BDNF Gene Val66met Polymorphism Associated Grey Matter Changes in Human Brain

Çağdaş Eker[1], Ömer Kitis[2], Erol Ozan[1], Hamza Okur[3],
Ozlem Donat Eker[1], Mehmet Akif Ersoy[1], Fisun Akdeniz[1],
Simavi Vahip[1,4], Nurten Akarsu[3], Ali Saffet Gonul[1,2,4,5]

ABSTRACT:
BDNF gene val66met polymorphism associated grey matter changes in human brain

Objective: A frequent single nucleotide polymorphism (SNP) in the targeting region of the human brain-derived neurotrophic factor (BDNF) gene (val66met) has been associated with abnormal intracellular trafficking and regulated secretion of BDNF in cultured neurons transfected with met allele. Moreover, normal individuals with met alleles have poor episodic memory performance, reduced hippocampal volume and reduced physiological engagement during memory encoding and retrieval. However, the effects of this SNP on other brain areas and functions have not been studied well. In this study, we aimed to explore the effects of BDNF val66met polymorphism on human brain without defining a region of interest a priori.
Methods: Twenty-eight healthy volunteers were studied by voxel-based morphometry according to their genotype. The imaging tool was 1.5T MRI scanner and images were analyzed by Statistical Parametric Mapping 2 software.
Results: The met carriers had decreased grey matter in left uncus, Brodmann Area (BA) 36, right inferior parietal lobule, BA 40 and left occipital lobe, BA 18 compared to those of val homozygote volunteers. On the other hand, they had more grey matter in right inferior frontal gyrus, BA10 and left inferior temporal gyrus, BA 20.
Conclusion: BDNF val66met polymorphism has a significant effect on brain structures which are involved in the working memory network of healthy people. However, further structural and functional imaging studies are needed to understand the effects of this SNP better in the physiology of working memory of healthy people and pathophysiology of psychiatric disorders.

Key words: BDNF, frontal cortex, parietal cortex, working memory, voxel-based morphometry

Klinik Psikofarmakoloji Bülteni 2005;15:104-111

ÖZET:
İnsan beyninde BDNF geninin val66met polimorfizmine bağlı gri madde değişiklikleri

Amaç: Beyinden köken alan nörotropfik faktörü (BDNF) kodlayan genin val66met, tek nükleotid polimorfizmine (TNP) sık rastlanmaktadır ve bu durum met alleli ile transfekte edilmiş hücre kültürü nöronlarında BDNF'nin hücre-içi iletimi ve salgılanmasının düzenlenmesindeki anormallikle ilişkilidir. Dahası, met alleli taşıyan sağlıklı bireylerin epizodik bellek performansları düşüktür; hippokampal hacimleri ile bellek kodlaması ve geri çağırılması sırasındaki fizyolojik uyumları azalmıştır. Bununla birlikte TNP'nin diğer beyin bölgeleri ve işlevleri üzerindeki etkilerine yönelik çalışmalar yeterli değildir. Biz, bu çalışmada BDNF val66met polimorfizminin insan beyni üzerindeki etkilerini önceden bir ilgi alanı belirlemeden araştırmayı amaçladık.
Yöntem: Yirmisekiz sağlıklı gönüllüye ait görüntüler üzerinde genotiplerine göre voksel-tabanlı morfometri ile çalışıldı. Görüntüleme cihazı olarak 1.5 T MRG tarayıcısı kullanıldı ve görüntüler Statistical Parametric Mapping 2 programı ile analiz edildi.
Bulgular: Met taşıyıcılarının sol uncus, Brodmann Alanı (BA) 36, sağ inferior parietal lobül, BA 40 ve sol oksipital lob ile BA 18'deki gri cevher hacimleri, val homozigot gönüllülerine göre daha düşüktü. Öte yandan sağ inferior frontal girus, BA 10 ve sol inferior temporal girus ile BA 20'deki gri cevher hacimleri daha yüksek saptandı.
Sonuç: BDNF val66met polimorfizmi, sağlıklı bireylerin işleyen bellek ağında yer alan beyin yapıları üzerinde belirgin bir etkiye sahiptir. Bununla birlikte bu TNP'nin sağlıklı bireylerdeki işleyen bellek fizyolojisi ve psikiyatrik hastalıklardaki patofizyoloji üzerine etkilerini anlamak için daha fazla yapısal ve işlevsel beyin görüntüleme çalışmasına gereksinim vardır.

