The Effects of the Catechol-O-methyltransferase val158met Polymorphism on White Matter Connectivity in Patients with Panic Disorder

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Research report

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To date, study results of the association COMT val158met (rs4680) polymorphism and panic disorder have been inconsistent.

**Racial difference**
- Valine (Val) allele-Caucasian (Hamilton et al., 2002; Rothe et al., 2006)
- Methionine (Met) allele-Asian (Woo et al., 2002, 2004)

**fMRI results**
- Val allele-fearful face-Rt. Amygdala (Domschke et al., 2008)
- Met allele-unpleasant picture-limbic and prefrontal activation (Smolka et al., 2005)
COMT val158met polymorphism

- Met allele carrier $\rightarrow$ low COMT activity $\rightarrow$ increased catecholamines activity (Lachman et al., 1996; Chen et al., 2004)

- Met allele $\rightarrow$ higher sympathetic activity (a lower skin temperature)

![Graph showing skin temperature differences between Val/Val and Met carriers in normal control subjects and patients with panic disorder.](image-url)
COMT val158met polymorphism

- In the domain of emotional functioning, individuals with a Met allele carrier
  - higher endocrine and subjective responses to stress (Jabbi et al., 2007)
  - higher harm avoidance (Enoch et al., 2003)
  - Increased neuroticism (Enoch et al., 2003; Stein et al., 2005)
  - higher trait anxiety (Woo et al., 2004)
  - lower extraversion (Stein et al., 2005)
  - higher pain sensitivity along with reduced μ-opioid receptor response (Zubieta et al., 2003)
  - increased aggression/hostility (Han et al., 2006; Lachman et al., 1998; Rujescu et al., 2003; Volavka et al., 2004)

- Val allele carrier
  - Higher anxiety related scores in female val carriers (Kim et al., McGrath et al., 2004)
COMT val158met polymorphism and fMRI

A. Ventrolateral PFC

B. Rt. amygdala

C. Lt. dorsal hippocampus
Brain White Matter Connectivity & Panic Disorder

- Greater FA in PD group
- Also FA positively correlated with clinical severity
COMT val158met Polymorphism

White Matter Connectivity

Panic Disorder
Hypothesis

- 1st study to investigate WM connectivity in relation to the COMT val158met polymorphism in panic disorder

I. To test COMT genotype influence the WM connectivity of panic disorder

II. To explore whether the relationship between WM abnormalities & clinical severity of panic disorder in influenced by COMT genotype
Subjects

- 26 patients with panic disorder (9 men, 17 women; age 37.00 ± 9.85)
  - Only patients with primary panic disorder were included
  - Secondary lifetime diagnoses were major depression in 5 patients
  - Generalized anxiety disorder in 3 patients
- 26 healthy control subjects (10 men, 16 women; age, 37.62 ± 10.18)
- All subjects were right handed
- Exclusion criteria
  - Life time history of schizophrenia, bipolar disorder, PTSD, OCD and eating disorder
  - History of alcohol or drug abuse and dependence
  - Mental retardation
  - Current or past serious medical or neurological illness
  - Contraindications to magnetic resonance (MR) scanning including metal implants, pregnancy or severe claustrophobia
- SSRI, Benzodiazepines within 1 week (3.38 ± 3.89 days)
• Panic Disorder Severity Scale (PDSS)
• Albany Panic and Phobia Questionnaire (APPQ)
• Anxiety Sensitivity Inventory-Revised (ASI-R)
MRI acquisition

- Diffusion data was acquired on a 3T GE Signa HDxt scanner (GE Healthcare, Milwaukee, WI, USA).
- Diffusion-weighted images were acquired using an echo planar imaging (EPI) sequence, with the following parameters: repetition time (TR) 17,000 ms, echo time (TE) 108 ms, field of view (FOV) 24 cm, 144x144 matrix, 1.7 mm slice thickness, voxel size 1.67x1.67x1.7 mm³.
- A double echo option was used to reduce eddy-current related distortions.
- To reduce impact of EPI spatial distortions, an 8 channel coil and array spatial sensitivity encoding techniques (ASSET, GE Healthcare) with a sensitivity encoding (SENSE) speed-up factor of 2 was used.
- Seventy axial slices parallel to the anterior commissure–posterior commissure (AC–PC) line covering whole brain were acquired in 51 directions with b=900 s/mm².
- Eight baseline scans with b=0 s/mm² were also acquired. Diffusion-Tensor Images (DTIs) were estimated from the Diffusion-Weighted Images using the Least-Squares method (approximate scan time=17 min)
Tract-based spatial statistics (TBSS)

