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Title: Relationship between the catechol-O-methyl transferase Val108/158Met genotype and brain volume in treatment-naive major depressive disorder: voxel-based morphometry analysis

Article Type: Research Article

Keywords: major depressive disorder, Val108/158Met COMT, caudate, VBM

Abstract: Catechol-O-methyltransferase (COMT) is a methylation enzyme engaged in the degradation of dopamine and noradrenaline by catalyzing the transfer of a methyl group from S-adenosylmethionine. An association was found between the Valine (Val) 108/158Methionine (Met) COMT polymorphism (rs4680) and major depressive disorder (MDD). The authors prospectively investigated the relationship between the Val108/158Met COMT genotype and voxel-based morphometry findings for patients with first-episode and treatment-naïve MDD and healthy subjects (HS). Thirty MDD and 48 HS matched for age and gender were divided by the COMT genotype. Diagnostic effects, effects of COMT genotype, and the genotype-diagnosis interaction effects in relation to brain morphology in the Val/ Met and Val/Val individuals were evaluated using a voxel-based analysis of high-resolution MRI findings. Among the Val/Met individuals, the volume of the bilateral caudate was significantly smaller for MDD than for HS. In the Val/Val individuals, the caudate volume was comparable between MDD and HS. Significant genotype-diagnosis interaction effects on brain morphology were noted in the right caudate.

Dear Sir:

Thank you very much for giving the kind comments and the helpful suggestions on our manuscript. We have seriously considered the comments. To respond the comments, we have revised our manuscript. I hope I have fully and satisfactorily responded to all of them. We will note the revised points in detail as follows.

Reviewer #1: Overall: the authors investigate the relationship between the COMT val/met polymorphism and brain volumes in depression. The study is novel as no previous study investigated COMT and brain size in relation to depression. The results are compelling and worth being published in this journal. Major revisions are however required.

Abstract:

1. It provides sufficient information but the authors should provide the refSNP (rs) numbers associated to the SNP included in this manuscript (this applies to the introduction of the manuscript too).

Our reply

According to the comments above, we added the the refSNP (rs) numbers “rs 4680” in the **Abstract** and **Introduction**.

Introduction:

2. I would recommend a major reorganization of the introduction, for instance by talking about COMT polymorphisms and depression prior to describining the VBM technique.

Our reply

According to the comments above, we moved the following sentence “Over the past decade, researchers have examined depression-associated anomalies in the volume of brain structures using the voxel-based morphometry (VBM) method, which is a systematic imaging analysis tool for evaluating gray matter (GM) and white matter density and volume across multiple regions of interest (ROI) (Ashburner and Friston, 2000). Unlike conventional ROI approaches, the VBM using SPM (Statistical Parametric Mapping) can detect tissue density changes in small or anatomically

ill-defined brain regions (Ashburner and Friston, 2000).” from the second paragraph to the third paragraph in the **Introduction**.

3. Benefits of this technique in comparison to Freesurfer for instance would be helpful.

Our reply

According to the comments above, we added the following sentence in the **Introduction**; Recently FreeSurfer (Martinos Center, Boston, MA) also have been used widely. Although FreeSurfer can segment subcortical regions separately (Fischl et al., 2002), an equipped surface-based statistical method with FreeSurfer cannot analyze the subcortical regions. One advantage of SPM compared to FreeSurfer is to be able to perform statistical analysis in both cortex and subcortical nuclei, which are segmented as GM, at the same time.

4. Further, the authors could improve their explanation of the relationship between dopamine and depression and COMT and depression (for instance there a few studies on white matter and neural activity in relation to COMT variants and mood disorders).

Our reply

According to the comments above, we added the following sentence regarding the relationship between the depression and the dopamine and COMT in the **Introduction**; The dopaminergic system in brain has been implicated in the modulation of the pathophysiology of major depressive disorder (MDD) and the mechanisms of antidepressant drugs (Nestler and Carlezon, 2006; Nutt et al., 2006). The COMT genotype also may relate to the pathophysiology of MDD; a multicenter European study found an association between the high-activity COMT Val allele, particularly the Val/Val genotype, and MDD (Massat et al., 2005), although some case-control association studies of COMT have demonstrated the negative results (Cusin et al., 2002; Frisch et al., 1999; Kunugi et al., 1997). In addition, a few studies of MDD have reported the influences of COMT genotype on white matter connectivity with diffusion tensor image (Hayashi et al., 2014; Seok et al., 2013) and the prefrontal cortex activation with functional MRI (Opmeer et al., 2013).

5. The authors mention the effects of medication on the brain at the very end of the introduction. This should be addressed when discussing the brain abnormalities

associated with depression. For instance, could medication affect the brain morphology and expression of the COMT gene? The authors may mention the connection between illness chronicity and brain volume, and possibly the relationship between COMT gene expression levels. Age and gender related differences in MDD and genetic influence should also be briefly discussed.

Our reply

According to the comments above, we deleted the sentence "We recruited first-episode and treatment-naive MDD to observe the effect specifically to the COMT genotype because antidepressants may alter brain volumes (Malberg et al., 2000)" from the **Introduction**, and we added the following sentence regarding the medication effects on the brain morphology and expression of the COMT gene as a strength of our study in the **Discussion**; To our knowledge, this study provides the first evidence of the relationship between the COMT genotype and the brain morphology of the first-episode MDD, but not the chronic stage of MDD. Furthermore, the strength of this study lies in the recruitment of the drug-naïve MDD. The previous studies indicate that the alterations in brain volume may occur during the course of MDD and after antidepressant treatments (Hamilton et al., 2008; Malberg et al., 2000; Zeng et al., 2012). In addition, a blood plasma concentration of metabolites of dopamine, homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenlglycol (MHPG) alters after antidepressant treatments, which also suggests the possibility to affect the COMT gene expression levels after antidepressant treatments (Davila et al., 2006). Therefore, the morphological changes of the caudate in this study might be related to the acute state of MDD, but not the effects caused by the antidepressant treatments or the illness chronicity of MDD.

