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The COMT Val158Met Polymorphism and Temporal Lobe Morphometry in Healthy Adults

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Abstract

We examined the relationship between COMT Val158Met genotype and temporal lobe volumes, including the caudate as a control region. 31 healthy subjects completed 1.5T brain MRI and genotyping. After controlling for demographics, Val158 allele homozygotes exhibited significantly smaller temporal lobe and hippocampal volumes, with a trend for smaller amygdala volumes.

Keywords

Magnetic Resonance Imaging; Genetic Polymorphisms; Hippocampus; Temporal Lobe

1. INTRODUCTION

Catechol-*O*-methyl transferase (COMT) is a methylation enzyme engaged in the degradation of norepinephrine and dopamine. Of particular interest is a functional single nucleotide polymorphism resulting in the substitution of methionine (Met) for valine (Val) at codon 158 (Val158Met) (Lachman et al., 1996). The Met158 enzyme exhibits approximately one-fourth the activity of the Val158 enzyme, presumably resulting in greater synaptic levels of dopamine.

Structural neuroimaging studies examining this polymorphism are limited. Studies in healthy individuals show no effect of this polymorphism on total gray or white matter volumes (Zinkstok et al., 2006) or frontal lobe volume (Ho et al., 2005). However, individuals with schizophrenia but not healthy controls who were COMT Val homozygotes exhibited reduced volumes of the left amygdala and right temporal lobe (Ohnishi et al., 2006).

We tested for an association between COMT Val158Met genotype and hippocampus, amygdala, and temporal lobe volumes in healthy adults. Despite that others found such a relationship only in individuals with schizophrenia (Ohnishi et al., 2006), their findings guided our hypothesis that Val homozygotes would exhibit smaller temporal volumes than Met allele

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carriers. We also measured the caudate as a control region that we hypothesized would not be related to genotype.

2. METHODS

2.1. Sample

Participants were recruited from the community by advertisement for inclusion as healthy control subjects in a study examining the pathophysiology of Bipolar Disorder. Eligibility criterion included age of 18–49 years. Exclusion criteria included: 1) any psychiatric disorder history, including substance abuse or dependence, as detected using the National Institute of Mental Health Diagnostic Interview Schedule (Robins et al., 1981); 2) uncontrolled medical illness; 3) use of psychotropic medications; 4) pregnancy; and 5) any MRI contraindication. Subjects provided written informed consent before study procedures were performed. The study was approved by the Duke University Health System Institutional Review Board.

2.2. Magnetic Resonance Imaging and Image Analysis

MR imaging of the brain was performed on a 1.5 T system (Signa, GE Medical Systems, Milwaukee, WI). Scan preparation, as well as the pulse sequence for the axial dual-echo fast spin-echo (FSE) used acquisition for morphometry of the caudate, and cerebrum, has been previously described (Beyer et al., 2004). An additional axial IR-prepped 3D series was used for measuring the temporal structures, with pulse sequence parameters of TE = minimum full echo, TI = 300ms, 16 KHz, a 256 x 256 matrix, 1.5-mm section thickness, 1 excitation and a 24cm field of view.

The MR images were processed at the Neuropsychiatric Imaging Research Laboratory (NIRL) by analysts blinded to subject demographic data and genotype. A NIRL-modified version of MrX software was used for tissue segmentation following previously described methods (Payne et al., 2002). Cerebrum was measured as part of this method as a proxy for total brain volume, and did not include the brain stem or cerebellum. Methods for measurement of the hippocampus (Taylor et al., 2005) and caudate (Beyer et al., 2004) have been previously described.

The temporal lobe tracing is performed in the NIRL-developed GRID program and applied to an automated segmentation image (Van Leemput et al., 1999) to obtain gray and white matter tissue estimates. Before tracing, a standard realignment procedure was completed, aligning the images to a plane that bisected the brain into right and left cerebral hemispheres. A sagittal realignment was done individually for each temporal lobe so the axis of the temporal lobe was horizontal.

The temporal lobe was traced moving anteriorly in the coronal view, starting on the slice where the crus fornix is full and visible. Tracing started at the most inferior aspect of the vertical fissure of the Sylvian fissure, crossing the temporal stem at its shortest distance. It continued superior-medially to include the medial gray matter of the temporal lobe. Once the white matter of the temporal stem was absent, the temporal lobe was disconnected from the remainder of the cerebrum, and continued to be traced anteriorly until gone.

