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ORIGINAL PAPER

Relationship between VEGF-related gene polymorphisms and brain morphology in treatment-naïve patients with first-episode major depressive disorder

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Abstract

Vascular endothelial growth factor (VEGF) is involved in the development of major depressive disorder (MDD). Recently, a genome-wide association study has revealed that four VEGF-related single nucleotide polymorphisms (SNPs) (i.e., rs4416670, rs6921438, rs6993770 and rs10738760) were independently associated with circulating VEGF levels. The current study investigated the relationship between brain volume and these four SNPs in first-episode drug-naïve MDD patients. A total of 38 first-episode drug-naïve MDD patients and 39 healthy subjects (HS) were recruited and underwent high-resolution T1-weighted imaging. Blood samples were collected from all the participants for serum VEGF assays and VEGF-related SNPs genotyping. Genotype–diagnosis interactions related to whole-brain cortical thickness and hippocampal subfield volumes were evaluated for the four SNPs. The results revealed a genotype–diagnosis interaction only for rs6921438 (i.e., the MDD patients and HS with the G/G genotype versus the MDD patients and HS with A-carrier genotype) in the subiculum of the left hippocampus ($p < 0.05$), and not the other SNPs. There was a volume reduction in the left subiculum of G/G genotype patients compared with the other groups. The "hypochondriasis" scores of the HAMD-17 scale were significantly higher in the G/G genotype patients than the A-carrier genotype patients. The association was observed between VEGF-related SNP rs6921438 and subiculum atrophy in first-episode drug-naïve MDD patients.

Keywords Major depressive disorder · Vascular endothelial growth factor (VEGF) · VEGF-related gene polymorphisms · Brain morphology · Hippocampal subfields volume · Surface-based morphometry

Introduction

Vascular endothelial growth factor (VEGF) was originally identified as a vascular permeability factor and it belongs to the group of signal proteins involved in the regulation of physiological and pathological angiogenesis [1]. However, VEGF has been shown to have neuroprotective, neurogenesis and vasculogenesis potential in the central nervous system [2, 3]. For example, it enhances axonal outgrowth, cell survival [4], synaptic plasticity [5], synaptic transmission [6], and it protects hippocampal neurons against glutamate-mediated toxicity [7].

In recent years, the role of VEGF in psychiatric diseases, particularly in major depressive disorder (MDD) has been gradually emphasized. Many recent studies also suggested that VEGF is involved in the development of MDD [8, 9]. Iga et al. demonstrated that the peripheral leukocyte VEGF mRNA levels of MDD patients were significantly higher than those of healthy subjects (HS) at baseline, and their reduction after 8 weeks of treatment may predict clinical improvement [10]. Lee et al. reported that MDD patients exhibited significantly increased plasma VEGF levels compared with HS [11]. According to the review by Duric and

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Duman, stress alters cortisol, several neurotrophic factors including VEGF, and cell signaling, which introduces cell loss, neuronal atrophy and remodeling, and finally leads to brain structural changes in MDD [12]. Taken together, it is possible that VEGF plays a role in pathophysiology of MDD.

Despite the link between VEGF and major depression, few studies have demonstrated that VEGF polymorphisms are associated with an increased risk for depression. Recently, four single nucleotide polymorphisms (SNPs), including rs4416670, rs6921438, rs6993770 and rs10738760, were identified to explain approximately 50% of the heritability of circulating VEGF levels through a genome-wide association study (GWAS) [13]. Among them, an association between SNP rs4416670 and an increased depressive risk was reported and this finding may help to establish potential links between VEGF genetic factors and MDD [14].

