequency SP (ifSP) group, i.e., subjects who had two SPs; and (3) low-frequency SP (lfSP) group, i.e., those who had only one or none SP. The study included 40 hfSP patients, 24 ifSP patients, 13 lfSP patients, 17 hfSP controls, 26 ifSP controls and 23 lfSP controls. In order to assess the cumulative SP effect, group comparisons were performed according to the cumulative approach for clinical, mean CT of the 34 regions of interest per hemisphere, and mean DT MRI measures of WM tracts. All statistics were assessed using analysis of variance (ANOVA) models followed by post-hoc pairwise comparisons adjusted for age and education and false discovery rate (FDR) corrected for multiple comparisons. Differences in categorical variables were assessed using the Chi-square test. In case of significant findings in the cumulative model, we tested whether the results were driven by the effect of a single-gene SP. This was performed using additional ANOVA models between groups of subjects stratified according to each single-gene SP, i.e., if only one SP group showed the same significant alteration as the cumulative model, this was considered an effect of the single-gene SP rather than the accumulation of SPs, and the finding was not reported. The probability level of $P < 0.05$ was considered statistically significant. For statistical analysis, SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL) was used.

Power analysis showed that our sample sizes achieved a 99% power to detect differences between the means of the CT of the right rostral middle frontal gyrus (selected as the region expected to be altered in the MDD population in accordance with previous literature [Chang et al., 2011; Khundakar et al., 2009; Miller and Cohen, 2001]) *vs.* the alternative of equal means using an *F* test with a 0.05 significance level. The size of the variation in the means represented their standard deviation, which was 0.05.

RESULTS

Demographic, clinical and genetic features

Demographic, clinical and genetic features of MDD patients and controls are reported in Table I. All patients and controls were similar for age, gender, and WMH load. Compared with controls, MDD patients showed lower education, higher depression scores, and more frequent family history for MDD. Compared with controls, MDD patients had more prevalent Val66Val polymorphism of the *BDNF* gene, while there was a higher prevalence of

Met carriers of the *COMT* Val158Met polymorphism and L' carriers of the *SERT* 5-HTTLPR polymorphism in patients relative to controls. The probability of each patient to be simultaneously a Val homozygote for *BDNF*, Met carrier for *COMT*, and L' carrier for *SERT* (i.e., hfSP) was significantly higher in comparison to controls. Combination of any two SPs was more prevalent in patients than in controls, although the statistical significance was lower than that of all three SPs (data not shown). No other SP combination showed a statistically significant difference between patients and controls.

Demographic and clinical features of patients and controls with different SP frequencies are reported in Table II. Patients and controls with different SP frequencies were similar for age and gender. Patient groups were also similar for age at onset and all showed lower education and higher scores of depression compared with healthy controls. Significant difference was found between the hfSP and lfSP patient groups, as well as between ifSP and lfSP patient groups for the HDRS item 18 score (diurnal variations, Supporting Information Table II). The patient groups had a similar distribution of personality disorders, except for the borderline personality disorder (more frequent in ifSP and lfSP groups compared with hfSP patients), and the narcissistic personality disorder (more frequent in the lfSP compared with the ifSP group, Supporting Information Table III). Finally, the hfSP patient group had a significantly more frequent family history for MDD compared to all groups of controls and compared to the ifSP patients.

Cortical thickness

hfSP patients showed reduced CT of: the bilateral rostral middle frontal gyrus compared to both hfSP and lfSP controls; the right lateral occipital gyrus compared to hfSP controls; and the left frontal pole compared to ifSP controls. Compared to lfSP controls, hfSP and lfSP patients showed cortical thinning of the left inferior parietal cortex (Table III; Fig. 1). Cortical thinning of the right precentral gyrus in lfSP patients compared to hfSP controls was driven by the *SERT* gene only (specifically by the S homozygotes carriers) rather than by the cumulative effect, and therefore not reported as a finding of the cumulative effect.

Volumetry

HfSP patients showed smaller left hippocampal volume compared to hfSP controls and smaller right hippocampal volume compared to lfSP controls (Table III).

Tractography

Compared to hfSP controls, hfSP and lfSP MDD patients showed a decreased FA of the entire CC, with a more severe involvement of the CC body (Table III; Fig. 1).