- TBSS version 1.2 in FMRIB Software Library (FSL version 4.1, Oxford, U.K) according to the standard procedure
- Preprocessing: skull stripping and eddy current correction
- FA images were created by fitting a tensor model to the raw diffusion data
- All subjects’ FA data were then aligned into the standard space (Montreal Neurologic Institute 152 standard)
- All transformed FA images were combined and applied to the original FA map, resulting in a standard-space version FA map
- All transformed FA images were averaged to create a mean FA image, which was then thinned (skeletonized) to create a mean FA skeleton
- The skeleton was thresholded by FA <0.2 (TBSS default) to include only major fiber bundles
Tract-based spatial statistics (TBSS)

- FSL Randomise program (Nichols and Holmes, 2002)
  - Statistical analysis was performed voxel by voxel to detect regions of significant differences of FA among two genotype groups (nonparametric permutation tests with a correction for multiple comparisons)
  - General linear model tested with 5000 permutations, and the significance level was set at p<0.05
- Multiple comparisons were corrected with threshold-free cluster enhancement (TFCE)
- Only the clusters of more than 50 contiguous voxels were considered in the analysis
- To assess correlation analysis, the DTI data were analyzed using the TBSS General Linear Model (GLM) regression analysis with PDSS, APPQ, and ASI-R as a factor
• Preparation
  – Genomic DNA was extracted from blood (stored frozen) using G-DEX™ II Genomic DNA Extraction Kit (Intron, Korea)

• SNP Genotyping
  – The detection of SNP was based upon analysis of primer extension products generated from previously amplified genomic DNA using a chip-based MALDI-TOF mass spectrometry platform (Sequenom, San Diego, CA, USA)

• PCR
  – The PCR reaction was performed in a volume of 5 µl containing 1X PCR buffer (Solgent, Korea), 2.5 mM MgCl₂, 0.2 mM each dNTP, 0.1 U hTaq Polymerase (Solgent, Korea), and 200 nM of each primers, and 4.0 ng of genomic DNA
  – Primers COMT-F (5’-ACGTTGGATGTTTTCCAGGTCTGA- CAACGG) and COMT-R (5’-ACGTTGGATGACCCAGCGGATGGTG- GATT)
  – The reaction consisted of denaturation at 95°C for 15 min, followed by 45 cycles of 95°C for 20 sec, 56°C for 30 sec, and 72°C for 1 min, with a final extension at 72°C for 3 min
Results

- Genotype distributions of all 52 subjects were in accordance with Hardy–Weinberg equilibrium ($\chi^2=2.37$, df=1, p=0.12)

- *No statistically significant association* between panic disorder and COMT val158met polymorphism in this study (HC, AA: n=5, AG: n=9, GG: n=12; panic disorder, AA: n=3, AG: n=9, GG: n=14; $\chi^2=0.65$, df=2, p=0.72)

- For further analysis, genotypes were analyzed using a *dominant genetic model* according to functionality and previous studies of panic disorder in Asian population (AA/AG: n=12, GG: n=14)
### Sociodemographic and clinical characteristics

#### Table 1
Sociodemographic and clinical characteristics for panic disorder patients.

<table>
<thead>
<tr>
<th></th>
<th>AA and AG (n=12)</th>
<th>GG (n=14)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male/female</td>
<td>5/7</td>
<td>4/10</td>
<td></td>
<td>0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>34.92 ± 10.60</td>
<td>38.79 ± 9.18</td>
<td>−1.00</td>
<td>0.33</td>
</tr>
<tr>
<td>Agoraphobia</td>
<td>8</td>
<td>8</td>
<td></td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PDSS score (mean ± SD)</td>
<td>11.17 ± 4.95</td>
<td>10.86 ± 5.25</td>
<td>0.15</td>
<td>0.88</td>
</tr>
<tr>
<td>APPQ score (mean ± SD)</td>
<td>42.00 ± 35.29</td>
<td>44.43 ± 34.88</td>
<td>−0.18</td>
<td>0.86</td>
</tr>
<tr>
<td>ASI-R score (mean ± SD)</td>
<td>53.75 ± 24.41</td>
<td>39.38 ± 18.89</td>
<td>1.65</td>
<td>0.11</td>
</tr>
</tbody>
</table>


<sup>a</sup> Fisher's exact test.
Results

Threshold-free cluster enhancement method
5000 permutations $p<0.05$, corrected for FWE