Morphological alterations of cortical and subcortical regions have been revealed in structural neuroimaging studies of depressive patients [15, 16]. The hippocampus, a component of limbic structures, has been proven to regulate the impact of emotional experiences and to control general affective states [17]. The abnormalities of total hippocampus and its subfields have been identified to be associated with mood disorders, particularly with MDD [18–24]. These brain alterations may be the results of brain development and neurodegeneration under the regulation of genetic and epigenetic factors. In previous studies, the link between genetic determinants and brain volume has been proven (e.g., brain-derived neurotrophic factor (BDNF) gene, serotonin transporter (5-HTT) gene, norepinephrine transporter (NET) gene, methylenetetrahydrofolate reductase (MTHFR)/catechol-O-methyltransferase (COMT) gene, piccolo presynaptic cytomatrix protein (PCLO) gene) [25–31]. However, to the best of our knowledge, no previous studies have examined the neuroimaging changes associated with VEGF-related SNPs in MDD patients. Thus, we hypothesized that VEGF-related SNPs may be associated with serum VEGF level which affects brain morphometry regarding the pathophysiology of MDD. In short, the aim of this neuroimaging study was to comprehensively examine the association between VEGF-related SNPs and morphometric brain abnormalities in first-episode drug-naïve MDD patients.

Materials and methods

Participants

Thirty-eight first-episode drug-naïve MDD patients and 39 HS were recruited. Participants with MDD were diagnosed by a psychiatrist (A.K., who has 13 years of experience in psychiatry diagnosed) using the fully Structured Clinical Interview for Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition, Text revision (DSM-IV-TR). The exclusion criteria for MDD patients included any history of neurological diseases or other physical diseases, comorbid psychiatric disorders, and the use of drugs (steroids, aspirin, or nonsteroidal anti-inflammatory drugs) that affect the immune system and previous episodes of depressive disorders. The depression severity of each patient was assessed with the 17-item Hamilton Rating Scale for Depression (HAMD-17) [32]. We also evaluated subcategories of HAMD-17 by grouping the items into six factors: core (items 1, 2, 7, 8, 10 and 13), sleep (items 4,5,6), activity (items 7 and 8), psychic (items 9 and 10), somatic anxiety (items 11,12,13) and delusion (items 2, 15 and 20), as described by Serretti et al. [33]. The HS group consisted of staff from our institution, their relatives (by marriage) and close friends. Biologically related relatives were excluded. They were interviewed by the same psychiatrist using the Structured Clinical Interview for DSM-IV, non-patient edition. Eligible healthy subjects had no history of serious medical or neuropsychiatric illness, no use of drugs (steroids, aspirin, or nonsteroidal anti-inflammatory drugs) and no family history of major psychiatric or neurological illnesses among their first-degree relatives.

This study was performed in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board at the University of Occupational and Environmental, Japan. All participants were fully explained and provided written informed consent before beginning the study procedures.

Genotyping

Genomic DNA from peripheral blood was isolated using the QIAamp DNA Mini-Kit (QIAGEN, Tokyo, Japan), according to the manufacturer's protocol. Four VEGF-related SNPs (rs4416670, rs6921438, rs6993770 and rs10738760) were genotyped using polymerase chain reaction (PCR). The PCR products were enzymatically purified. The results of genotyping were confirmed by sequencing reactions using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies Corporation). The sequencing primers used were the same as the PCR primers. The sequences were read using an Applied Biosystems 3730xl DNA Analyzer (Life Technologies Corporation). The sequencing output data were then compared with the reference sequence. The result of genotyping is shown in Table 1. We categorized the genotypes of each SNP into the C/C genotype and T-carrier genotype for rs4416670; G/G genotype and A-carrier genotype for rs6921438; A/A genotype and T-carriers for rs6993770; G/G genotype and A-carrier genotype for rs10738760.

Table 1 SNP characteristics in MDD patients and HS

SNP	Chromosome	Minor allele	Genotype distribution ($n=77$)		HWE	
			MDD patients	HS	MDD patients	HS
rs4416670	6	C	CC/CT/TT:16/14/8	CC/CT/TT:6/25/8	0.158	0.075
rs6921438	6	A	GG/AG/AA:14/17/7	GG/AG/AA:10/21/8	0.649	0.618
rs6993770	8	T	AA/AT/TT:11/20/7	AA/AT/TT:12/23/4	0.691	0.149
rs10738760	9	A	GG/AG/AA:10/16/12	GG/AG/AA:16/18/5	0.338	0.986

SNP single nucleotide polymorphism, *MDD* major depressive disorder, *HS* healthy subjects, *HWE* Hardy–Weinberg equilibrium

Serum VEGF assay

Peripheral blood samples were collected from all participants, sera were extracted and stored in different storage tubes at −80 °C until they were assayed. The serum levels of VEGF were measured using the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