Anahtar sözcükler: BDNF, frontal korteks, parietal korteks, işleyen bellek, voksel-tabanlı morfometri

Klinik Psikofarmakoloji Bülteni 2005;15:104-111

Ege University School of Medicine Department of Psychiatry, Izmir-Turkey[1], Ege University School of Medicine Department of Radiology, Neuroradiology Department Izmir-Turkey[2], Hacettepe University School of Medicine, Gene Mapping Laboratory, Pediatric Hematology Unit, Department of Pediatrics[3], Ege University Center for Brain Research, Izmir-Turkey[4], Institut de Physique Biologique, 4 rue Kirschleger Universite Louis Pasteur Strasbourg, France[5]

Yazışma Adresi / Address reprint requests to:
Ali Saffet Gonul, MD, Associate Prof., Ege University School of Medicine Department of Psychiatry, Izmir-Turkey

Telefon / Phone: +90-232-339-8804
Faks / Fax: +90-232-339-8804

Elektronik posta adresi / E-mail address:
saffet@med.ege.edu.tr,
gonul@alsace.u-strasbg.fr

Kabul tarihi / Date of acceptance:
15 Ağustos 2005 / August 15, 2005

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), which is a member of the neurotrophin family, is widely expressed in the adult mammalian brain (1). It plays a critical role in the long-term survival, differentiation and outgrowth of neurons during development and maintenance of neuronal systems in adult life (2-5). It is also thought that BDNF is one of the key factors in neurotrophin theory which has proposed that the failure of neurogenesis and neuronal plasticity causes various psychiatric diseases like schizophrenia and major depression.

The gene of BDNF is localized to chromosome 11p14.1 (6) and has a single nuclear polymorphism (SNP) at nucleotide position 196/758 which results in amino acid change at codon 66 Valine (Val) → Methionine (Met) (Val66Met) of the proBDNF molecule. The SNP is located in a section of BDNF

precursor protein that is cleaved away by proteases on the cell surface, rendering the amino acid change absent from mature BDNF (7). Cultured hippocampal neurons transfected with Met-BDNF show reduced depolarization induced secretion and fail to localize BDNF to secretory granules and dendritic process (7,8). Normal individuals with Met alleles have poor episodic memory performance, and reduced hippocampal physiological engagement during memory encoding and retrieval, studied by functional magnetic resonance imaging (fMRI) (8). Furthermore, N acetyl aspartate level which is accepted as an indirect measure of neuronal integrity and synaptic abundance showed a linear reduction with increasing number of Met alleles in the hippocampus of normal individuals (8). Recent reports showed that Met allele may have a negative effect on hippocampal volumes which may be more pronounced in patients with schizophrenia (9,10). The effect of this SNP on other regions of the brain is still not known well.

Voxel-base morphometry (VBM) is a new approach for performing an explanatory analysis without the need to define structures a priori (11,12). In addition, VBM analysis allows structural investigation of functional regions, such as portion of frontal cortex that may be difficult to define anatomically. Potential disadvantages however, include the need to control for the large number of comparison made in a structural study of whole brain, with the potential loss of statistical power that entails.

In this study, we aimed to explore the effects of BDNF val66met polymorphism on human brain anatomy without defining a region of interest a priori. This approach allows us to define new anatomical areas that are going to be subject of future studies designed to investigate the effects of this SNP in patients with major psychiatric disorder like major depressive disorder or schizophrenia. We also studied the hippocampus which is known to be affected most with this SNP separately with the "small volume comparison" option of Statistical Parametric Mapping (SPM) software.