COMT AA/AG genotype (FA) > GG genotype (FA) in Panic disorder
# Regions of group difference

<table>
<thead>
<tr>
<th>Cluster size (voxels)</th>
<th>Peak coordinates (mm)</th>
<th>Z</th>
<th>Anatomical locations</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>3139</td>
<td>37, –41, 4</td>
<td>6.33</td>
<td>Posterior thalamic radiation, right</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>26, –31, 23</td>
<td>5.18</td>
<td>Posterior corona radiata, right (adjacent to right retrolenticular part of internal capsule)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>26, –15, 34</td>
<td>5.08</td>
<td>Superior corona radiata, right (frontal lobe white matter)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>31, –17, 35</td>
<td>4.53</td>
<td>Superior longitudinal fasciculus, right</td>
<td>0.009</td>
</tr>
<tr>
<td>241</td>
<td>41, –18, –12</td>
<td>4.93</td>
<td>Sagittal stratum, right (including the inferior longitudinal fasciculus and the inferior fronto-occipital fasciculus)</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>47, –14, –20</td>
<td>4.32</td>
<td>Temporal lobe white matter, right (adjacent to right sagittal stratum)</td>
<td>0.042</td>
</tr>
</tbody>
</table>
Correlation between the anxiety-rating scale scores and FA in COMT rs4680 AA/AG genotype group in panic disorder

Threshold-free cluster enhancement method 5000 permutations \( p < 0.05 \), corrected for FWE
Regions of correlation

Table 3
Regions showing significant correlation between the anxiety-rating scale scores and fractional anisotropy (FA) in COMT rs4680 AA/AG genotype group in patients with panic disorder.

<table>
<thead>
<tr>
<th>Anxiety-rating scale</th>
<th>Cluster size (voxels)</th>
<th>Peak coordinates (mm)</th>
<th>Anatomical locations</th>
<th>$p^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPQ</td>
<td>119</td>
<td>25, –29, 24</td>
<td>Posterior corona radiata, right</td>
<td>0.006$^c$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31, –35, 14</td>
<td>Retrolenticular part of internal capsule, right</td>
<td>0.006$^c$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31, –39, 16</td>
<td>Posterior thalamic radiation, right</td>
<td>0.007$^c$</td>
</tr>
<tr>
<td>ASI-R</td>
<td>71</td>
<td>25, –27, 24</td>
<td>Posterior corona radiata, right</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31, –35, 14</td>
<td>Retrolenticular part of internal capsule, right</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31, –39, 16</td>
<td>Posterior thalamic radiation, right</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Discussion

1st study to examine WM connectivity using diffusion tensor imaging in relation to the COMT val158met variant in patients of panic disorder

Increased FA values in COMT rs4680 AA/AG genotype compared to GG genotype in panic disorder

- Right posterior thalamic radiation (including the optic radiation), right posterior corona radiata, right superior corona radiata, right SLF, and right sagittal stratum (including the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus)

Positive correlation between clinical data (APPQ, ASI-R) and FA of the clusters showing significant group difference
COMT 158met allele

- higher endocrine & subjective responses to stress (Jabbi et al., 2007)
- higher harm avoidance (Enoch et al., 2003)
- increased neuroticism (Stein et al., 2005)
- higher trait anxiety (Woo et al., 2004)
- higher pain sensitivity along with reduced $\mu$-opioid receptor response (Zubieta et al., 2003)
- increased aggression/hostility (Han et al., 2006; Lachman et al., 1998; Rujescu et al., 2003; Volavka et al., 2004)
- limbic and prefrontal activation elicited by unpleasant stimuli in healthy subjects (Smolka et al., 2005)
- higher sympathetic activity in patients with panic disorder (Kang et al., 2010)
Higher FA value on several WM regions
Positive correlation between FA & clinical data

- Altered WM connectivity in COMT AA/AG genotype group of panic disorder patients
- The physiological basis of the pathologic alterations in the FA remains unclear; however, increased FA of WM has been suggested as a **pathological process modifying tissue-integrity** (Rosas et al., 2006)
- Increase in FA
  - Increased **density**, more **myelination** of fibers, and greater **directional coherence**, which could contribute to more **rapid or greater information transmission** functionally (Shimony et al., 1999)
WM around the thalamus

Posterior thalamic radiation & retrolenticular part of the internal capsule

- **Thalamus**
  
  - Important neuroanatomical structures in panic disorder in terms of *fear network* (Gorman et al., 2000)
  
  - higher *glucose metabolism* (Sakai et al., 2005) and *gray matter volume reduction* (Asami et al., 2009) in patients with panic disorder
  