Furthermore, we added the following sentence regarding the effects of the age and gender on the expression of the COMT gene as a limitation in the **Discussion**; Third, we could not confidently exclude the potential effect of age and gender on the current results, although we set the age and gender as covariates in the VBM analysis. The previous studies suggested the possibility of age and gender related differences in COMT genotype effects; the COMT activity was reported to be highest at an early age (in particular from age 6 to 20) (Massat et al., 2005; Wahlstrom et al., 2007), and an estrogen and a primary female sex hormone down-regulated the COMT expression (Xie et al., 1999).

6. Further, could the authors please state their main hypotheses.

Our reply

According to the comments above, we added the following sentence in **Introduction**; Therefore, we hypothesized that the COMT genotype also affects a brain morphology of MDD.

Methods:

7. It is unclear whether the authors included participants of all ages. What was the age range of the participants? Please include in the participant description.

Our reply

According to the comments above, we added the age ranges in the **Table 1**.

8. Within the Genotyping section: more information should be provided in terms of assay for genotyping, reference to the taqman technique and amplification processes. Relative references should be included.

Our reply

According to the comments above, we added the following sentence regarding the genotyping in the **Introduction**; Genotyping for the COMT Val108/158Met polymorphism used direct sequencing of regions (Hayashi et al., 2014). In brief, DNA was isolated from peripheral blood mononuclearcells by the QIAamp DNA Mini-Kit (QIAGEN, Tokyo, Japan). Genotyping was carried out with their polymerase chain reaction (PCR) SNP genotyping system using BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies Japan, Tokyo, Japan). The DNA was read using a BMG Applied Biosystem 3730xI DNA Analyzer (Life Technologies Japan, Tokyo, Japan). We used a forward primer (TCACCTCTCCTCCGTCCCAA) and a reverse primer (ACAAGGCCCCCACTCTGTCCCT) for the COMT Val108/158Met polymorphism.

9. In the statistical analyses seciton the authors should explain whether the genotype x diagnoses analyses were performed on brain region volumes found to be difference between HC and MDD patients or on the whole brain.

Our reply

We performed all of VBM analysis based on the whole brain. We added the following sentence in 2.4. Statistical Analyses in the **Methods**; All of these contrasts were performed on the whole brain.

10. A. Also why did the authors consider the ratio between the caudate volume and the total GM volume?

Our reply

To confirm the findings by the VBM analysis, we also added the quantitative analyses using the caudate volume ratio. We added the following sentence in 2.4. Statistical Analyses in the **Methods**; To confirm the findings, we added the region of interest analyses regarding the caudate volume that observed in the VBM analysis.

10. B. Also how did the authors deal with intracranial volume differences across participants given the possibly large age range?

Our reply

According to the comments above, we re-examined the caudate volume ratios using an ANCOVA to control the effect of age. We added the following sentence in 2.4. Statistical Analyses in the **Methods**; An ANCOVA was used to compare the group differences, while controlling the effect of age.

Results:

11. Section 3.7 could be transformed in a table and doesn't need to be included.

Our reply

According to the comments above, we added **Table 3** and shortened the previous description in the section *3.7. Volume analysis of the caudate* in the **Results**.

Discussion:

12: I would recommend to change the structure of the discussion, by talking about 1. Abnormalities in brain regions associated with striatal regions in depression, 2. Results of lesion studies in the caudate (please define what kind of lesions if you do, trauma? Illness-related?). Also please provide a more recent reference than Mendez et al. 1989 or Parashos (references from 2000 onwards are preferable). 3. discuss the relationship between emotional regulation/processing and striatal regions. Also please provide a

more critical appraisal of the current findings in reference to Pizzagalli et al for example. It is unclear why the authors provide such reference when they do not discuss how the "genetic" populations differed in relation to their clinical presentation. 4. Discuss what the current findings mean for MDD or mood. 5. I would encourage the authors to address the topic of other genetic variants associated with dopamine and MDD further.

Our reply

According to the comments above, I rewrote the paragraph in **Discussion** "Previous studies reported that damage to the caudate elicits depressive symptoms beyond those generally associated with brain damage. For example, damage to the caudate resulting from cerebrovascular disease in the absence of other neural anomalies has been found to result in symptoms of depression. Furthermore, there are some MRI studies concerning the relationship between MDD and the caudate. Diminished caudate volume is one of the most consistently replicated abrrations found in studies of MDD brain volume. Gabbay et al. have reported a caudate metabolic aberration in MDD using MR spectroscopy. In addition, Pizzagalli et al. found that the anhedonic symptoms and depression severity were associated with a reduced bilateral caudate volume in unmedicated MDD. Our finding of caudate volume reduction in first-episode and treatment-naive patients with MDD also supports the hypothesis that the caudate plays a causal role in depression." by talking the following descriptions which the reviewer recommended.

12-1. Abnormalities in brain regions associated with striatal regions in depression

According to the comments above, we added the following sentence to mention the abnormality of caudate in MDD; "Previous morphometric studies and functional neuroimaging studies have documented smaller caudate volume as well as impaired metabolism and blood flow in the caudate in the MDD (Drevets, 2000; Gabbay et al., 2007; Parashos et al., 1998).".

12-2. Results of lesion studies in the caudate (please define what kind of lesions if you do, trauma? Illness-related?). Also please provide a more recent reference than Mendez et al. 1989 or Parashos (references from 2000 onwards are preferable).

According to the comments above, we added the following sentences regarding the relationship between depression and illness-related change in the caudate; "The damage to the caudate resulting from the basal ganglia-related disorders, such as Parkinson's disease and Huntington's disease, has also been found to result in symptoms of

depression (Wagner et al., 2011).”. In addition, we deleted the references of [Mendez et al. 1989] and [Parashos et al. 1998].

12-3. discuss the relationship between emotional regulation/processing and striatal regions. Also please provide a more critical appraisal of the current findings in reference to Pizzagalli et al for example. It is unclear why the authors provide such reference when they do not discuss how the "genetic" populations differed in relation to their clinical presentation.

According to the comments above, we added the following sentences; “Regarding the emotional regulation, the caudate is known to constitute a central component of the brain reward system (Breiter et al., 1997). A decreased responsiveness to reward constitutes a central characteristic of MDD (Hasler et al., 2004; Tremblay et al., 2005).” Furthermore, according to the comments above, we would like to delete the following sentence, which is confusing; In addition, Pizzagalli et al. found that the anhedonic symptoms and depression severity were associated with a reduced bilateral caudate volume in unmedicated MDD.