METHODS

Subjects and Evaluation

Twenty-eight healthy volunteers were recruited for this study. All of the volunteers were screened with Structured Clinical Interview for DSM-IV (SCID) by one of us (13). Neither of them had been diagnosed with any axis I psychiatric conditions including substance use disorder (with the exception of nicotine addiction). The following subjects were also excluded: subjects with serious or unstable medical illness or head trauma associated with loss of consciousness, subjects with a history of seizure disorder or any other organic mental disorder; subjects with a family history of any axis I psychiatric disorder. Each participating volunteer provided written informed consent after receiving a complete description of the study, and the study was approved by the Institutional Review Board.

MRI Acquisition

The imaging was performed on a 1.5 Tesla MR unit (Magnetom Vision Symphony Upgrade, Siemens, Erlangen, Germany) with a circularly polarized head coil. Three-dimensional (3-D) T1-weighted images, using a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence, were acquired with the following parameters: echo time (TE) = 3.93 ms, repetition time (TR) = 1600 ms, 2 mm coronal slices without gap, flip angle = 15°, NEX = 1, field of view (FOV) = 25 cm.

MRI Data Analysis

Image analysis was performed by using SPM 2 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, London; http://www.fil.ion.ucl.ac.uk/spm/) running in MATLAB 6.5 (Mathworks, Sherborn, MA). Image processing (VBM) was based on the method reported by Ashburner and Friston (11). As the details given below, this process involves spatially normalizing of all the images to the same streotactic space, extracting the grey matter from normalized images, smoothing and finally performing statistical analysis.

Spatial normalization achieved by registration each of the image to the same SPM2 Montreal Neurological Institute T1 template image, by minimizing the residual sum of square differences between them with an initial 12-parameter affine transformation, followed by 16 nonlinear iteration using 7 X 8 X 7 discrete cosine transform basis function. Images were written out in 1 X 1 X 1mm resolution. Normalized images

then segmented into the grey matter, white matter, cerebrospinal fluid, and skull/scalp compartments by using an automated and operator-independent process. The segmentation of normalized MR images in SPM uses a clustering algorithm identifying a voxel density of a particular tissue type combined with a priori knowledge of the spatial distribution of these clusters in healthy subjects. The segmentation step also incorporates an image-density nonuniformity correction (11) to address image-density variations caused by different positions of cranial structures within the MRI head coil. The segmented grey and white matters were smoothed with a 12-mm full-width, half-maximum isotropic Gaussian kernel to accommodate individual variability in the sulcal and gyral anatomy. By smoothing the data, the partial volume effect was used to create a spectrum of grey-white matter intensities. Grey- or white- matter density is equivalent to the weighted average of the grey- or white-matter voxels located in the volume defined by the smoothing kernel. Because previous studies showed a fair correlation between the regional grey- or white-matter density identified with VBM and their volumes measured by the conventional manual tracing method (14-16), the regional grey- and white matter density can be considered to represent the local amount of grey and white matter.

A mask image for hippocampus was formed with software MRicro (http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html). For the mask image, a mean image of 28 volunteers was obtained by SPM. The borders of hippocampus were manually traced in the best seen planes. The traced area was accepted as 1 and background as 0. Finally, masked image transformed to analyze format for small volume comparison.

Genotyping

Blood samples were collected from all volunteers and DNA subsequently extracted. A total of DNA was extracted by using MagNa Pure LC (Roche Molecular Biochemicals, Germany) an automated nucleic acid purification system and MagNa Pure LC DNA Isolation Kit I (Roche Molecular Biochemicals, Germany) according to the manufacturer instructions. 200 µL whole blood has been used as the starting amount. Real Time PCR assay and probe melting point hybridization analysis were used to detect allelic changes (Figure 1). The primers used in this study were demonstrated in (Table 1) and were purchased from used Tib Mol Biol, (Germany). BDNF_F and BDNF_R were used to amplify 184bp fragment containing G‡A transition responsible for val66met change. Specific probes (detection probe BDNF LC640 and anchor probe BDNF FLU (Table 1) were included in the PCR mixture. The detection probe was 5' labeled with LC-Red 640 and 3' phosphorylated, and the anchor probe was 3' labeled with fluorescein. The probes were designed in a distance of two nucleotides. PCR was performed on

