Sensorimotor Gating of Schizophrenia Patients Depends on Catechol O-Methyltransferase Val$^{158}$Met Polymorphism

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It has been recently shown that Catechol O-methyltransferase (COMT) Val$^{158}$Met polymorphism strongly influences prepulse inhibition (PPI) of the acoustic startle response (ASR) in healthy human volunteers. Given that schizophrenia patients exhibit impairment in PPI and that COMT is a putative susceptibility gene for schizophrenia, we investigated the impact of the COMT Val$^{158}$Met polymorphisms on PPI in schizophrenic inpatients. We analyzed COMT Val$^{158}$Met polymorphisms and assessed startle reactivity, habituation, and PPI of ASR in 68 Caucasian schizophrenic inpatients. Clinical symptoms were measured with the Positive and Negative Syndrome Scale (PANSS). Patients carrying the Val$^{158}$Met Met/Met allele showed elevated PPI levels whereas startle reactivity and habituation did not differ from the other two genotypes. These preliminary results imply that PPI is influenced by COMT Val$^{158}$Met genotype in schizophrenia as well. In concert with other findings, our data suggest that PPI is a polygenic trait.

Key words: prepulse inhibition/acoustic startle response/sensorimotor gating/catechol O-methyltransferase/Val$^{158}$Met/COMT/schizophrenia/polymorphism

Introduction

Prepulse inhibition (PPI) of the acoustic startle response has been established as an operational measure of sensorimotor gating, and reductions in PPI have been consistently demonstrated in patients with schizophrenia. PPI is defined as a reduction of the startle reflex response that occurs when a loud startle stimulus is preceded by a weak nonstartling stimulus. It was proposed that the mechanism underlying PPI regulates sensory input by filtering out irrelevant or distracting stimuli to prevent sensory information overflow. Human and animal studies suggest that PPI is critically modulated by dopamine neurotransmission at several stages of the cortico-striato-pallido-pontine (CSPP) circuitry that has been shown to process PPI. Consequently, it has been postulated that the supposed alterations of dopamine function in schizophrenia may also account for the PPI deficit seen in these patients. Catechol O-methyltransferase (COMT) is a key dopamine catabolic enzyme that presents a significant modulator of prefrontal cortical function, and initially the COMT gene was suggested as a candidate for schizophrenia susceptibility. However, association studies did reveal divergent results, but the Val$^{158}$Met polymorphism seems to have a strong impact at least on cognitive functioning especially in “prefrontal” neurocognitive tasks in schizophrenia. Recently, it has been shown that the COMT Val$^{158}$Met polymorphism strongly affects PPI in healthy human volunteers. Individuals homozygous for the Met allele displayed higher PPI levels than Val homozygotes, while heterozygotes were intermediate. This pattern is in line with previous results on the influence of the COMT Val$^{158}$Met polymorphism on executive functioning in healthy individuals and schizophrenia patients. Additionally, it has been shown that PPI is linked with planning and strategy formation in healthy humans.

Because we have previously shown that PPI in schizophrenia patients depends on the linked T102C and A-1438G polymorphisms of the serotonin-2A receptor (5-HT$^{2A}$R), we reanalyzed our patient sample to investigate also the impact of the COMT Val$^{158}$Met polymorphism on PPI in schizophrenia. Because Roussos et al have shown that healthy homozygous carriers of the Met allele display the highest PPI levels, we predict a comparable pattern in our schizophrenia patients.

Methods and Materials

Participants

Seventy-one schizophrenia inpatients admitted to the Psychiatric Hospital of the University of Bonn were
considered eligible for the study if the following criteria were met: a diagnosis of schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), age between 18 and 65 years, and Caucasian ethnicity. Patients were excluded if they had a history of a neurological disease, substance dependency, or a severe somatic disease. Every patient was evaluated by a Structured Clinical Interview according to DSM-IV. Clinical symptoms were measured with the Positive and Negative Syndrome Scale (PANSS).17 Fourteen patients were unmedicated, 12 patients received typical antipsychotics, and 44 patients were treated with one or more atypical drugs. Demographic and clinical data of the patients grouped according to their COMT Val158Met genotype are shown in table 1. This study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn. After receiving a written and oral description of the aim of this study, all participants gave written informed consent statements before inclusion.