12-4. Discuss what the current findings mean for MDD or mood.

According to the comments above, we added the following sentences; “Our findings of caudate volume reduction in first-episode and treatment-naïve MDD also support the hypothesis that the caudate plays a causal role in MDD. In addition, our results also suggest that the difference of dopamine levels might affect caudate volume reduction in MDD.”.

12-5. I would encourage the authors to address the topic of other genetic variants associated with dopamine and MDD further.

According to the comments above, we added the following sentences; “In addition, our results also suggest that the difference of dopamine levels affect caudate volume reduction in MDD. Therefore, the other genetic variants such as TREK1 (Dillon et al., 2010) and dopamine receptor genotype (Dannlowski et al., 2013), which may be thought to associate with both dopamine and MDD, may also affect the caudate volume in MDD.”.

13: Overall I could encourage the authors to shorten the section on dopamine and rather focus on val vs met carriers-related findings. Specifically when the authors talk about

"COMT" genotype they could mention whether it's val/val or val/met that affect the dopaminergic flux (see Carr and Sesack, 2000).

Our reply

According to the comments above, we shortened the previous sentence as following; The previous animal studies indicate that Val/Met individuals with higher dopamine levels in the PFC also have lower dopamine levels in the caudate compared to the Val/Val individuals, because the dopamine flux in the PFC indirectly has the opposite effect on downstream dopaminergic targets, particularly the striatum (Carr and Sesack, 2000; Takahata and Moghaddam, 2000). These previous findings raise the possibility that the COMT genotype also has an indirect effect on the striatal dopamine level. Therefore, we hypothesize that lower dopamine levels associated with Val/Met (as opposed to Val/Val) causes the neurodevelopmental difference in the caudate of MDD.

14: When the authors mention that "the neurotoxic damage associated with the environmental factors that causes MDD" it would be good to know "which factors" the authors are referring to.

Our reply

According to the comments above, we added the following sentence in the **Discussion**; neurotoxic damage associated with the environment factors, such as the stressful life-events, which may relate to the neurodevelopmental difference in MDD (Frodl et al., 2010).

15: I would shorten the very last section starting from "a previous study…" as it appears to be a repetition of the results section and rather focus on the strengths and relevance of the current findings. The last paragraph of this paragraph deserves to be at the beginning of the discussion and not at the end.

Our reply

According to the comments above, to describe the strengths of this study, we added the following sentence as the second paragraph in the **Discussion**; To our knowledge, this study provides the first evidence of the relationship between the COMT genotype and the brain morphology of the first-episode MDD, but not the chronic stage of MDD. Furthermore, the strength of this study lies in the recruitment of the drug-naïve MDD. The previous studies indicate that the alterations in brain volume may occur during the

course of MDD and after antidepressant treatments (Hamilton et al., 2008; Malberg et al., 2000; Zeng et al., 2012). In addition, a blood plasma concentration of metabolites of dopamine, homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenlglycol (MHPG) alters after antidepressant treatments, which also suggests the possibility to affect the COMT gene expression levels after antidepressant treatments (Davila et al., 2006). Therefore, the morphological changes of the caudate in this study might be related to the acute state of MDD, but not the effects caused by the antidepressant treatments or the illness chronicity of MDD.

Language:
16. please proofread the paper (there are a few typos e.g. abrrations) and the last page of the discussion needs to be rewritten. In the highlights please change "MDD grey matter" for example by saying "in MDD patients" or "in MDD". The authors should use the term valine/methionine just before using the acronyms val and met at the beginning of the abstract, not at the end of it. In the abstract would recommend to say "volume is comparable between MDD patients and HS" rather than talk about "same reduction". In the results section please change the sentence "no voxel could survive after correction" and state "no voxel survived the correction for multiple comparisons".

Our reply

We revised the manuscript according to the all reviewer's comments.

Reviewer #2: This article reports a voxel-based analysis of MRI data examining the effect of the COMT Val158Met polymorphism on cerebral morphology in a cohort of first-episode, drug-naïve individuals with MDD and comparison healthy control subjects. The authors did not observe a primary effect of COMT genotype. However, using both voxel-based analyses and measures of caudate volume, the authors report a difference in caudate volume (particularly right caudate) between depressed and control subjects, but only in the Val/Met genotype group.

Comments:
1. In the introduction, the authors state an association between the COMT val/val genotype and MDD, citing a single reference. This is a bit misleading, as there are

other studies, some of which did not find that relationship. Please consider reporting that published results are mixed, citing as appropriate.

Our reply

According to the comments above, we added the following sentence in the **Introduction**; although some case-control association studies of COMT have demonstrated the negative results (Cusin et al., 2002; Frisch et al., 1999; Kunugi et al., 1997).

2. MRI occurred within a month of diagnosis. Did participants receive antidepressant treatment (either medication or psychotherapy) during this interval? If so, details should be included in the manuscript.

Our reply

All participants underwent MRI before receiving antidepressant treatment. We added the following sentence in **Methods**; All MDD underwent the brain MRI before receiving antidepressants medication or psychotherapy.

3. The authors appeared to exclude subjects with the Met/Met genotype as these homozygous individuals were rare. Was there a reason they were excluded rather than combined with the heterozygous subjects (ie, creating a "Met carrier" group)? If they were excluded from analyses, they probably do not need to be discussed in the demographics section and should be removed from Table 1 with statistical analyses being rerun.

Our reply

In this study, the HS and MDD with Met/Met were excluded from the analysis because of the small sample size. So, we added the following sentences in the **Methods**; “HS and MDD with Met/Met were excluded from the analysis because of the small sample size” and “we could not evaluate morphological changes in MDD with Met/Met because of the small sample size. In future, larger studies including MDD with Met/Met are required to confirm our findings. ” in the **Discussion** as the limitation. In addition, according to the comments above, we deleted the Met/Met individuals in the demographics section 3.1. in the **Results** and **table 1**.

4. It is unclear why total GM volume is used to create the caudate volume ratio rather than total intracranial volume, which is a better and more commonly used proxy for head size.