MRI acquisition

Brain imaging examinations were performed with 3T MRI (Signa EXCITE 3T; GE Healthcare, Waukesha, WI, USA) with an 8-channel brain coil. Diffusion tensor images were performed with the following parameters: TR/TE = 12,000/83.3 ms, slice thickness = 4 mm, no gap, field of view = 26 cm, number of excitations = 1, and spatial resolution = 1 × 1 × 4 mm. Diffusion gradients (*b* value of 1000 s/mm^2) were applied simultaneously. The diffusion properties were measured in 25 directions. Using the Grad Warp software program [34] for intensity inhomogeneity with the "N3" function [35], we corrected all images for image distortion due to gradient non-linearity.

A radiologist (S.K., who has 21 years of experience in neuroradiology) who reviewed the conventional MRI data (including T2-weighted images) reported no gross abnormalities (e.g., infarcts, hemorrhages, or brain tumors) in any of the study participants.

Image processing

Whole-brain analyses using surface-based morphometry (SBM)

We estimated the regional cortical thickness using FreeSurfer software version 6.0 (http://www.freesurfer.net/fswiki/FreeSurferWiki). The technical details of cortical thickness analysis have been described elsewhere [36]. Using a common spherical coordinate system [37], the data were re-sampled for all participants. The cortical map for each participant was smoothed with a 10-mm kernel at full width at half-maximum (FWHM) for cortical analyses.

Volumetry of hippocampal subfields

FreeSurfer software version 6.0 was used to calculate hippocampal subfield volumes (http://www.freesurfer.net/fswiki/HippocampalSubfields) [38]. The hippocampal subfields were segmented using a Bayesian inference approach and a novel atlas algorithm of the hippocampal formations that was primarily built upon ultra high-resolution ex vivo MRI data from autopsy brains. The calculated hippocampal subfields included the Cornu Ammonis (CA)1, CA3, CA4, granule cell layer of dentate gyrus (GC-DG), fimbria, subiculum, presubiculum, parasubiculum, hippocampal fissure molecular layer, hippocampus–amygdala transition area (HATA), hippocampal tail, and whole hippocampus (Fig. 1). We calculated the total hippocampal volume in each hemisphere by summing the volumes of all subfields except for that of the fimbria, presubiculum, parasubiculum, molecular layer,

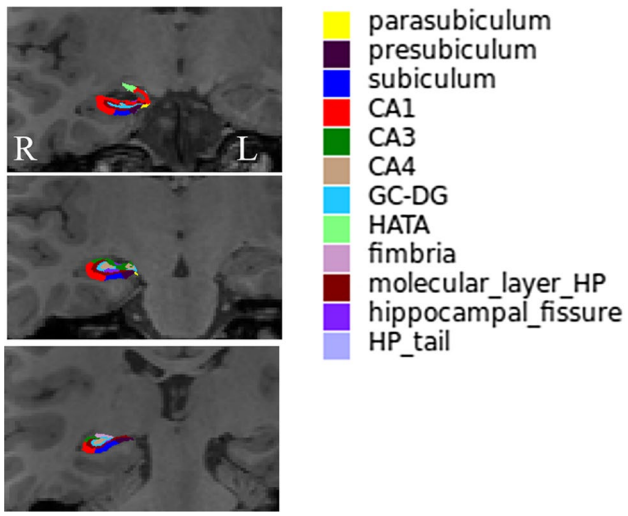

Fig. 1 Representative subdivision of the hippocampal subfields. The mask of each region was overlapped on the coronal T1-weighted images. Color classification: parasubiculum = yellow; presubiculum = black; subiculum = blue; CA1 = red; CA3 = dark green; CA4 = brown; granule cell layer of dentate gyrus (GC-DG) = sky blue; hippocampus–amygdala transition area (HATA) = green; fimbria = purple; molecular layer of the hippocampus (HP) = dark brown; hippocampal fissure = dark purple; hippocampal tail = gray

hippocampus–amygdala transition area and hippocampal fissure. The technical details have been described in a previous report [39].