Table 1: Primer and probe sequences used in this study. Nucleotide in bold and underlined shows the position of G‡A transition producing val66met common coding variant

Primers	Primer Sequences
BDNF_F	5'- ACTCTGGAGAGCGTGAATGG - 3'
BDNF_R	5'- CCAAAGGCACTTGACTACTGA- 3'
Probes	**Probe Sequences**
BDNF_FLU	AAGAGGCTTGACATCATTGGCTGACACT
BDNF_LC640	CGAACACGTGATAGAAGAGCTGTTGGAT

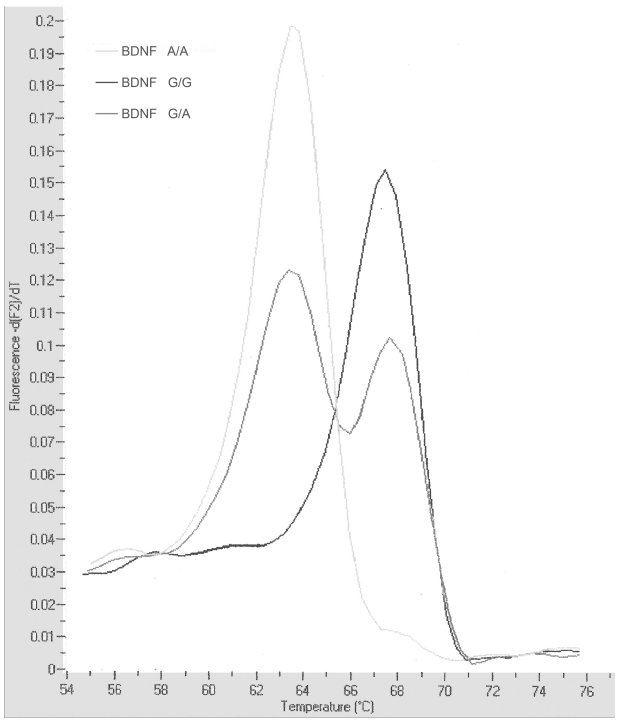

Figure 1: Melting peak analysis of BDNF val66met SNP change (G‡A). The figure demonstrate homozygous, G/G (val/val); and A/A (met/met) as well as heterozygous G/A (val/met) peaks.

LightCycler Instrument (Roche Diagnostics, Germany) in capillary glass tubes. PCR mixture contained 4,8 μL PCR Grade H2O, 0,8 μL 25 mM MgCl2 (last concentration 2mM), 0,5 μL BDNF_F (10 pmol/μL), 0,5 μL BDNF_R (10 pmol/μL), 0,2 μL BDNF_FLU (10 pmol/μL), 0,2 μL BDNF_ LC640 (10 pmol/μL) and 1 μL Light Cycler DNA Master Hybridization Probe Mix (Roche Diagnostics, Germany) and 2 μL sample DNA was added to the mixture. Cycling conditions consisted of an initial denaturation at 95°C for 10 minutes, followed by 45 cycles with denaturation at 95°C for 10 seconds, annealing at 53°C for 10 seconds, and extension at 72°C for 12 seconds. After completion of the amplification process, melting step was performed and samples of the reaction mixture were denatured at 95°C for 0 s, held at 52°C for 1:30 minutes and then slowly heated to 78°C at a ramp rate 0.2°C/seconds. During this process, declining of fluorescence was continuously monitored. Fluorescence channel 2 / 1 (F2/1) was selected as reading parameter. Melting curves were converted melting peaks by plotting the negative derivative of the fluorescence with respect to temperature (Figure 1).

Statistical Analysis

Processed images were analyzed with SPM2, employing the framework of general linear model (17). A design matrix was used in which age and gender were used as covariates of no interest and genetic polymorphism was considering parameter of interest. Two contrasts were calculated, testing for a positive and negative of grey matter volume with parameter of interest. Significance was set a p-value of p=0.025 (for each tail) with a minimum cluster size of 200 voxels. Points of maximum correlation were converted from Montreal Neurological Institute to Talariach coordinates using nonlinear transformation (18). Comparison of sociodemographic variables of volunteers was done by non-parametric tests.