DISCUSSION

This is the first study investigating the cumulative effect of the three major genes (i.e., *BDNF*, *COMT* and *SERT*) known to be associated with the risk of developing depression on brain structural integrity of MDD patients and healthy controls. We addressed the possible synergistic effect of these gene polymorphisms by stratifying patients and controls in groups depending on the number of SPs (defined as those with higher prevalence in our patients than in controls).

We observed that the probability to be *BDNF*-Val homozygote, *COMT*-Met carrier, and *SERT*-L' carrier was significantly higher in patients compared to controls and for this reason we named this group as the hfSP group.

The cumulative effect of genetic polymorphisms on MDD clinical features

Among patients, the hfSP patient group had a significantly higher probability to have at least one MDD affected family member when compared to ifSP patient group and all control groups, suggesting that these genetic factors might explain a proportion of heritability. Clinically, higher HDRS scores on diurnal variations were the only feature influenced by the SP accumulation, with these disturbances being more severe with increasing number of SPs. This finding is not surprising due to the role that each investigated gene plays in the circadian system. The circadian system is a 24-hour internal timing mechanism for the brain and the body, which regulates behavior, physiology, and mood. Serotonin is known to modulate the response of the circadian system to the light by mediating the modification of the period and phase of the central clock through the behavioral arousal and through the feedback from the locomotor activity. Although the role of the *BDNF* and *COMT* on diurnal variations has been less investigated, the Val158Met polymorphism has been found to be associated with increased sleepiness in healthy controls and narcoleptic patients [Dauvilliers et al., 2001]; and the interaction between *BDNF* plasma levels, hypothalamus-pituitary-adrenal axis, and sex steroids has been found to be critical in clinical conditions with modified homeostasis/adaptation, such as functional hypothalamic amenorrhea [Drakopoulos et al., 2015]. Against this background, it is noteworthy that an accumulation of SPs of these genes in hfSP patients has an effect on diurnal variations greater than that of a single gene.

The Cumulative Effect of Genetic Polymorphisms on MDD Brain Structural Integrity

Our MRI study provided three major findings: (1) The effect of the genetic accumulation on the fronto-occipital CT in the hfSP group of patients; (2) The effect of MDD on the parietal CT and CC integrity of patients, independent

TABLE II. Demographic and clinical features of MDD patients and healthy controls stratified according to the frequency of susceptibility polymorphisms

	MDD Patients		Controls		F/χ^2	P	hfSP	ifSP	hfSP	ifSP	hfSP	ifSP
N	40		24		13		17		26		23	
Age [years]	43.5 ± 10.1 (24–62)		45.5 ± 11.7 (22–60)		46.3 ± 10.0 (28–63)		47.3 ± 8.8 (28–59)		44.2 ± 11.1 (23–63)		45.4 ± 12.1 (24–62)	
Age at onset	34.8 ± 11.2 (11–59)		39.1 ± 12.5 (21–59)		37.8 ± 13.5 (12–53)		–		–		–	
Women	30 (75%)		19 (79.2%)		11 (84.6%)		11 (64.7%)		22 (84.6%)		20 (87%)	
Education	3.2 ± 1.0 (1–5)§		3.2 ± 1.0 (1–5)§		3.1 ± 1.0 (2–5)§		4.4 ± 1.1 (2–6)		4.4 ± 1.3 (2–6)		4.4 ± 1.1 (2–6)	
WMH load [ml]	0.13 ± 0.6 (0–3.8)		0.21 ± 0.6 (0–2.8)		0.17 ± 0.4 (0–1.3)		0.09 ± 0.2 (0–0.6)		0.10 ± 0.2 (0–0.8)		0.04 ± 0.1 (0–0.6)	
FH	22 (55.0%)		6 (25.0%)		5 (38.5%)		1 (5.9%) [°]		4 (15.4%) [°]		2 (8.7%) [°]	
HDRS	22.6 ± 4.9 (11–30)		23.2 ± 4.5 (16–36)		23.0 ± 5.6 (12–33)		–		–		–	
BDI	29.5 ± 11.5 (7–59)§		29.6 ± 13.9 (5–56)§		34.3 ± 11.1 (15–51)§		3.2 ± 5.3 (0–21)		1.3 ± 2.1 (0–9)		2.6 ± 2.5 (0–9)	
Time between the first psychiatric consultation and the MRI visit	73.5 ± 95.0 (1–420)		57.2 ± 65.5 (1–240)		77.9 ± 72.1 (5–240)		–		–		–	

Values denote means ± standard deviations (range or frequency). §=significantly different compared with each group of controls; [°]= significantly different compared with hfSP patients. Significance is $P < 0.05$, otherwise [°] denotes $P < 0.001$. P values refer to ANOVA or χ^2 test, as appropriate (see Methods for further details). Age at onset is the age at the first symptoms, as reported by the patient.