Statistical analysis

For the analysis of demographic and clinical characteristics, analysis of variance (ANOVA) was performed to test for differences in age among four groups, in terms of the SNPs and diagnoses (HS and MDD patients). Fisher's exact test was used to evaluate the between-group differences in gender. A permutation test was used to compare the total and subcategory HAMD-17 scores between MDD patients grouped by genotype. The Mann–Whitney U test and Kruskal–Wallis test were used to compare the VEGF levels between groups. We assessed the relationship between HAMD-17 scores as well as its subcategory scores and VEGF levels in MDD group using Spearman's rank correlation.

Voxel-wise statistical analysis was performed using a surface-based analysis using the FreeSurfer statistical tool QDEC after 10-mm FWHM kernel smoothing. A general linear model was then applied at each vertex. The morphological changes in the cortical thickness were assessed using a full factorial model with the diagnosis and genotype status being used as independent variables. Age, gender and HAMD-17 scores were included as covariates of no interest in all analyses to control for confounding variables. The following *t* test comparisons for 2×2 factorial designs were performed: diagnosis effects (MDD patients versus HS), genotype effect, and genotype–diagnosis interaction. In addition, age and gender were set as "nuisance factors" to control for confounding variables. It is plausible that the HS and MDD patients included in this study may exhibit different cortical evolution rates; thus, the DODS (different offsets, different slopes) was employed. To correct for multiple comparisons, we used a Monte Carlo simulation for the cluster analysis. The cluster-forming threshold was set at $p < 0.05$. Clusters were then tested against an empirical null distribution of maximum cluster size built using synthesized Z-distributed data across 10,000 permutations, producing clusterwise *p* values that were fully corrected for multiple comparisons.

The volume of each hippocampal subfields between four groups (divided based on genotype and diagnosis) were compared using ANOVA, followed by Tukey's post hoc test.

We used multiple linear regression analysis to evaluate the effect of genotype–diagnosis interaction on hippocampal subfield volumes. Age and gender were entered as covariates in the analyses. Individual hippocampal subfield volumes were normalized for intersubject variation in head size by dividing hippocampal subfield volumes by the total intracranial volume.

Statistical analyses were considered to be significant at $p < 0.05$. All statistical analyses were performed using EZR software version 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [40], which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of the SNPs and demographic clinical data

In this sample, the distributions of the genotypes among both the HS and MDD patients were in agreement with Hardy–Weinberg equilibrium (Table 1). No significant differences were observed between the MDD patients and HS, in terms of the distributions of age (46.7 ± 15.7 vs. 41.4 ± 11.2, $p = 0.09$) and gender ($p = 0.13$).

Whole-brain analyses using SBM

Diagnosis effects: MDD patients vs. HS

Irrespective of genotype, no significant differences in cortical thickness were observed between the MDD patients and HS.

Genotype effects

For the four VEGF-related SNPs, no brain areas were found to have significant differences in the cortical thickness among the genotypes in the MDD patients and HS groups ($p > 0.05$, FWE-corrected).

Genotype–diagnosis interaction

For the four VEGF-related SNPs, no significant genotype–diagnosis interaction was observed in relation to brain morphology (cortical thickness).

Volumetry of hippocampal subfields

Diagnosis effects: MDD patients vs. HS

Regardless of the genotype, no significant differences in total hippocampal volume or hippocampal subfield volumes were observed between the MDD patients and HS.

Genotype effects

For the four VEGF-related SNPs, no SNPs were found to influence hippocampal subfield volumes in the MDD patients and HS (there was no effect of genotype).

Genotype–diagnosis interaction

In the multiple linear regression models adjusted for age and gender, we identified a genotype–diagnosis interaction of rs6921438 (the MDD patients and HS with the G/G genotype versus the MDD patients and HS who are A-carriers) only in the left subiculum ($p < 0.05$) (Table 2). For rs6921438, the volume of the left subiculum was significantly different among the four groups ($p = 0.039$), and a post hoc test showed that the volume of the left subiculum was significantly smaller in the MDD patients with the G/G genotype ($2.49 \times 10^{-04} \pm 2.86 \times 10^{-05}$) than in the MDD patients with the A-carrier genotype ($2.75 \times 10^{-04} \pm 2.97 \times 10^{-05}$) (Table 3). We observed no genotype–diagnosis interaction associated with the volume of any hippocampal subfields for the other three VEGF-related SNPs.