Our reply

According to the comments above, we recalculated the caudate volume ratios using the total intracranial volume. We added the following sentence in the ***2.4. Statistical Analyses***; We calculated the caudate volume ratios (caudate volume to total intracranial volume).

5. In the text and in Table 2, the results are presented in only one contrast: MDD< HS or Val/Val < Val/Met. Were the opposite contrasts examined? Were there no significant differences?

Our reply

Our result demonstrated no significant volume reduction in any brain regions in HS compared to MDD and in Val/Val individuals compared to Val/Met. We added the following sentences; "No brain regions showed significant volume reduction in HS compared to MDD." and "No brain regions showed significant volume reduction in Val/Met individuals compared to Val/Val individuals." in the **Results**.

6. The results for diagnosis effects in Val/Val individuals and Val/Met individuals as presented in Table 2 and sections 3.4/3.5 appear to be reversed.

Our reply

The results in Table 2 were incorrect. We revised **Table 2**.

7. Do the authors have any thoughts on the laterality of the findings?

Our reply

We consider that the laterality of COMT genotype effects is small in this study because we found the tendency that COMT genotype also affected the left caudate volume reduction in MDD. We added the following sentences in the **Discussion**; "The COMT genotype–diagnosis interaction effects were also detected in the left caudate, although no voxel survived the correction for multiple comparisons. These results may suggest that the COMT genotype also affects the volume reduction of the bilateral caudate in MDD.".

8. In the discussion, the authors state that, "…we can also hypothesize that lower dopamine levels…cause neurodegeneration in the caudate of MDD." I am not sure this is an accurate description of what they are observing. Neurodegeneration implies a progressive loss of volume - something that is not observed in a cross-sectional study and would be unlikely in a midlife adult cohort. Would it be more likely to be a neurodevelopmental difference?

Our reply

According to the comments above, we exchanged the word “neurodegeneration” to “neurodevelopmental difference” through the **Text**.

9. I would like to see an expansion of the authors' discussion about the possible explanation of why COMT's effect on dopamine levels may affect caudate volume differentially in depressed and nondepressed adults.

Our reply

According to the comments above, we added the following sentence in **Discussion**; The current study demonstrated that COMT genotype affects the caudate volume differentially between MDD and HS. This may be due to the difference of dopamine levels in the caudate between MDD and HS. Meyer et. al. reported that striatal dopamine transporter binding and dopamine reuptake are reduced in MDD compared to HS (Meyer et al., 2007). Because the dopamine has antioxidant and neuroprotective effects (Vernaleken et al., 2007), we assume that , in the caudate, the lower levels of dopamine in MDD with Val/Met might increase vulnerability to neurotoxic damage associated with the environmental factors, such as the stressful life-events, which is one of the pathophysiological basis of MDD (Frodl et al., 2010).

10. The SPM figures don't appear to be particularly helpful and could likely be deleted or included only as supplementary figures.

Our reply

According to the comments above, we deleted SPM figures.

*Title Page showing full Author and Address Details

Title:

Relationship between the catechol-O-methyl transferase Val108/158Met genotype and brain volume in treatment-naive major depressive disorder: voxel-based morphometry analysis

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Highlights

- We analyzed the relationship between grey matter volume reduction in MDD and COMT genotype.
- We found MDD have decreased caudate volume.
- We found that COMT genotype affects the caudate volume reduction in MDD.

Comment [w1]: Reviewer #1: Language

Relationship between the catechol-O-methyl transferase Val108/158Met genotype and brain volume in major depressive disorder

Abstract

Catechol-O-methyltransferase (COMT) is a methylation enzyme engaged in the degradation of dopamine and noradrenaline by catalyzing the transfer of a methyl group from S-adenosylmethionine. An association was found between the Valine (Val) 108/158Methionine (Met) COMT polymorphism (rs4680) and major depressive disorder (MDD). The authors prospectively investigated the relationship between the Val108/158Met COMT genotype and voxel-based morphometry findings for patients with first-episode and treatment–naïve MDD and healthy subjects (HS). Thirty MDD and 48 HS matched for age and gender were divided by the COMT genotype. Diagnostic effects, effects of COMT genotype, and the genotype–diagnosis interaction effects in relation to brain morphology in the Val/ Met and Val/Val individuals were evaluated using a voxel-based analysis of high-resolution MRI findings. Among the Val/Met individuals, the volume of the bilateral caudate was significantly smaller for MDD than for HS. In the Val/Val individuals, the caudate volume was comparable between MDD and HS. Significant genotype–diagnosis interaction effects on brain

Comment [w1]: Reviewer #1: 16

Comment [w2]: Reviewer #1: 16

Comment [w3]: Reviewer #1: 1

Comment [w4]: Reviewer #1: 16

morphology were noted in the right caudate.

Key words: major depressive disorder, Val108/158Met COMT, caudate, VBM

1. Introduction

Catechol-O-methyltransferase (COMT) catalyzes the first step in a major degradation pathway of the catecholamine neurotransmitters: dopamine, noradrenaline, and adrenaline (Matsumoto et al., 2003). The COMT gene resides on the q11 region of chromosome 22 (Grossman et al., 1992), where a functional missense mutation causes a single G-to-A base-pair substitution, resulting in a single nucleotide polymorphism in exon 4. This polymorphism results in the substitution of methionine (Met) for valine (Val) at codons 108/158 (rs4680) (Lachman et al., 1996). Met/Met individuals have a 4-fold decrease in enzymatic activity relative to Val/Val individuals, resulting in higher dopamine levels (Lachman et al., 1996). Val/Met individuals exhibit intermediate levels of enzymatic activity (Weinshilboum et al., 1999).