RESULTS

Genotyping showed that twenty of the volunteers are val/val carrier while eight of the volunteers are met/val carriers. There was no homogenous met carrier in the group (The BDNF met polymorphism is relatively common in the human population with prevalence for heterozygote between 20-30% and prevalence for the homozygote at □4% (8,9)). The two groups divided according to their genotype were similar in the aspect of age, sex and education (Table 2).

Table 2: Demographical data of healty volunteers included in BDNF comparison

	Val/Val (N=20)		Met-Carriers (N=8)		Comparison	
	Mean	**SD**	**Mean**	**SD**	**U**	**p[a]**
Age	28.4	7.6	26.4	3.4	75	p>0.05
Education (years)	13.4	2.9	15.1	2.1	52	p>0.05
	Male	Female	Male	Female		p[b]
Gender	5	15	2	6		df=1 p>0.05

SD: Standard deviation, a: Mann-Whitney U test, b: Chi-square test

Table 3: Regional Coordinates of Areas of Differential Gray Matter Volume

Brain Region	Talariach Coordinates			Brodmann Area	Cluster Size	t	p*
	X	Y	Z				
Val/Val > Met-car							
	-6	-99	14	Left Occipital Lobe,Cuneus, BA 18	818	3.0	0.003
	-24	-2	-33	Left Limbic Lobe,Uncus, BA 36	305	2.64	0.007
	34	-40	45	Right Inferior Parietal Lobule, BA 40	309	2.47	0.010
	37	6	-36	Right temporal Cortex, BA 21	333	2.19	0.014
Met-car > Val/Val	44	45	1	Right Inferior Frontal Gyrus, ,BA 10	889	4.18	<0.001
	-60	-38	-20	Left Inferior Temporal Gyrus, BA 20	250	3.97	<0.001

*: uncorrected

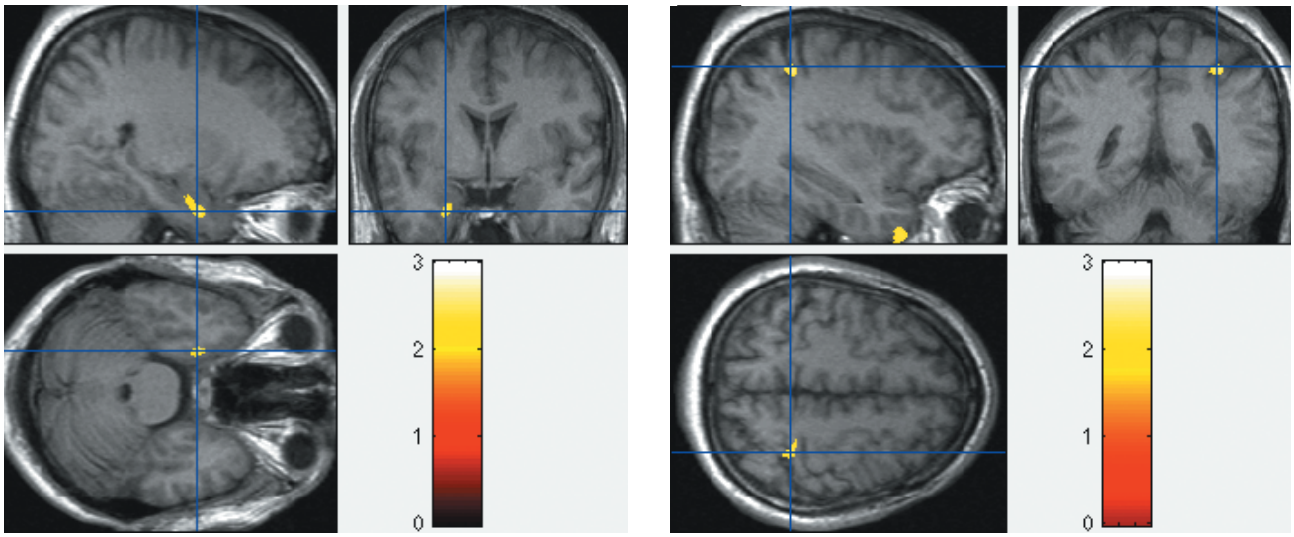

Figure 2. Val carriers have more grey matter volume in left uncus, BA 36 (A) and right inferior parietal lobule, BA 40 and inferior temporal cortex, BA 21 (B).