Table 2 The genotype–diagnosis interaction on hippocampal subfield volumes

Hippocampal subfields	rs4416670	rs6921438	rs6993770	rs10738760
Left				
CA1	0.242	0.151	0.266	0.773
CA3	0.416	0.429	0.973	0.562
CA4	0.382	0.745	0.571	0.780
GC-DG	0.459	0.770	0.361	0.484
Subiculum	0.403	0.041*	0.630	0.288
Molecular layer	0.536	0.160	0.413	0.316
Hippocampal tail	0.452	0.557	0.192	0.385
Whole hippocampus	0.558	0.149	0.668	0.360
Right				
CA1	0.590	0.781	0.116	0.832
CA3	0.950	0.857	0.575	0.282
CA4	0.374	0.910	0.725	0.950
GC-DG	0.343	0.969	0.438	0.688
Subiculum	0.148	0.654	0.636	0.880
Molecular layer	0.515	0.836	0.202	0.745
Hippocampal tail	0.518	0.430	0.916	0.809
Whole hippocampus	0.384	0.798	0.276	0.967

All data are *p* values. Age and gender are entered as covariates in analyses

CA cornu ammonis, *GC-DG* granule cell layer of dentate gyrus

*Significant genotype–diagnosis interaction ($p < 0.05$)

HAMD-17 scores and peripheral/serum VEGF levels in SNP rs6921438

We obtained the subcategory HAMD-17 scores from all 38 MDD patients. No significant differences in total HAMD-17 scores were observed between the patients with the G/G genotype and the patients who are A-carriers ($p = 0.89$). For the factor analysis of the HAMD-17 scale, the "hypochondriasis" scores were significantly higher in the G/G individuals than the A-carrier individuals ($p < 0.05$) (Table 4).

No difference in serum VEGF levels between the MDD and the HS groups was observed. In the MDD patients, the VEGF levels were lower in the G/G individuals than in the A-carrier individuals, but there were no significant differences between them ($p = 0.92$). In the HS, VEGF levels were significantly increased in G/G individuals compared to A-carrier individuals ($p = 0.03$) (Table 5). We found no relationship between HAMD-17 scores, its subcategory scores and serum VEGF levels in the MDD patients (Table 6).

Discussion

To the best of our knowledge, this study provides the first evidence of a relationship between brain morphology and VEGF-related SNPs in MDD patients. The strength of this study involves the recruitment of first-episode, drug-naïve patients with MDD. In this study, hippocampal subfield analysis demonstrated a genotype–diagnosis interaction of rs6921438 in the left subiculum. The volume reduction of the left subiculum in the MDD patients compared to HS was significantly larger in G/G individuals than in A-carrier individuals. Although we did not find that SNP rs6921438 influenced any hippocampal subfield volumes in the patients and HS (there was no effect of genotype), we showed a significant genotype–diagnosis interaction. These results may support the conclusion that SNP rs6921438 is associated with atrophy of the subiculum in MDD patients. Furthermore, we also found that the "hypochondriasis" score was significantly higher in the G/G patients than in the A-carrier patients. In other words, the results partially support our hypothesis that the VEGF-related SNPs affect brain morphometry which is related to the pathophysiology of MDD.

The hippocampus has been thought to play an important role in MDD. Previous meta-analyses have revealed hippocampal volume reduction in MDD [15, 24, 41]. Furthermore, hippocampal volume may be associated with a risk of MDD [42] and depressive symptom severity [43]. Anatomically, the hippocampus is further divided into the various hippocampal subfields as follows: cornu ammonis (CA1, CA2, CA3, and CA4), dentate gyrus (DG), and subiculum. The subiculum is located in the inferomedial area of the hippocampus and serves as a connection between the CA1 and

Table 3 The volume of hippocampal subfields based on genotype groups regarding the rs6921438