Amygdala processing also used GRID, beginning with portions of the hippocampus first being traced and removed from the brain image. The amygdala was traced in the coronal plane, with the first slice being where the angular bundle clearly separates the medial amygdala border from the entorhinal cortex. This is the middle portion: tracing proceeded posteriorly, then anteriorly. Posteriorly, the angular bundle and its projections created the medial and inferio-medial boundaries. Tracing continued posteriorly until the end of the amygdala, as signaled by complete replacement of the amygdala by the hippocampus cutout, which also served as

the posterior-inferior border. Anteriorly, the CSF was the medial border as the angular bundle became indistinct. Most superior-medial boundaries were formed by the CSF, but occasionally this border was formed by the white matter tract of the angular bundle. White matter tracts served as the superior-lateral border and inferior-lateral borders, with extensions of the temporal horn also aiding in the definition of the inferior-lateral border. Anterior tracing was complete when the optic chiasm ceased to be a continuous structure. The amygdala was typically traced on 8–12 slices using 1.88 mm interpolated slice thickness.

Reliability was established by repeated measurements on multiple scans before raters were approved to process study data. Intraclass correlation coefficients (ICC's) were as follows: total cerebrum = 0.997, left hippocampus = 0.8, right hippocampus = 0.7, left amygdala = 0.9, right amygdala = 0.8, left and right caudate = 0.9, left and right temporal lobe = 0.9.

2.3. Genetic Analyses

DNA was extracted from fresh and frozen blood and stored according to previously reported methods (Rimmeler et al., 1998). An aliquot of DNA was used for COMT genotyping following methods previously published (Lachman et al., 1996), using PCR amplification with a Taqman by-design assay (Applied Biosystems) that recognized the single nucleotide polymorphism (SNP) which defines the Val158 Met polymorphism (rs4680). The samples were examined with an ABI7900 DNA analyzer and the genotypes determined with the SDS software package (Applied Biosystems). Greater than 95% genotyping efficiency was required before data were submitted for further analysis.

2.4. Statistical Analyses

Brain volumes were adjusted for total cerebral volume by dividing the raw regional volume by the total cerebral volume and used as the primary measure for each region. Following strategies used in previous studies (Ohnishi et al., 2006), subjects were dichotomized based on the presence or absence of any Met158 allele. Two-tailed Student *t*-tests were used to examine group differences in continuous variables, and Fisher's exact test was used to examine group differences in categorical variables, with race being dichotomized to Caucasian and non-Caucasian. Models using repeated measures ANOVA were created for regions that were significantly different between groups in at least one hemisphere in univariate analyses. Adjusted regional brain volume was the dependent variable and genotype, age, race, sex, and hemisphere were independent variables. Initial models included a genotype by hemisphere interaction term; if this term was not significant, it was removed and the model rerun. As statistically significant findings on univariate tests would be confirmed through models controlling for covariates, no corrections for multiple comparisons were performed.

3. RESULTS

Our sample consisted of 31 individuals with a mean age of 30.8 years (SD = 8.5y, range 20–47 years); five (16.1%) were male. Six were Val/Val homozygotes, four were Met/Met homozygotes, and 21 were heterozygotes. There was no evidence of deviation from Hardy-Weinberg equilibrium.

There were no significant differences in demographic variables between the genotype groups (Table 1). The racial distribution was diverse: of the 6 Val allele homozygotes, 2 were Caucasian, 2 were African-American, and 2 were Asian. Of the 25 Met allele carriers, 13 were Caucasian, 10 African-American, 1 Asian, and 1 of mixed ancestry (Caucasian/African-American).

In univariate analyses, temporal lobe volumes but not caudate or cerebral volumes were smaller in Val allele homozygotes (Table 1). Subsequent models examined the relationship between genotype and adjusted volumes of regions significantly different in univariate analyses: hippocampus, amygdala, and total and gray matter temporal lobe volumes. These models included age, sex, race, and hemisphere as covariates, along with a genotype by hemisphere interaction term that did not reach a level of statistical significance in any model, although there was a trend for the hippocampus ($F_{1,29} = 4.06, p=0.0534$). When the interaction term was removed and the models rerun, genotype was significantly associated with temporal lobe total ($F_{1,26} = 5.50, p = 0.0277$) and gray matter volume ($F_{1,26} = 6.36, p=0.0187$), as well as with hippocampus volume ($F_{1,26} = 5.59, p = 0.0258$), but not amygdala volume ($F_{1,26} = 3.07, p=0.0931$).