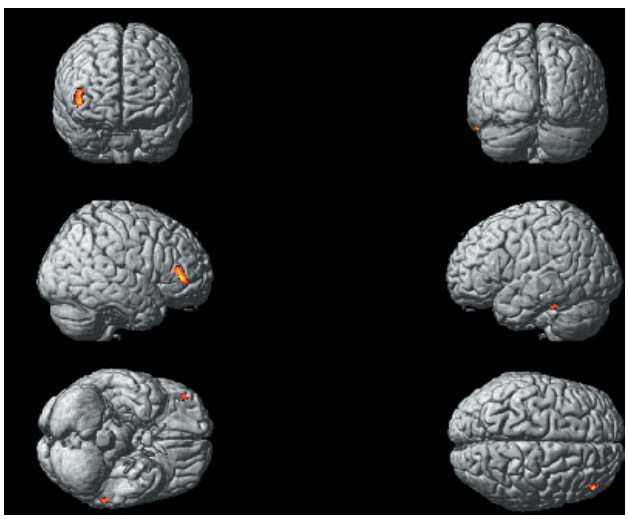

Figure 3: Whole brain rendering showing regions of increased grey matter volume (right inferior frontal lobe and left temporal cortex) of met-carriers volunteers comparing to val homozygotes.

The val carrier volunteers have more grey matter in the left uncus, Brodmann Area (BA) 36; right inferior parietal lobule, BA 40; inferior temporal cortex, BA 21 and left occipital lobe, BA 18 (not shown in the figure) compared to those of met homozygote volunteers (Table 3, Figure 2). On the other hand, met-carriers have more grey matter in right inferior frontal gyrus, BA 10 and left inferior temporal gyrus, BA 20 (Table 2, Figure 3). No significant volume difference was detected in hippocampus with and without mask image.

DISCUSSION

The action of neurotrophic factors are varied and complex since they have major regulatory roles from early development up to adulthood. Indeed, neurotrophic factors were initially known to be involved in survival, outgrowth and differentiation of neurons during development (reviewed in 19). However, high neurotrophic factor concentrations persist in various areas of adult brain, suggesting that these molecules have physiological roles beyond fetal life. Indeed, neurotrophic factors and their receptors are expressed in brain areas exhibiting a high degree of plasticity and, their expression regulated by neuronal activity. Moreover, they regulate directly or indirectly synaptic transmission. In the adult brain, the coinciding activity in pre-synaptic inputs and post-synaptic cell induces short and long term changes, with further change in synaptic efficacy.

Among neurotrophic factors, BDNF is certainly one the best studied molecule for its effect synaptic plasticity. Non-human studies showed that BDNF is involved in learning and memory. In addition, BDNF gene expression is markedly enhanced by tetanic stimulation that induced long-term potentiation (20,21) and during spatial memory tests (22,23). Inhibition of BDNF signaling in rodents by gene knockout or infusion of BDNF antibody impairs spatial learning and memory (24,25). Evidence that BDNF is

induced as an immediate early gene after neuronal stimulation and its secretion depends on neuronal activity rather than tonic secretion is substantial (7,21).

One BDNF gene SNP which results an amino acid substitution (val met) is associated in altered intracellular trafficking and regulated secretion of BDNF protein in primary cortical neurons and neurosecretory cells (7,8). This study further showed that BDNF met carriers has differences in their brain morphometry compared to those of BDNF val homozygotes. The differences were mainly observed in the areas of frontal and parietal cortices together with temporal cortex. These areas are associated with episodic and working memory in humans or primates (26,27). The disadvantage of BDNF met carriers in episodic memory has been demonstrated before, by both memory tests and associated functional MRI studies (8,28). Met carriers exhibit relatively diminished hippocampal engagement in comparison with val homozygotes during both encoding and retrieval process. These findings were supported by later reports of lower hippocampal volumes in met carriers (9,10).