Abbreviations: BDI = Beck Depression Inventory; FH = positive Family History for psychiatric disorders; h/i/l-fSP = high/intermediate/low-frequency susceptibility polymorphisms; HDRS = Hamilton Depression Rating Scale; MDD = Major Depression Disorder; WMH = white matter hyperintensity. Education scale: 1 = no school; 2 = primary school; 3 = high school; 4 = college; 5 = university degree; 6 = master degree or doctoral degree.

TABLE III. Significant regional mean CT measurements, volumes of hippocampus, and diffusion tensor MR measures of white matter tracts in MDD patients and healthy controls stratified according to the frequency of susceptibility polymorphisms

		MDD Patients			Controls				
		hfSP	ifSP	lfSP	hfSP	ifSP	lfSP	<i>F</i>	<i>P</i>
<i>Regional mean CT measurements</i>									
Frontal lobe									
Rostral middle frontal gyrus	L	2.20 ± 0.11*#	2.23 ± 0.13	2.22 ± 0.09	2.28±.08	2.25 ± 0.07	2.30 ± 0.09	5.39	0.002
Rostral middle frontal gyrus	R	2.19 ± 0.12*° #°	2.24 ± 0.14	2.22 ± 0.11	2.30 ± 0.09	2.23 ± 0.08	2.31 ± 0.08	4.72	0.004
Frontal pole	L	2.17 ± 0.86†	2.38 ± 0.54	2.51 ± 0.22	2.65 ± 0.21	2.61 ± 0.20	2.43 ± 0.55	4.86	0.003
Occipital lobe									
Lateral occipital cortex	R	2.05 ± 0.14#	2.10 ± 0.16	2.04 ± 0.16	2.18 ± 0.12	2.10 ± 0.11	2.16 ± 0.11	3.91	0.01
Parietal lobe									
Inferior parietal cortex	L	2.32 ± 0.12*	2.37 ± 0.12	2.29 ± 0.13*	2.37 ± 0.12	2.35 ± 0.08	2.41 ± 0.09	3.98	0.01
<i>Volumetry</i>									
Hippocampus	R	3.61 ± 0.39*	3.72 ± 0.40	3.61 ± 0.51	3.98 ± 0.36	3.80 ± 0.33	4.00 ± 0.37	3.23	0.01
Hippocampus	L	3.56 ± 0.35#	3.61 ± 0.38	3.60 ± 0.38	3.93 ± 0.44	3.66 ± 0.28	3.84 ± 0.32	3.04	0.01
<i>DT MR measures of white matter tracts</i>									
FA of CC		0.55 ± 0.02#	0.56 ± 0.03	0.55 ± 0.03#	0.58 ± 0.01	0.56 ± 0.02	0.56 ± 0.02	2.57	0.06
FA of the body of CC		0.53 ± 0.02#	0.53 ± 0.03	0.52 ± 0.04#	0.55 ± 0.01	0.53 ± 0.02	0.54 ± 0.02	2.73	0.047

Values are means ± standard deviations [mm]. * = significantly different compared with lfSP controls; † = significantly different compared with ifSP controls; # = significantly different compared with hfSP controls. Significance is $P < 0.05$, otherwise ° denotes $P < 0.001$. P values refer to ANOVA model adjusted for age and education, false-discovery rate corrected for multiple comparisons.

Abbreviations: CC = corpus callosum; DT MR = Diffusion Tensor Magnetic Resonance; FA = fractional anisotropy; h/i/l-fSP = high/intermediate/low-frequency susceptibility polymorphisms; L = left; MDD = Major Depressive Disorder; R = right.

of the genetic accumulation; (3) The integrity of the fronto-occipital cortex and the CC in hfSP control group.