Table 1. Demographic and Clinical Characteristics of Schizophrenia Patients Grouped According to Their COMT Val158Met Genotype (Means and SD of Means in Parentheses, Sex, and Smoking in Frequency Data)

<table>
<thead>
<tr>
<th>COMT Val158Met Genotype</th>
<th>Met/Met</th>
<th>Val/Met</th>
<th>Val/Val</th>
<th>Total</th>
<th>F/χ²</th>
<th>df/dferr</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13 (19.1%)</td>
<td>38 (55.9%)</td>
<td>17 (25.0%)</td>
<td>68 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>32.2 (7.1)</td>
<td>34.6 (11.3)</td>
<td>37.1 (11.3)</td>
<td>34.8 (10.6)</td>
<td>0.77</td>
<td>2/65</td>
<td>.47</td>
</tr>
<tr>
<td>Years of education</td>
<td>15.0 (3.3)</td>
<td>14.6 (3.0)</td>
<td>13.6 (2.8)</td>
<td>14.4 (3.0)</td>
<td>0.85</td>
<td>2/65</td>
<td>.43</td>
</tr>
<tr>
<td>Men in %</td>
<td>53.8</td>
<td>73.7</td>
<td>64.7</td>
<td>67.6</td>
<td>1.83</td>
<td>2</td>
<td>.40</td>
</tr>
<tr>
<td>Smoker in %</td>
<td>61.5</td>
<td>52.6</td>
<td>52.9</td>
<td>54.4</td>
<td>0.33</td>
<td>2</td>
<td>.85</td>
</tr>
<tr>
<td>Patients with a first episode in %</td>
<td>69.2</td>
<td>65.8</td>
<td>58.8</td>
<td>64.7</td>
<td>0.39</td>
<td>2</td>
<td>.82</td>
</tr>
<tr>
<td>Age of onset</td>
<td>28.2 (5.5)</td>
<td>31.0 (9.9)</td>
<td>31.1 (11.1)</td>
<td>30.5 (9.5)</td>
<td>0.46</td>
<td>2/65</td>
<td>.63</td>
</tr>
<tr>
<td>Duration of illness (y)</td>
<td>4.0 (7.3)</td>
<td>3.9 (7.2)</td>
<td>5.9 (11.4)</td>
<td>4.4 (8.4)</td>
<td>0.37</td>
<td>2/65</td>
<td>.69</td>
</tr>
<tr>
<td>Number of episodes</td>
<td>1.7 (1.2)</td>
<td>2.0 (1.7)</td>
<td>2.3 (2.2)</td>
<td>2.0 (1.8)</td>
<td>0.43</td>
<td>2/65</td>
<td>.65</td>
</tr>
<tr>
<td>Medication status in % (unmedicated/typical/atypical antipsychotic)</td>
<td>23/15/62</td>
<td>18/24/58</td>
<td>29/6/65</td>
<td>22/18/60</td>
<td>2.90</td>
<td>4</td>
<td>.58</td>
</tr>
<tr>
<td>Daily chlorpromazine equivalentsa</td>
<td>256 (225)</td>
<td>279 (199)</td>
<td>259 (201)</td>
<td>269 (202)</td>
<td>0.09</td>
<td>2/65</td>
<td>.91</td>
</tr>
<tr>
<td>PANSS positive</td>
<td>20.5 (6.9)</td>
<td>17.2 (7.4)</td>
<td>19.3 (9.2)</td>
<td>18.3 (7.7)</td>
<td>1.00</td>
<td>2/65</td>
<td>.38</td>
</tr>
<tr>
<td>PANSS negative</td>
<td>22.6 (9.4)</td>
<td>19.9 (7.2)</td>
<td>21.3 (6.3)</td>
<td>20.7 (7.5)</td>
<td>0.67</td>
<td>2/65</td>
<td>.51</td>
</tr>
<tr>
<td>PANSS general</td>
<td>41.2 (12.6)</td>
<td>39.4 (15.3)</td>
<td>43.2 (12.5)</td>
<td>40.6 (14.1)</td>
<td>0.39</td>
<td>2/65</td>
<td>.68</td>
</tr>
<tr>
<td>PANSS total</td>
<td>86.3 (22.6)</td>
<td>76.7 (27.9)</td>
<td>83.8 (25.8)</td>
<td>80.2 (26.4)</td>
<td>0.80</td>
<td>2/65</td>
<td>.45</td>
</tr>
</tbody>
</table>