Comment [w5]: Reviewer #1: 1

The dopaminergic system in brain has been implicated in the modulation of the pathophysiology of major depressive disorder (MDD) and the mechanisms of antidepressant drugs (Nestler and Carlezon, 2006; Nutt et al., 2006). The COMT genotype also may relate to the pathophysiology of MDD; a multicenter European study found an association between the high-activity COMT Val allele, particularly the Val/Val genotype, and MDD (Massat et al., 2005), although some case-control association studies of COMT have demonstrated the negative results (Cusin et al., 2002;

Frisch et al., 1999; Kunugi et al., 1997). In addition, a few studies of MDD have reported the influences of COMT genotype on white matter connectivity with diffusion tensor image (Hayashi et al., 2014; Seok et al., 2013) and the prefrontal cortex activation with functional MRI (Opmeer et al., 2013). Therefore, we hypothesized that the COMT genotype also affects a brain morphology of MDD. To our knowledge, no study has reported the positive effect of the COMT genotype on brain morphology of MDD. Although Pan et al. have reported negative relationship between COMT genotype and brain morphology of MDD, this study was limited to evaluate only geriatric patients (>60 years) in the chronic stage of depression and use a regions of interest (ROI) method (Pan et al., 2009).

Over the past decade, researchers have examined depression-associated anomalies in the volume of brain structures using the voxel-based morphometry (VBM) method, which is a systematic imaging analysis tool for evaluating gray matter (GM) and white matter density and volume across multiple ROI (Ashburner and Friston, 2000). Unlike conventional ROI approaches, the VBM using SPM (Statistical Parametric Mapping) can detect tissue density changes in small or anatomically ill-defined brain regions (Ashburner and Friston, 2000). Recently FreeSurfer (Martinos Center, Boston, MA) also have been used widely. Although FreeSurfer can segment subcortical regions

Comment [w6]: Reviewer #2: 1

Comment [R7]: Reviewer #1: 4

Comment [R8]: Reviewer #1: 6

Comment [R9]: Reviewer #1: 2

separately (Fischl et al., 2002), an equipped surface-based statistical method with FreeSurfer cannot analyze the subcortical regions. One advantage of SPM compared to FreeSurfer is to be able to perform statistical analysis in both cortex and subcortical nuclei, which are segmented as GM, at the same time.

Comment [R10]: Reviewer #1: 3

This study aimed to investigate the relationship between COMT genotype and volumetric differences in first-episode and treatment-naïve MDD and healthy subjects (HS) using VBM analysis of brain magnetic resonance imaging (MRI) data.

2. Methods

2.1. Participants

The protocol of this prospective study was approved by the Institutional Review Board of the institution. All participants provided written informed consent to participate in the study. Thirty, first-episode, right-handed, and treatment- naïve MDD were recruited. A psychiatrist (K.H. with 7 years of experience in psychiatry) diagnosed a major depressive episode by using the Structured Clinical Interview according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV-TR criteria. The severity of depression was evaluated using the17-item Hamilton Rating Scale for Depression (HAMD). Only those with the HAMD total score of ≥14 were eligible for

the study. Exclusion criteria included any history of neurological diseases or other physical diseases, and the presence of comorbidities with other disorders (no evidence of schizoaffective disorder, bipolar disorder, Axis II, personality disorders, and mental retardation).

Forty-eight HS were also recruited via an interview conducted by the same psychiatrist using the Structured Clinical Interview for DSM-IV, nonpatient edition. None of them had a history of serious medical or neuropsychiatric illness or a family history of major psychiatric or neurological illness among their first-degree relatives.

The 30 patients with MDD and the 48 HS were divided by the COMT genotype: 15 MDD with Val/Val, 14 MDD with Val/Met, 1 MDD with Met/Met, 28 HS with Val/Val, 17 HS with Val/Met, and 3 HS with Met/Met (Table 1). The HS and MDD with Met/Met were excluded from the analysis because of the small sample size.

Comment [w11]: Reviewer #2:3

2.2. Genotyping

Seventy-eight subjects from the neuroimaging study provided a blood sample, from which DNA was extracted according to standard laboratory protocols. These samples were genotyped for the COMT Val108/158Met polymorphism. Genotyping for the COMT Val108/158Met polymorphism used direct sequencing of regions (Hayashi et al.,

2014). In brief, DNA was isolated from peripheral blood mononuclearcells by the QIAamp DNA Mini-Kit (QIAGEN, Tokyo, Japan). Genotyping was carried out with their polymerase chain reaction (PCR) SNP genotyping system using BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies Japan, Tokyo, Japan). The DNA was read using a BMG Applied Biosystem 3730xI DNA Analyzer (Life Technologies Japan, Tokyo, Japan). We used a forward primer (TCACCTCTCCTCCGTCCCAA) and a reverse primer (ACAAGGCCCCCACTCTGTCCCT) for the COMT Val108/158Met polymorphism.

Comment [w12]: Reviewer #1: 8

2.3. MRI and Image Processing for VBM

All MDD underwent the brain MRI before receiving antidepressants medication or psychotherapy. MRI data were obtained using a 3.0-Tesla scanner (Signa EXCITE 3 T; GE Medical Systems, Milwaukee, Wisconsin) with a three-dimensional fast-spoiled gradient-recalled acquisition with steady state (3D-FSPGR). The following parameters were used: repetition time ms/echo time ms/inversion time, 10/4.1/700; flip angle, 10; field of view, 24 cm; section thickness, 1.2 mm; resolution, 0.47 × 0.47 × 1.2 mm. All images were corrected for image distortion due to gradient non-linearity using "GradWarp" (Jovicich et al., 2006) and for intensity inhomogeneity using "N3" (Sled et

Comment [w13]: Reviewer #2: 2

al., 1998). Image processing for VBM, SPM8 was used. The 3D-FSPGR images in native space were spatially normalized and cerebrospinal fluid images; and modulated for intensity using the DARTEL (Diffeomorphic Anatomical Registration Through Exponential Lie Algebra) toolbox on SPM8 (Ashburner, 2009). The DARTEL was proposed by Ashburner as an alternative method for normalization in the SPM package (Ashburner, 2007). In an intensity modulation step, voxel values of the segmented images were multiplied by the measure of warped and unwarped structures derived from the nonlinear step of the spatial normalization. This step converted the relative regional GM density into absolute GM density, expressed as the amount of GM per unit volume of brain tissue before spatial normalization. The resulting modulated GM and white matter images were smoothed with an 8-mm Gaussian kernel.