Considering the first point, compared to controls, only the hfSP patient group showed a reduced CT of the bilateral rostral middle frontal gyrus, left frontal pole, right lateral occipital gyrus and hippocampus bilaterally. The rostral middle frontal gyrus, part of the dorsolateral prefrontal cortex (DLPFC), is a critical region for executive functions, working memory, goal-directed actions, abstract reasoning, attentional control and for the regulation of negative emotions through reappraisal/suppression strategies [Miller and Cohen, 2001]. DLPFC involvement, mainly in the middle frontal regions, has been previously suggested as crucial in MDD cases by studies that have not considered the effect of genes [Chang et al., 2011; Khundakar et al., 2009]. In previous genetic reports, all genes considered in our study showed an effect on this region. Cortical thinning of the DLPFC has been found to be related to the *BDNF* Val168Met polymorphism in MDD patients [Legge et al., 2015]. A reduced *SERT* binding in this region has been observed in people at high risk to develop depression [Frokjaer et al., 2009]. DLPFC activation, associated with a poor emotional processing, has been observed to increase with the number of *COMT* Met-alleles [Mier et al., 2010], and a specific decreased activation of the middle frontal gyrus during a working memory task was observed in MDD patients and healthy *COMT*-Met carriers [Opmeer et al., 2013]. Furthermore, compared to hfSP controls only, the hfSP patient group showed a

cortical thinning of the right lateral occipital cortex. Recently, there has been increasing evidence that MDD is associated with altered gamma-aminobutyric acid (GABA) neurotransmission with MDD patients having low plasma and CSF GABA levels. MR spectroscopy investigations in MDD demonstrated reduced GABA levels in prefrontal regions and occipital cortex [Hasler et al., 2007; Sanacora et al., 1999]; in these regions a decreased cell density of GABAergic neurons has been observed in autopsy studies [Maciag et al., 2010; Rajkowska et al., 2007]. Our findings indicate that the effect of MDD on CT of the prefrontal and occipital regions might be mediated more by the cumulative effect of all the three genes rather than by each of them separately.

To our knowledge, up to date, only two studies have examined the synergic effect of *BDNF*, *COMT* and *SERT* on cerebral structure in healthy subjects or MDD patients, and both focused on hippocampal volume. However, findings are contradictory with one study showing a high association between the cumulative model and hippocampal total and subregional volume loss in healthy subjects [Rabl et al., 2014], and the other showing no effect on hippocampal structure in a population of MDD patients and controls [Phillips et al., 2015]. In keeping with previous findings [Rabl et al., 2014], in our population we observed a cumulative genetic effect on the hippocampus in MDD patients. Due to the central role of amygdala and thalamus (together with the hippocampus) in the interaction between the medial prefrontal cortex and the limbic

Figure 1.

Regional cortical thinning and white matter tract damage of MDD patients compared to healthy controls stratified according to the frequency of susceptibility polymorphisms (SP). Colours indicate comparisons between high-frequency (first row) and low-frequency (second row) patients vs. healthy controls with specific SP frequency: red= comparison vs. both high- and low-frequency SP controls (e.g., these are the regions where both

high- and low-frequency SP controls showed increased CT and WM integrity compared with patients); green= vs. high-frequency SP controls; blue= vs. intermediate-frequency SP controls; yellow= vs. low-frequency SP controls. Results are overlaid on the Montreal Neurological Institute template and shown at $p < 0.05$ corrected for False Discovery Rate. R = Right; L = Left.

system [Price and Drevets, 2010], the lack of significant effects in these regions was unexpected. This could be due to some limitations of the Freesurfer technique in segmenting appropriately the subcortical structures [Morey et al., 2009]. In addition, it is also likely that MDD is associated with subregional alterations of these structures which may be better depicted using parcellation or shape analyses.

Concerning the second point, hfSP and lfSP patient groups showed cortical thinning of the left inferior parietal lobe compared to lfSP controls and altered WM microstructure of the entire CC (with a prominent involvement of the body) compared to hfSP controls. Recent studies revealed altered connectivity of the frontoparietal network in MDD patients [Kaiser et al., 2015] and parietal lobe in people at risk to develop the disease [Mannie et al., 2010]. Furthermore, reduced FA of the CC, mainly in the body, has been previously observed in patients with the first MDD episode, geriatric depression, and chronic recurrent MDD [Murphy and Frodl, 2011]. Interestingly, these