  - Increased FA in WM around thalamus in other anxiety disorder (Yoo et al., 2007)

* Higher FA in the WM around the thalamus, and positive correlation of the FA value in the WM around the thalamus with clinical characteristics such as *APPQ* and *ASI* scores in AA/AG genotype of panic disorder.
More rapid or greater information transmission at level of thalamus (part of fear network) among panic disorder with AA/AG genotype → higher phobic symptoms & anxiety sensitivity

Visual dependence in patients with panic disorder (Caldirola et al., 2011; Jacob et al., 1995)

- panic disorder subjects with AA/AG genotype have more rapid information transmission with filtering failures of visual stimuli in this study
Relative internal norepinephrine (NE) overload

- COMT 158met allele
  - lower COMT activity & poor degradation of catecholamines → relative **internal** norepinephrine (NE) overload

- NE-producing neurons in *locus ceruleus* → diencephalic and forebrain regions *including thalamus*
  - considered as mediating **fear and fear responses** (Hsiao and Potter, 1990)

- Lower COMT activity in COMT AA/AG genotype → higher level of NE around the WM of the thalamus (part of fear network) → increased FA and more rapid or increased information transmission → higher trait anxiety and anxiety sensitivity in panic disorder
Right SLF in parietal lobe

- Altered FA in SLF: depression (Kieseppa et al., 2010; Zou et al., 2008)

- SLF
  - Major bidirectional association tract connecting large parts of the frontal cortex with the parietal, occipital and temporal lobes
  - Parietal portion of SLF in present results focus on the abnormality of the WM connectivity in frontal-subcortical circuits connecting with dorsolateral prefrontal cortex (Schmahmann and Pandya, 2007) and suggest its possible association with AA/AG genotype in panic disorder
• Sagittal stratum ~ associated with other anxiety disorders (Baur et al., 2011)

• The inferior longitudinal fasciculus
  – Connecting occipital ↔ temporal cortex
  – main component of the visual-limbic pathway
  – The dysfunction of this tract might affect the neuromodulatory effect of amygdala on visual cortex resulting altered transmitting of valenced signals (Phillips et al., 2003)
Right sagittal stratum (inferior longitudinal fasciculus + inferior fronto-occipital fasciculus)

- The inferior fronto-occipital fasciculus
  - connects the occipital lobe ↔ infero- & dorsolateral regions of the frontal lobe
  - possible involvement in *emotional visual function* (Ffytche and Catani, 2005)
- Abnormal modulation of *emotional stimuli specific to vision* in AA/AG genotype compared to GG genotype in panic disorder
Right hemisphere

- Previous studies with asymmetries with greater right hemispheric activation of panic disorder
  - cerebral metabolic rates (Nordahl et al., 1998)
  - electroencephalography (Wiedemann et al., 1999).
  - *activation of oxygenated hemoglobin* on the *right lateral prefrontal cortex* was reported in the *COMT AA genotype* of panic disorder patients (Tanii et al., 2009)

- Increased right hemisphere activation
  - Acute emotional reaction (Davidson et al., 1990)
  - Acute activation of the avoidance-withdrawal system in negatively valenced situations (Wiedemann et al., 1999)

- AA/AG genotype: genetic predisposition *vulnerable to negative emotions* resulting in *increased information transmission in right hemisphere* in panic disorder
PDSS vs APPQ, ASI-R

- PDSS scores were not significantly correlated with the FA value
  - PDSS scores refer to more specific panic symptom severity of panic disorder (Furukawa et al., 2009)
  - APPQ scores reflect broader anxiety including fear of agoraphobic situations, fear of activities that produce somatic sensations, and fear of social situations (Rapee et al., 1994)
  - Anxiety sensitivity: trait-like characteristic that predisposes individuals to the development of anxiety disorders (Schmidt et al., 2006)

- Increased FA value in AA/AG genotype → “broad anxiety” or “anxiety sensitivity” of panic disorder rather than panic specific symptoms
Limitations

- Sample size in each group was small
- Influence of medication and comorbidity with depression could not be corrected completely
Conclusion

• Association between altered WM connectivity & COMT val158met polymorphism in panic disorder

• Increased FA
  – Posterior thalamic radiation, posterior and superior corona radiata, SLF, and sagittal stratum in right hemisphere in COMT rs4680 AA/AG genotype

• WM connectivity
  – Possible endophenotype to reflect genetic function (COMT gene) more closely in panic disorder
Thank you for your attention