2.4. Statistical Analyses

For the analysis of demographic and clinical characteristics of participants, an ANOVA was performed to compare differences in age, total GM volume, and years of education among HS with Val/Val, HS with Val/Met, MDD with Val/Val, and MDD with Val/Met. Chi-square test was used in terms of sex among these 4 groups. A two-tailed t-test was used to compare the total of HAMD 17 scores between MDD with

Val/Val and with Val/Met.

In a VBM analysis, statistical analyses were performed using the SPM8 software. Morphological changes in GM were assessed using a full factorial model with diagnosis and genotype status (Val/Val or Val/Met) as independent variables. Age, sex, and total GM volume were included as covariates of no interest into all analyses to control for confounding variables. The following t-test comparisons for 2×2 factorial designs were made: (a) diagnosis effects; MDD versus HS, (b) COMT genotype effects; Val/Val individuals versus Val/Met individuals, (c) diagnosis effects in Val/Val individuals; MDD with Val/Val versus HS with Val/Val, (d) diagnosis effects in Val/Met individuals; MDD with Val/Met versus HS with Val/Met, (e) genotype–diagnosis interaction effects; diagnosis effects in Val/Val individuals versus diagnosis effects in Val/Met individuals. All of these contrasts were performed on the whole brain. These analyses yielded statistical parametric maps {SPM (t)} based on a voxel-level height threshold of $p < 0.001$. We used topological discovery rate (FDR) correction in SPM8, which means the inference on clusters based on cluster size, controlling the fraction of false positive clusters among all clusters on average (Chumbley et al., 2010; Chumbley and Friston, 2009). The significance level was set at FDR-corrected $p < 0.05$.

Comment [w14]: Reviewer #1:9

To confirm the findings, we added the region of interest analyses regarding the

caudate volume that observed in the VBM analysis. We calculated the caudate volume ratios (caudate volume to total intracranial volume). The caudate area was identified by using Talairach Daemon (Lancaster et al., 2000) and automated anatomical labeling (Tzourio-Mazoyer et al., 2002) through WFU PickAtlas version 3.0.4 (Maldjian et al., 2004; Maldjian et al., 2003). An ANCOVA was used to compare the group differences, while controlling the effect of age.

Comment [KW15]: Reviewer #1: 10A

Comment [w16]: Reviewer #2: 4

Comment [w17]: Reviewer #1: 10B

Statistical analyses were performed using the statistical software package StatView 5.0 (SAS Institute, Cary, NC). A p-value of <0.05 was assumed to indicate a statistically significant difference, the SPM8 analysis excepted.

3. Results

3.1. Demographic and clinical data

There was no significant difference among the 4 groups (HS with Val/Val, HS with Val/Met, MDD with Val/Val, and MDD with Val/Met) with regard to the distribution of sex, age, and total GM volume (Table 1). There was a significant difference between HS and MDD regard to the years of education ($p < 0.01$), although there were no significant differences in the years of education between HS with Val/Val and those with Val/Met ($p = 0.32$) and between MDD with Val/Val and those with Val/Met ($p =$

0.70) (Table 1). The mean of the total HAMD17 score was not significantly different between MDD with Val/Val and those with Val/Met (p = 0.59) (Table 1).

3.2. Diagnosis effects (MDD versus HS)

The volume of the bilateral caudate and right superior temporal gyrus was smaller for MDD than for HS (right caudate: FDR-corrected p = 0.003; left caudate: FDR corrected p = 0.003; left superior temporal gyrus: FDR-corrected p = 0.048) (Table 2). In addition, MDD demonstrated the volume reduction in the left superior frontal gyrus (uncorrected p = 0.032) (Table 2), although no voxel survived the correction for multiple comparisons (FDR-corrected p = 0.206). No brain regions showed significant volume reduction in HS compared to MDD.

Comment [w18]: Reviewer #1: 16

Comment [KW19]: Reviewer #2: 5

3.3. COMT genotype effects (Val/Val individuals versus Val/Met individuals)

Val/Val individuals demonstrated the volume reduction in the right inferior frontal gyrus compared to Val/Met individuals (uncorrected p = 0.044), although no voxel survived the correction for multiple comparisons (FDR-corrected p = 0.316). No brain regions showed significant volume reduction in Val/Met individuals compared to Val/Val individuals.

Comment [w20]: Reviewer #1: 16

Comment [KW21]: Reviewer #2: 5

3.4. Diagnosis effects in Val/Val individuals (MDD with Val/Val versus HS with Val/Val)

Among of Val/Val individuals, MDD demonstrated the volume reduction in the left superior frontal gyrus (uncorrected p = 0.038), although no voxel survived the correction for multiple comparisons (FDR-corrected p = 0.535).

Comment [w22]: Reviewer #1: 16

3.5. Diagnosis effects in Val/Met individuals (MDD with Val/Met versus HS with Val/Met)

Among of Val/Met individuals, the volume of the bilateral caudate head and body was significantly smaller in MDD than in HS (right: FDR-corrected P = 0.001; left: FDR-corrected P = 0.001) (Table 2). In addition, MDD demonstrated the volume reduction in the left superior frontal gyrus (uncorrected p = 0.028) (Table 2), although no voxel survived the correction for multiple comparisons (FDR-corrected p = 0.206).

Comment [w23]: Reviewer #1: 16

3.6. COMT genotype–diagnosis interaction (diagnosis effects in Val/Val individuals versus diagnosis effects in Val/Met individuals)

In comparison with the diagnosis effects in Val/Val individuals, the diagnosis effects

in Val/Met individuals demonstrated significantly larger volume reduction in the right caudate head and body (FDR-corrected P = 0.001) (Table 2). The left caudate head and body was also demonstrated larger volume reduction (uncorrected p = 0.020) (Table 2), although no voxel survived the correction for multiple comparisons (FDR-corrected p = 0.085).

Comment [w24]: Reviewer #1: 16

In comparison with the diagnosis effects in Val/Met individuals, the diagnosis effects in Val/Val individuals demonstrated larger volume reduction in the left superior frontal gyrus (Brodmann area 8), one part of dorsolateral prefrontal cortex (DLPFC) (uncorrected p = 0.010), however, no voxel survived the correction for multiple comparisons (FDR-corrected p = 0.119).