changes have been also observed in healthy adolescents with a familial risk for MDD [Huang et al., 2011]. The CC is the largest myelinated inter-hemispheric WM structure, and lesions to it are known to alter inter-hemispheric integration related to cognition and emotional regulation [Kiehl et al., 2010]. Specifically, the CC body section contains fibers linking the primary motor cortices, premotor cortices and associative regions, such as those of the parietal cortex [Catani et al., 2002]. Alterations of the primary motor cortices have been found to be associated with the typical disturbances of fine motor behavior, gross locomotor activity, or ideation of these patients [Bennabi et al., 2013]. Based on the consistent reports of reduced CC FA independent of illness duration or MDD staging, and the recent data showing alterations of the frontoparietal networks in MDD patients [Kaiser et al., 2015], our findings suggest that the impaired integrity of the CC and the parietal cortex could be structural correlates of MDD that are, at least partially, independent of genetic accumulation.

However, our data also show that in these regions each group of controls is not equal in the way it is “protected” from MDD, with lfSP controls showing greater inferior parietal thickness, and hfSP controls showing increased WM organization in the body of CC.

It is noteworthy that ifSP and lfSP patient groups showed more frequent comorbidity with the borderline personality disorder, compared with hfSP patients and the lfSP patient group has more frequent comorbidity with the narcissistic personality disorder relative to the ifSP patients. Therefore, we cannot exclude that both MDD and structural damage in the ifSP and lfSP patients may be, at least partially, related to their personality disorders in addition to their genetic profiles. However, since we investigated the presence of personality disorders only in 79% of patients and in none of the controls, we could not further investigate this aspect in the present study.

Another intriguing finding of this study is that the hfSP control group had a preserved integrity of the rostral middle frontal cortices, right lateral occipital cortex, and CC, and higher education when compared to patient groups. These findings can be interpreted based on what we know about brain and cognitive reserves. The brain reserve protects from pathology and limits its clinical outcome. The original concept of brain reserve is quantitative, i.e., more neurons or synapses to lose, less impact of structural damage. This idea is supported by a set of seminal studies in healthy subjects and patients with dementia suggesting that prevalence or incidence of cognitive impairment is lower in individuals with larger brains [Schofield et al., 1997]. As recently suggested by Sibille [2013] in a model on age, neuroplasticity and vulnerability to depression, the cumulative altered expression of SPs may trigger the onset of several cellular alterations, leading to an integrity loss of specific structural regions, and the amount of these abnormalities may potentially determine if and when decreased function reaches a certain threshold and the pathophysiological output occurs. The greater brain reserve in hfSP controls would postpone this threshold with a consequent delay of the disease onset. In addition, in our sample, the higher education level of hfSP controls compared to the groups of patients may represent an environmental protective factor (i.e., cognitive reserve). Thus, it is tempting to speculate that, in the presence of high frequency SP accumulation, both these reserve typologies may interact to prevent development of depression. On the other hand, a different explanation may rather consider cortical thinning as a susceptibility factor, which, when associated to high risk genetic profile, leads to the onset of depressive symptoms. Future longitudinal studies on larger populations of healthy subjects with a genetic risk to develop MDD are needed to clarify these findings.

Strengths and Limitations of the Study

A number of factors increases the reliability of our findings. The patient population is clinically well defined.

Cortical and WM alterations have been investigated with advanced MRI techniques, such as a surface-based approach and tractography which are able to detect subtle changes, and a sound statistical methodology with correction for multiple comparisons and confounding variables. However, there are also some caveats to be considered when interpreting our findings. First, the relatively small sample size when we stratified the groups according to the number of SPs. Second, the ifSP patient and control groups did not show specific patterns of brain features; this is likely due to the fact that ifSP groups were very heterogeneous in terms of SPs. Third, our analysis was not controlled for treatment type, dosage and duration.

CONCLUSIONS

In conclusion, we detected the cumulative effect of *SERT*, *BDNF* and *COMT* functional polymorphisms on brain morphological features in MDD patients and controls. In patients, such a cumulative effect is evident in the rostral middle frontal cortex, frontal pole, and lateral occipital cortex. These findings suggest that the accumulation of the observed SPs might raise susceptibility for MDD more than each polymorphism separately. This hypothesis needs to be confirmed by genome-wide association studies on a larger patient population. Finally, the hfSP control group may represent a good model for increasing our knowledge of protective factors that may play a role in preventing the development of depression.

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