4. DISCUSSION

Val158 allele homozygotes exhibited significantly smaller hippocampal and temporal lobe volumes, with a trend for an association between genotype and amygdala volume. We could not support that the genotype effect was limited to a specific hemisphere. As predicted, we did not find an association between caudate volume and genotype. These findings were not explained by demographic differences.

Identification of a genotype effect in healthy individuals differs from previously published studies. Individuals at risk of psychosis exhibit polymorphism-related frontal lobe differences, as seen in children with 22q11.2 deletion syndrome (Kates et al., 2006) and individuals at risk of schizophrenia due to family history (McIntosh et al., 2006). Val allele homozygotes with schizophrenia exhibit smaller temporal structures (Ohnishi et al., 2006), but similar associations were not seen in that study's control group. Other studies including healthy individuals have shown either no association with genotype (Ho et al., 2005) or only age-related changes (Zinkstok et al., 2006).

The predominantly female composition of our sample is important given reports of gender-specific effects of COMT genotype on brain structure (Kates et al., 2006; Zinkstok et al., 2006). From the present study, it is unclear if our findings are specific to women, or seen in both sexes. Equally important is that COMT allele frequency differs between races (Palmatier et al., 1999) and differences in racial representation can contribute to artifactual findings (Risch and Merikangas 1997). This risk is lowered in the current study as there was no significant difference in genotype representation between racial groups. This study examines a small sample size, with few Val homozygous subjects. Larger studies are needed to determine if these findings are representative of the greater population of Val/Val homozygous individuals, if gender or racial differences exist, and should obtain more equal numbers of subjects with and without the Met allele.

A possible explanation for our findings is dopamine's effect on brain maturation. Dopaminergic innervation changes during the course of development in the frontal and temporal lobes (Rosenberg and Lewis 1995). The lower synaptic dopamine levels associated with the higher-activity COMT Val158 variant may be associated with differential brain development.

Our results, although preliminary, support that COMT polymorphisms are related to structural brain differences in healthy adults. This requires further study, including measures of potentially associated cognitive and functional correlates. Such work should also continue to be extended to individuals with psychiatric illnesses.