Hippocampal subfields	MDD patients		Healthy subjects		*p* value
	G/G	A-carrier	G/G	A-carrier	
Left					
CA1	$3.66\times10^{-4}\pm4.21\times10^{-5}$	$3.93\times10^{-4}\pm4.31\times10^{-5}$	$3.88\times10^{-4}\pm3.53\times10^{-5}$	$3.83\times10^{-4}\pm4.63\times10^{-5}$	0.31
CA3	$1.22\times10^{-4}\pm1.59\times10^{-5}$	$1.25\times10^{-4}\pm1.48\times10^{-5}$	$1.28\times10^{-4}\pm2.01\times10^{-5}$	$1.25\times10^{-4}\pm1.77\times10^{-5}$	0.86
CA4	$1.53\times10^{-4}\pm1.55\times10^{-5}$	$1.56\times10^{-4}\pm1.48\times10^{-5}$	$1.57\times10^{-4}\pm1.99\times10^{-5}$	$1.57\times10^{-4}\pm1.44\times10^{-5}$	0.86
GC-DG	$1.76\times10^{-4}\pm1.88\times10^{-5}$	$1.80\times10^{-4}\pm1.85\times10^{-5}$	$1.83\times10^{-4}\pm2.22\times10^{-5}$	$1.84\times10^{-4}\pm1.53\times10^{-5}$	0.63
Subiculum	$2.49\times10^{-4}\pm2.86\times10^{-5}$	$2.75\times10^{-4}\pm2.97\times10^{-5}$	$2.76\times10^{-4}\pm2.07\times10^{-5}$	$2.71\times10^{-4}\pm2.91\times10^{-5}$	0.039*
Molecular layer	$3.25\times10^{-4}\pm3.51\times10^{-5}$	$3.50\times10^{-4}\pm3.51\times10^{-5}$	$3.46\times10^{-4}\pm2.68\times10^{-5}$	$3.46\times10^{-4}\pm2.81\times10^{-5}$	0.12
Hippocampal tail	$3.14\times10^{-4}\pm4.56\times10^{-5}$	$3.33\times10^{-4}\pm5.71\times10^{-5}$	$3.25\times10^{-4}\pm5.54\times10^{-5}$	$3.22\times10^{-4}\pm3.97\times10^{-5}$	0.68
Whole hippocampus	$1.98\times10^{-3}\pm1.88\times10^{-4}$	$2.11\times10^{-3}\pm1.93\times10^{-4}$	$2.11\times10^{-3}\pm1.91\times10^{-4}$	$2.09\times10^{-3}\pm1.52\times10^{-4}$	0.16
Right					
CA1	$3.93\times10^{-4}\pm5.17\times10^{-5}$	$4.04\times10^{-4}\pm5.17\times10^{-5}$	$3.88\times10^{-4}\pm4.30\times10^{-5}$	$4.01\times10^{-4}\pm5.16\times10^{-5}$	0.80
CA3	$1.35\times10^{-4}\pm2.16\times10^{-5}$	$1.38\times10^{-4}\pm1.94\times10^{-5}$	$1.35\times10^{-4}\pm1.96\times10^{-5}$	$1.34\times10^{-4}\pm1.39\times10^{-5}$	0.88
CA4	$1.59\times10^{-4}\pm2.21\times10^{-5}$	$1.63\times10^{-4}\pm1.98\times10^{-5}$	$1.60\times10^{-4}\pm1.52\times10^{-5}$	$1.62\times10^{-4}\pm1.45\times10^{-5}$	0.95
GC-DG	$1.84\times10^{-4}\pm2.65\times10^{-5}$	$1.88\times10^{-4}\pm2.32\times10^{-5}$	$1.89\times10^{-4}\pm1.75\times10^{-5}$	$1.89\times10^{-4}\pm1.67\times10^{-5}$	0.90
Subiculum	$2.60\times10^{-4}\pm3.52\times10^{-5}$	$2.72\times10^{-4}\pm3.45\times10^{-5}$	$2.72\times10^{-4}\pm2.28\times10^{-5}$	$2.73\times10^{-4}\pm3.12\times10^{-5}$	0.64
Molecular layer	$3.44\times10^{-4}\pm4.32\times10^{-5}$	$3.54\times10^{-4}\pm4.16\times10^{-5}$	$3.48\times10^{-4}\pm2.79\times10^{-5}$	$3.56\times10^{-4}\pm3.33\times10^{-5}$	0.75
Hippocampal tail	$3.35\times10^{-4}\pm6.26\times10^{-5}$	$3.45\times10^{-4}\pm4.49\times10^{-5}$	$3.44\times10^{-4}\pm4.43\times10^{-5}$	$3.61\times10^{-4}\pm6.03\times10^{-5}$	0.49
Whole hippocampus	$2.09\times10^{-3}\pm2.56\times10^{-4}$	$2.15\times10^{-3}\pm2.26\times10^{-4}$	$2.13\times10^{-3}\pm1.64\times10^{-4}$	$2.17\times10^{-3}\pm2.03\times10^{-4}$	0.65