Comment [w25]: Reviewer #1: 16

3.7. Volume analysis of the caudate

The bilateral caudate volume ratios were shown in Figure 1 and Table 3. The bilateral caudate volume ratios were significantly smaller for the MDD with Val/Met than for HS with Val/Met (right: p < 0.01; left: p < 0.01). No significant differences were found between the MDD with Val/Val and HS with Val/Val (right: p = 0.42; left: p = 0.35).

Comment [KW26]: Reviewer #1: 11

4. Discussion

In this study, the VBM demonstrated that MDD were characterized by smaller caudate volumes compared with HS. In addition, the significant COMT genotype–diagnosis interaction effects were found in the right caudate which means the caudate volume reduction in MDD compared to HS in Val/Met individuals are larger than in Val/Val individuals. The COMT genotype–diagnosis interaction effects were also detected in the left caudate, although no voxel survived the correction for multiple comparisons. These results may suggest that the COMT genotype affects the volume reduction of the bilateral caudate in MDD.

Comment [w27]: Reviewer #1: 16

Comment [KW28]: Reviewer #2: 7

To our knowledge, this study provides the first evidence of the relationship between the COMT genotype and the brain morphology of the first-episode MDD. Furthermore, the strength of this study lies in the recruitment of the drug-naïve MDD. The previous studies indicate that the alterations in brain volume may occur during the course of MDD and after antidepressant treatments (Hamilton et al., 2008; Malberg et al., 2000; Zeng et al., 2012). In addition, a blood plasma concentration of metabolites of dopamine, homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenlglycol (MHPG) alters after antidepressant treatments, which also suggests the possibility to affect the COMT gene expression levels after antidepressant treatments (Davila et al., 2006). Therefore, the morphological changes of the caudate in this study might be related to the acute state of

MDD, but not the effects caused by the antidepressant treatments or the illness chronicity of MDD.

The caudate have been thought to play a casual role in MDD. Previous morphometric studies and functional neuroimaging studies have documented smaller caudate volume as well as impaired metabolism and blood flow in the caudate in MDD (Drevets, 2000; Gabbay et al., 2007; Parashos et al., 1998). The damage to the caudate resulting from the basal ganglia-related disorders, such as Parkinson's disease and Huntington's disease, has also been found to result in symptoms of depression (Wagner et al., 2011). Regarding the emotional regulation, the caudate is known to constitute a central component of the brain reward system (Breiter et al., 1997). A decreased responsiveness to reward constitutes a central characteristic of MDD (Hasler et al., 2004; Tremblay et al., 2005). Our findings of caudate volume reduction in first-episode and treatment-naïve MDD also support the hypothesis that the caudate plays a causal role in MDD. In addition, our results also suggest that the difference of dopamine levels might affect caudate volume reduction in MDD. Therefore, the other genetic variants such as TREK1 (Dillon et al., 2010) and dopamine receptor genotype (Dannlowski et al., 2013), which may be thought to associate with both dopamine and MDD, may also affect the caudate volume in MDD.

Comment [R29]: Reviewer #1: 5

Comment [R30]: Reviewer #1: 15

Comment [R31]: Reviewer #1: 12

With regard to schizophrenia, there are some studies showing positive associations between COMT genotype and GM volume (Ohnishi et al., 2006). Among patients with chronic schizophrenia, those with Val/Val exhibited volume reduction in the bilateral anterior cingulate cortex and the left amygdala-uncus compared to those with Val/Met and Met/Met (Ohnishi et al., 2006). In addition, Ehrlich et al. reported that the volume of the bilateral amygdala and hippocampus is smaller in schizophrenics with Val/Val than those with Met/Met (Ehrlich et al., 2010). COMT appears to be concentrated in the extrasynaptic spaces, especially in the prefrontal cortex (PFC) and the medial temporal lobe (Matsumoto et al., 2003). These studies on schizophrenia suggest that higher COMT activity with a subsequent decrease in dopamine levels may cause neurodevelopmental difference within dopamine-innervated brain regions. Therefore, we can also hypothesize that lower dopamine levels associated with Val/Met (as opposed to Val/Val) causes the neurodevelopmental difference in the caudate of MDD.

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Our hypothesis may be supported by the previous studies. The previous animal studies indicate that Val/Met individuals with higher dopamine levels in the PFC also have lower dopamine levels in the caudate compared to the Val/Val individuals, because the dopamine flux in the PFC indirectly has the opposite effect on downstream dopaminergic targets, particularly the striatum (Carr and Sesack, 2000; Takahata and

Moghaddam, 2000). A postmortem study revealed lower dopamine synthesis rates, which resulted in lower dopamine levels, in projections from the midbrain to the striatum in Val/Met individuals than those in Val/Val individuals (Akil et al., 2003). In addition, an in vivo positron emission tomography study has shown that dopamine synthesis in the dopaminergic midbrain is lesser in Met/Met individuals than in Val carriers (Val/Val and Val/Met individuals) (Meyer-Lindenberg et al., 2005). Furthermore, a functional MRI study has revealed the behavioral advantage of Val/Val individuals compared to Met/Met individuals in terms of instrumental reversal learning, which is suggestive of lower level of dopamine in the striatum of Met/Met individuals (Krugel et al., 2009). Although these two studies (Krugel et al., 2009; Meyer-Lindenberg et al., 2005) did not directly compare Val/Met and Val/Val individuals, previous findings indicate that Val/Met individuals with an intermediate level of enzymatic activity (Weinshilboum et al., 1999) have lower dopamine levels in the striatum compared with Val/Val individuals.

Comment [R34]: Reviewer #1: 13

The current study demonstrated that COMT genotype affects the caudate volume differentially between MDD and HS. This may be due to the difference of dopamine levels in the caudate between MDD and HS. Meyer et. al. reported that striatal dopamine transporter binding and dopamine reuptake are reduced in MDD compared to

HS (Meyer et al., 2001). Because the dopamine has antioxidant and neuroprotective effects (Vernaleken et al., 2007), we assume that , in the caudate, the lower levels of dopamine in MDD with Val/Met might increase vulnerability to neurotoxic damage associated with the environmental factors, such as the stressful life-events, which is one of the pathophysiological basis of MDD (Frodl et al., 2010).