In this report we could not find volumetric difference in hippocampal formation which is known to be very important in episodic memory, between met carriers and val homozygotes. However, there are number of reasons that, might explain this negative finding. The first reason might be the small number of volunteers in this study and due to that the difference between the groups might not be reached to statistical significant level. The methodological differences between the studies might be the second reason to explain the no difference because region of interest analysis that was preferred previous studies assumes that, hippocampus is one variable but voxel based morphometry compares every voxel (1X1X1 mm in this study) in hippocampus. The third reason might be the other genetic variables like serotonin transporter gene s allele or Apolipoprotein E gene allele which are known to have impacts on hippocampal volume had not been controlled in this study (29,30). One other reason might be the lack of met homozygotes in the met-carriers group. It was previously showed that a significant linear reduction in hippocampal NAA levels which shows viability of neurons, with increasing number of met allele observed, indicating possible allele dose effect (8). Thus, a similar effect might be acceptable for hippocampal volume. The first and the third reasons should also be received as the limitations of this study.

The working memory which refers to a limited capacity system that is responsible for the temporary storage and processing of information while cognitive tasks are performed (31). These procedures are mainly processed in the neocortical regions, particularly prefrontal cortex and parietal cortex (26). The psychological tests showed that met carriers do not have lower scores for working memory compared to val homozygotes (8). However, in the same study during N back test, a test for working memory, met carriers showed abnormal limbic system activation but no difference in the prefrontal cortex. In this study, the finding of increased grey matter in the prefrontal cortex of met-carriers might be a compensation for decreased grey matter of parietal and temporal regions of working memory network in the brain. This kind of compensation might explain the normal results of the psychological tests and abnormal functional imaging data in previous studies. Thus, based on our findings, we proposed that brains of met-carriers might need greater frontal area to handle the working memory procedures because it can not use the network efficiently. These changes which were independent from age and sex should be occurred before adulthood. This time period might be from birth to end of adolescence when there is abundance of synaptic pruning and new organizations (which are highly depending on neurotrophic factors) are going on in the brain (32).

It is known that neurotrophic factors including BDNF play an important role in visual system and ocular dominance organization plasticity (33,34). However, to our knowledge, no study has evaluated BDNF val66met polymorphism in the visual system. Nevertheless, our finding of increased grey matter in BA 18 in val homozygotes gives us an idea that this SNP might be important in changing grey matter structures and related functions in the visual system.

As a conclusion, BDNF val66met polymorphism has a significant impact on brain structures in healthy people. These structures are mainly involved in the working memory network which is known to be not

working properly in psychiatric diseases like schizophrenia. Thus, further structural and functional imaging studies are needed to understand the effects of this SNP better in the physiology of working memory of healthy people and pathophysiology of psychiatric diseases.

References:

1. Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde YA. Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 1990;9: 2459-2464

2. Ernfors P, Kucera J, Lee KF, Loring J, Jaenisch R. Studies on the physiological role of brain-derived neurotrophic factor and neurotrophin-3 in knockout mice. Int J Dev Biol 1995;39: 799-807

3. Ward NL, Hagg T. BDNF is needed for postnatal maturation of basal forebrain and neostriatum cholinergic neurons in vivo. Exp Neurol 2000;162: 297-310

4. Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol 2001;63: 71-124

5. Sairanen M, Lucas G, Ernfors P, Castren M, Castren E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. J Neurosci 2005;25:1089-1094

6. Fang H, Chartier J, Sodja C, Desbois A, Ribecco-Lutkiewicz M, Walker PR, et al. Transcriptional activation of the human brain-derived neurotrophic factor gene promoter III by dopamine signaling in NT2/N neurons. J Biol Chem 2003;278: 26401-26409

7. Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 2004;24: 4401-4411

8. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;24: 257-269

9. Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, et al. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 2004;24: 10099-10102

10. Szeszko PR, Lipsky R, Mentschel C, Robinson D, Gunduz-Bruce H, Sevy S, Ashtari M, Napolitano B, Bilder RM, Kane JM, Goldman D, Malhotra AK. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Mol Psychiatry. 2005;10:631-636

11. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000;11:805-821

12. Ashburner J, Friston KJ. Why voxel-based morphometry should be used. Neuroimage 2001;14:1238-1243

13. First MB, Spitzer RL, Gibbon M, Williams JBW (). Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (SCID-CV). Washington, DC: American Psychiatric Press, 1997

14. Wright IC, Ellison ZR, Sharma T, Friston KJ, Murray RM, McGuire PK. Mapping of grey matter changes in schizophrenia. Schizophr Res 1999;35:1-14

15. De Bellis MD, Keshavan MS, Frustaci K, Shifflett H, Iyengar S, Beers SR, Hall J. Superior temporal gyrus volumes in maltreated children and adolescents with PTSD. Biol Psychiatry 2002;51:544-552

16. Kubicki M, Shenton ME, Salisbury DF, Hirayasu Y, Kasai K, Kikinis R, Jolesz FA, McCarley RW. Voxel-based morphometric analysis of gray matter in first episode schizophrenia. Neuroimage 2002;17:1711-1719

17. Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD, Frackowiak RSJ. Statistitical parametric maps in functional imaging: A general linear approach. Hum Brain Mapp 1995;2:189-210

18. Brett M (2002): The MNI brain and the Talairach atlas: http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml

19. Tapia-Arancibia L, Rage F, Givalois L, Arancibia S. Physiology of BDNF: focus on hypothalamic function. Front Neuroendocrinol 2004;25:77-107

20. Lu B, Gottschalk W. Modulation of hippocampal synaptic transmission and plasticity by neurotrophins. Prog Brain Res 2000;128:231-41

21. Poo MM. Neurotrophins as synaptic modulators. Nat Rev Neurosci 2001;2:24-32

22. Patterson SL, Grover LM, Schwartzkroin PA, Bothwell M. Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. Neuron 1992;9:1081-1088

23. Hall J, Thomas KL, Everitt BJ. Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. Nat Neurosci 2000;3:533-535

24. Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J Neurosci 2000;20:7116-7121

25. Linnarsson S, Bjorklund A, Ernfors P. Learning deficit in BDNF mutant mice. Eur J Neurosci 1997;9:2581-2587

26. Collette F, Van der Linden M. Brain imaging of the central executive component of working memory. Neurosci Biobehav Rev 2002;26:105-125

27. Tokuyama W, Okuno H, Hashimoto T, Xin Li Y, Miyashita Y. BDNF upregulation during declarative memory formation in monkey inferior temporal cortex. Nat Neurosci 2000;3:1134-1142

28. Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 2003;23:6690-6694

29. Frodl T, Meisenzahl EM, Zill P, Baghai T, Rujescu D, Leinsinger G, Bottlender R, Schule C, Zwanzger P, Engel RR, Rupprecht R, Bondy B, Reiser M, Moller HJ. Reduced hippocampal volumes associated with the long variant of the serotonin transporter polymorphism in major depression. Arch Gen Psychiatry 2004;61:177-183

30. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW. Patterns of brain activation in people at risk for Alzheimer's disease. N Engl J Med 2000;343:450-456

31. Baddeley A. Working memory: looking back and looking forward. Nat Rev Neurosci 2003;4:829-839

32. Paus T. Mapping brain maturation and cognitive development during adolescence. Trends Cogn Sci 2005;9:60-68

33. Akaneya Y, Tsumoto T, Hatanaka H. Brain-derived neurotrophic factor blocks long-term depression in rat visual cortex. J Neurophysiol 1996;76:4198-4201

34. Berardi N, Maffei L. From visual experience to visual function: roles of neurotrophins. J Neurobiol 1999;41:119-126