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References

- Beyer JL, Kuchibhatla M, Payne M, Moo-Young M, Cassidy F, MacFall JR, Krishnan KR. Caudate volume measurement in older adults with bipolar disorder. *International Journal of Geriatric Psychiatry* 2004;19:109–114. [PubMed: 14758576]
- Ho BC, Wassink TH, O'Leary DS, Sheffield VC, Andreasen NC. Catechol-O-methyl transferase Val158Met gene polymorphism in schizophrenia: working memory, frontal lobe MRI morphology and frontal cerebral blood flow. *Molecular Psychiatry* 2005;10:287–298.
- Kates WR, Antshel KM, AbdulSabur N, Colgan D, Funke B, Fremont W, Higgins AM, Kucherlapati R, Shprintzen RJ. A gender-moderated effect of a functional *COMT* polymorphism on prefrontal brain morphology and function in velo-cardio-facial syndrome (22q11.2 deletion syndrome). *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)* 2006;141B:274–280.
- Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996;6:243–250. [PubMed: 8807664]
- McIntosh AR, Baig BJ, Hall J, Job D, Whalley HC, Lymer GKS, Moorhead TWJ, Owens DGC, Miller P, Porteous D, Lawrie SM, Johnstone EC. Relationship of catechol-O-methyltransferase variants to brain structure and function in a population at high risk of psychosis. *Biological Psychiatry*. 2006Epub Sept 30
- Ohnishi T, Hashimoto R, Mori T, Nemoto K, Moriguchi Y, Iida H, Noguchi H, Nakabayashi T, Hori H, Ohmori M, Tsukue R, Anami K, Hirabayashi N, Harada S, Arima K, Saitoh O, Kunugi H. The association between the Val158Met polymorphism of the catechol-O-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia. *Brain* 2006;129:399–410. [PubMed: 16330500]
- Palmatier MA, Kang AM, Kidd KK. Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. *Biological Psychiatry* 1999;46:557–567. [PubMed: 10459407]
- Payne ME, Fetzter DL, MacFall JR, Provenzale JM, Byrum CE, Krishnan KRR. Development of a semi-automated method for quantification of MRI gray and white matter lesions in geriatric subjects. *Psychiatry Research Neuroimaging* 2002;115:63–77.
- Rimmler J, McDowell JG, Slotterback BD, Haynes CS, Menold MM, Rogala A, Speer MC, Gilbert JR, Hauser ER, Vance JM, Pericak-Vance MA. Development of a data coordinating center (DCC): Data quality control for complex disease studies. *American Journal of Human Genetics Suppl* 1998;63:A240.
- Risch N, Merikangas K. Genetic analysis of complex diseases. *Science* 1997;275:1329–1330.
- Robins LN, Helzer JE, Croughan J, Ratcliff KS. National Institute of Mental Health Diagnostic Interview Schedule. Its history, characteristics, and validity. *Archives of General Psychiatry* 1981;38:381–389. [PubMed: 6260053]
- Rosenberg DR, Lewis DA. Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: a tyrosine hydroxylase immunohistochemical analysis. *Journal of Comparative Neurology* 1995;358:383–400. [PubMed: 7560293]
- Taylor WD, Steffens DC, Payne ME, MacFall JR, Marchuk DA, Svenson IK, Krishnan KR. Influence of serotonin transporter promoter region polymorphisms on hippocampal volumes in late-life depression. *Archives of General Psychiatry* 2005;62:537–544. [PubMed: 15867107]
- Van Leemput K, Maes F, Vandermeulen D, Suetens P. Automated model-based tissue classification of MR images of the brain. *IEEE Transactions on Medical Imaging* 1999;18:897–908. [PubMed: 10628949]
- Zinkstok J, Schmitz N, van Amelsvoort T, de Win M, van den Brink W, Baas F, Linszen D. The *COMT* val(158)met polymorphism and brain morphometry in healthy young adults. *Neuroscience Letters* 2006;405:34–39. [PubMed: 16857316]

Table 1
COMT genotype differences in regional brain volumes adjusted for total cerebral volume

	<i>Val</i> homozygotes (N = 6)	<i>Met</i> carriers (N = 25)	t	Univariate p value
Age (years)	28.0 (7.6)	31.4 (8.7)	0.89	0.38
Sex (% female)	66.7% (4/6)	87.5% (21/24)	-	0.24
Race (% Caucasian)	33.3% (2/6)	52.0% (13/25)	-	0.65
Left temporal lobe	0.0695 (0.0042)	0.0737 (0.0034)	2.56	0.016
• Left temporal lobe, gray matter	0.0506 (0.0030)	0.0537 (0.0025)	2.60	0.015
• Left temporal lobe, white matter	0.0189 (0.0012)	0.0200 (0.0022)	1.11	0.279
Right temporal lobe	0.0689 (0.0026)	0.0737 (0.0047)	2.42	0.023
• Right temporal lobe, gray matter	0.0505 (0.0016)	0.0537 (0.0036)	2.16	0.040
• Right temporal lobe, white matter	0.0184 (0.0011)	0.0200 (0.0025)	1.51	0.141
Left amygdala	0.0016 (0.0002)	0.0018 (0.0002)	2.12	0.044
Right amygdala	0.0016 (0.0002)	0.0018 (0.0003)	1.69	0.104
Left hippocampus	0.0032 (0.0004)	0.0033 (0.0003)	0.57	0.573
Right hippocampus	0.0030 (0.0003)	0.0033 (0.0003)	2.75	0.010
Left caudate	0.0037 (0.0005)	0.0036 (0.0004)	0.28	0.7800
Right caudate	0.0039 (0.0003)	0.0040 (0.0005)	0.13	0.8996
Cerebrum (mL)	1174.4 (94.5)	1118.4 (109.0)	1.16	0.258

All univariate analyses of continuous variables had 29 degrees of freedom, data presented as mean (SD). Categorical variables analyzed using Fisher's exact test, comparing the ratio of women to men and the ratio of Caucasian subjects to minority subjects.