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Comment [R36]: Reviewer #2: 9

There were several limitations to this study. First, this study was limited by a small sample size, which may have prevented us from exploring potentially relevant interactions such as other genotypes that influence brain volume, and might also lead to the positive publishing bias. Second, we could not evaluate morphological changes in MDD with Met/Met because of the small sample size. In future, larger studies including MDD with Met/Met are required to confirm our findings. Third, we could not confidently exclude the potential effect of age and gender on the current results, although we set the age and gender as covariates in the VBM analysis. The previous studies suggested the possibility of age and gender related differences in COMT genotype effects; the COMT activity was reported to be highest at an early age (in particular from age 6 to 20) (Massat et al., 2005; Wahlstrom et al., 2007), and an estrogen and a primary female sex hormone down-regulated the COMT expression (Xie et al., 1999).

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Comment [R38]: Reviewer #1: 5

In conclusion, a polymorphism in the COMT gene appears to be associated with volume reduction in the caudate in MDD, which may be related to the manifestation of MDD. One can argue that the effects of one gene polymorphism cannot explain the morphological changes observed in association with MDD. Just like the effects of the Val158Met polymorphism, we agree that other polymorphisms of MDD susceptibility genes and genotype–genotype interactions may be linked to individual brain morphology. Future studies are required to explore the effects of other neuromodulatory genetic polymorphisms.

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Contributors

R. Yoshimura, S. Kakeda, and J. Nakamura designed the study. K. Hayashi, A. Katsuki, W. Umene-Nakano, and R. Yoshimura acquired the data, which K. Watanabe, O. Abe, S. Ide, and R. Watanabe analyzed. K. Watanabe, S. Kakeda, R. Yoshimura , and

Y. Korogi wrote the article, which all authors reviewed and approved for publication.

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Figure legends

Figure 1.

The results of volume analysis of the caudate

The bar charts show the caudate volume ratios (mean ± SD). Among Val/Met individuals, both the right and left caudate volume ratios in the MDD were significantly smaller than those in HS (right: $p < 0.01$; left: $p < 0.01$). Among Val/Val individuals, however, no significant differences were found between the MDD and HS (right: $p = 0.84$; left: $p = 0.38$).

Table 1

Demographic and Clinical Characteristics of Participants.

Comment [w39]: Reviewer #1: 7
Reviewer #2: 3

	HS (n = 45)		MDD (n = 29)		p value
	Val/Val (n = 27)	Val/Met (n = 18)	Val/Val (n = 15)	Val/Met (n = 14)	
Age, mean, (range, SD)	41.4 (20-65, 12.0)	40.7 (22-61, 10.3)	41.7 (22-59, 12.1)	49.3 (20-67, 13.2)	0.16
Female, numbers	8	4	7	6	0.20
Years of education, mean (SD)	16.8 (3.1)	15.9 (2.4)	13.2 (2.4)	13.6 (2.6)	<0.01
Total gray matter volume, mean (SD) (ml)	710.0 (69.7)	698.5 (44.5)	671.3 (70.0)	664.9 (56.9)	0.06
HAMD, mean of total scores (SD)			20.3 (4.3)	21.8 (7.2)	0.52

Val = Valine; Met = Methionine; SD = standard deviation; MDD = Major depression disorders; HS = healthy subjects;
HAMD = 17-item Hamilton Rating Scale for Depression

Table 2

Results of VBM analysis.

Anatomical regions	FDR corrected p (culuster level)	uncorrected p (culuster level)	Cluster size	T-value (Voxel level)	Talairach coordinates x	y	z
Diagnosis effects (MDD < HS)							
Right caudate	0.003	0.000	4288	4.68	12	9	-3
				4.28	21	20	8
				4.13	16	22	-3
Left caudate	0.003	0.000	4623	4.34	-12	10	-2
				4.31	-11	17	13
				4.26	-10	22	6
Left superior temporal gyrus	0.047	0.005	2071	4.11	-48	14	-4
				4.05	-55	14	-8
				3.90	-45	10	-2
Left superior frontal gyrus	0.206	0.032	1117	4.70	-25	67	-6
				4.29	-29	66	1
COMT-genotype effects							
right inferior frontal gyrus	0.316	0.044	967	4.37	55	32	0
Diagnosis effect in the Val/Met (MDD < HS)							
Right caudate	0.001	0.000	11241	5.62	12	12	-3
				5.52	12	19	-8
				5.45	13	22	4

Comment [w40]: Reviewer #2: 6

Left caudate	0.001	0.000	9630	5.34	-12	18	15
				5.01	-20	24	-6
				4.40	-12	12	-2
Left superior frontal gyrus	0.206	0.028	1177	4.25	-32	65	1
				3.97	-30	55	-7
Diagnosis effect in the Val/Val (MDD < HS)							
Left superior frontal gyrus	0.919	0.038	1027	4.26	-15	27	49
COMT genotype - diagnosis interaction effects							
Diagnosis effect in the Val/Val < Diagnosis effect in the Val/Met							
Right caudate	0.001	0.001	4597	4.48	12	11	-3
				4.45	12	21	-8
				3.43	12	3	-17
Left caudate	0.085	0.020	1349	4.09	-12	18	15
				3.85	-21	23	-1
				3.41	-9	23	-4
Diagnosis effect in the Val/Val > Diagnosis effect in the Val/Met							
Left superior frontal gyrus	0.119	0.010	1727	4.34	-18	29	55

Val = Valine; Met = Methionine; MDD = Major depression disorders; HS = healthy subjects; COMT = Catechol-O-methyltransferase

Table 3

Caudate volume ratios.

	HS	MDD	p value
Val/Val			
right	0.00115 (0.00010)	0.00116 (0.00009)	0.42
left	0.00118 (0.00012)	0.00117 (0.00010)	0.35
Val/Met			
right	0.00120 (0.00013)	0.00104 (0.00010)	< 0.01
left	0.00125 (0.00014)	0.00109 (0.00011)	< 0.01

The date shows the mean caudate volume ratios (caudate to total intracranial volume) and standard devitations.

Val = Valine; Met = Methionine; SD = standard deviation;
MDD = Major depression disorders; HS = healthy subjects

Comment [w41]: Reviewer #1: 11

Figure 1

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