The volumes were normalized and given under: mean ± s.d

CA cornu ammonis, *GC-DG* granule cell layer of dentate gyrus

*Significant genotype–diagnosis interaction ($p<0.05$)

Table 4 Demographic and clinical factors in subjects with G/G and A-carrier genotype regarding the rs6921438

	MDD patients			Healthy subjects		
	G/G ($n=14$)	A-carrier ($n=24$)	*p* value	G/G ($n=10$)	A-carrier ($n=29$)	*p* value
Male/female	10/4	10/14	0.15	8/2	20/9	0.69
Age (year)	46.4 ± 15.1	46.8 ± 16.4	0.95	38.2 ± 10.0	42.4 ± 11.6	0.31
HAMD-17						
Total (0–52)	21.5 ± 5.1	21.8 ± 6.6	0.89			
Subcategory (anxiety features)						
Psychic anxiety	2.07 ± 0.73	1.79 ± 1.22	0.53			
Somatic anxiety	1.71 ± 0.91	1.50 ± 0.72	0.53			
Gastrointestinal somatic symptoms	1.07 ± 0.62	1.13 ± 0.61	1.00			
General somatic symptoms	1.21 ± 0.58	0.83 ± 0.56	0.09			
Hypochondriasis	1.29 ± 0.91	0.50 ± 0.66	< 0.01*			
Insight	0.36 ± 0.50	0.29 ± 0.62	0.78			

MDD major depressive disorder, *HAMD-17* 17-item Hamilton Rating Scale for Depression

*Significant difference ($p<0.05$)

the entorhinal cortex. Regarding the CA1 and subiculum, the anterior region is associated with emotional functioning [44]. Numerous studies have provided evidence of a relationship between the subiculum and pathophysiology of MDD. A post-mortem study of depressed patients has demonstrated structural abnormalities in subicular neurons [45]. Another post-mortem study has also revealed a decrease in the spine density and arborization of apical dendrites in the subiculum [46]. A recent study of tissue from female monkeys with depression has revealed that the anterior CA1 and subiculum exhibit reduced volumes compared with those in normal monkeys [47]. In hippocampal shape studies utilizing MRI, CA1 and subiculum changes were reported in first-episode MDD patients [48, 49].

Table 5 Difference of VEGF levels between groups regarding the rs6921438

MDD patients		Healthy subjects		*p* value of Mann–Whitney *U* test
G/G	A-carrier	G/G	A-carrier	
51.07 ± 75.79		31.41 ± 24.91		0.46
34.94 ± 24.83	60.47 ± 92.96			0.92
		45.30 ± 33.67	26.62 ± 19.62	0.03*
34.94 ± 24.83		45.30 ± 33.67		0.47
	60.47 ± 92.96		26.62 ± 19.62	0.28

Data are VEGF levels (mean ± SD) pg/ml

MDD major depressive disorder

*Significant difference ($p < 0.05$)

Table 6 Relationship between the scores of HAMD-17 and Serum VEGF levels

	Scores (means ± s.d.)	Spearman's rank correlation coefficients	
		R	*p* value
HAMD-17 Total (0–52)	21.71 ± 6.02	− 0.138	0.41
Subcategory			
Core	10.29 ± 3.19	− 0.303	0.06
Sleep	3.32 ± 1.34	− 0.131	0.43
Activity	3.87 ± 1.34	− 0.075	0.65
Psychic	2.66 ± 1.42	− 0.271	0.99
Somatic anxiety	3.66 ± 1.56	0.018	0.91
Delusion	1.74 ± 1.11	− 0.182	0.27

MDD major depressive disorder, *HAMD-17* 17-item Hamilton Rating Scale for Depression

Subfield volumetry of hippocampus (SVH) is a novel method that facilitates the calculation of the hippocampal subfields volumes using 3D high-resolution T1-weighted imaging. In this study, SVH demonstrated the genotype–diagnosis interaction of rs6921438 in the left subiculum of first-episode MDD patients despite a lack of change in the total hippocampal volume. This result is consistent with those of previous studies of first-episode MDD patients [48] and in female monkeys with depression [47]. Hence, we could suppose that the hippocampal subfield abnormality does not always appear in parallel with whole hippocampal volume reduction and that the hippocampal subfield analysis used in this study may be a useful method for evaluating MDD pathogenesis.

While the reason why the SNP rs6921438 was associated with hippocampal structure differences in MDD patients remains unclear, it is essential to discuss the possible mechanism underlying these results. Two GWAS of VEGF levels, with a large number of participants (16,112 and 5,254), have both revealed that rs6921438 on chromosome 6p21.1 was independently associated with circulating VEGF levels [13, 50]. Moreover, accumulating evidence has indicated that VEGF affects neural cells and plays a significant role in hippocampal neurogenesis and neuroprotection [8, 12]. Cao et al. further demonstrated that elevated hippocampal VEGF levels in adult rats promote neurogenesis [51]. VEGF also modulates synaptic transmission [6], suggesting that the effects of this factor are multifaceted. VEGF signals through two high-affinity receptor tyrosine kinases (Flk-1 and Flt-1) [52, 53]. Flt-1 was distributed most densely in endothelial cells and neural progenitors of the hippocampus [54]. Flk-1 mediates the mitogenic effects of VEGF in vitro [3]. Indeed, Heine et al. demonstrated that rats exposed to chronic unpredictable stress exhibited reduced hippocampal VEGF protein levels compared to healthy control rats [55]. Therefore, this evidence for a neurotrophic model of hippocampal VEGF may support our hypothesis that VEGF level alteration, which is related to SNP, may ultimately result in atrophy of the hippocampus.

With respect to rs6921438, the "hypochondriasis" score was significantly increased in G/G patients (who exhibited reduced subiculum volumes) compared to A-carrier patients. The "hypochondriasis" item is categorized as an anxiety feature of the anxiety/somatization factor of the HAMD-17 scale. The anterior hippocampus (ventral hippocampus in rat) is required for anxiety-like behavior through the hippocampus–amygdala–medial prefrontal cortex circuit [56] and the fear response is increased when the ventral hippocampus is inactivated [57]. Thus, our results may also suggest a pivotal role for the anterior hippocampus in the expression of anxiety-related behavior in MDD patients.

Regarding of serum VEGF levels, no significant differences were noted between the MDD patients and HS. Furthermore, among the MDD patients, there were also no significant differences between G/G and A-carrier individuals for rs6921438. A previous review article has suggested that conflicting data exist regarding VEGF levels in depressed patients [8]. In this review article, increased VEGF levels in depressed patients compared to HS were reported in four studies. However, three other studies found no changes, and one demonstrated a decrease in VEGF levels. In addition, VEGF serum levels significantly vary at different times of

the day [58]. Therefore, there is a potential for variation in VEGF levels based on the time of day and sampling procedure, which may confound the results.

Our study has some limitations that should be acknowledged when interpreting the results. A small sample of patients was recruited from only one institution, which may result in sampling bias. However, it was difficult to recruit and retain first-episode drug-naïve MDD patients because many were administered antidepressants before they underwent MRI. In the future, the longitudinal prospective studies investigating the alterations of atrophy subiculum in MDD patients after treatment and the relationship with the response to antidepressants regarding VEGF-related SNPs could be interesting.

Conclusions

The VEGF-related SNP rs6921438 was associated with atrophy of the subiculum in first-episode drug-naïve MDD patients. The interpretation of the result should be prudent.

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Compliance with ethical standards

Conflict of interest The authors have reported no potential conflicts of interest. The authors have completed the form for the disclosure of potential conflicts of interest.

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