Note: COMT, catechol O-methyltransferase; PANSS, Positive and Negative Syndrome Scale.
*aAccording to Woods and Bazire.18,19 Unmedicated patients received the value zero.

Genotyping
In all patients, DNA for genotyping was isolated either from EDTA-anticoagulated blood or permanent cell cultures received after transforming the lymphocytes with Epstein-Barr virus. The isolation of the DNA followed the QIAGEN protocol for the Blood & Cell Culture DNA Maxi Kit (QIAGEN, Hilden, Germany). Polymerase chain reaction (PCR) was performed using 12.5 ng of DNA. The COMT Val158Met polymorphism was analyzed by a Taqman Assay. The procedure followed the protocol for Taqman Assays with the use of Taqman Universal PCR MasterMix, No AmpErase UNG (Applied Biosystems, Foster City, CA). Each assay consists of the unlabeled forward primer and the unlabeled reverse primer and 2 reporters that are dye labeled with FAM and VIC. The assays are designed for allelic discrimination of specific single-nucleotide polymorphisms. All alleles were scored in a single well by measuring the fluorescence at the end of the PCR using a Tecan Ultra 384 reader (Tecan, Crailsheim, Germany). Excitation and emission wavelengths for the FAM-labeled probes were 485 and 535 nm, and for the VIC-labeled probes 535 and 590 nm, respectively.

Startle Response Measurement
Our equipment, setup, and standard PPI testing procedures have been described in detail previously.20,21 Each examination began with a 4-minute acclimation period of 70-dB background noise that was continued throughout the session. Participants received 73 white noise sound pulses at an intensity of 116 dB (instantaneous rise/fall time) and a duration of 40 milliseconds separated by variable intertrial intervals between 8 and 22 seconds (mean 15 seconds). In 36 of the trials, the pulse was preceded by a 20-millisecond 86-dB white noise pre-pulse (instantaneous rise/fall time) with an interstimulus interval (ISI) of 120 milliseconds. The initial trial was a pulse-alone (PA) trial, which was separated for further analysis. All following trials were presented in a pseudorandomized order. The entire test session lasted about 20 minutes. To ensure that PPI was not influenced by smoking withdrawal, smoking ad libitum was permitted before testing.22 Trial exclusion and scoring criteria were identical to those used in previous studies20,21 Subjects with error trials >50% were excluded from data analysis. Based on this criterion, 3 patients (4.2%, 1 Met/Met, and 2 Val/Met carriers) were excluded from analysis.
**Results**

The COMT Val\textsuperscript{158}Met genotype frequency was distributed in accordance to the Hardy-Weinberg Equilibrium (HWE; $X^2 = 1.0, P = .32$). Given that the 5-HT\textsubscript{2A} T102C, A-1438G, and Y452H genotype frequencies were also distributed in accordance to the HWE in the present sample, genetic inhomogeneity of the analyzed population is unlikely.\textsuperscript{16} The groups did not differ regarding demographic and clinical data (see table 1).

In contrast to all other startle parameters, PPI was significantly affected by COMT genotype ($F_{2,65} = 3.05, P < .05, \eta^2 = 0.09$) (see figure 1 for PPI data and table 2 for the rest of the startle data). A Tukey HSD post hoc test revealed that homozygous carriers of the Met allele display significantly higher PPI levels compared with heterozygous carriers of the Val/Met genotype ($P < .05$, Cohen’s $d = 0.79$). Both homozygous groups (Met/Met vs Val/Val) did not significantly diverge with respect to PPI, but, however, the difference did show a moderate effect size ($d = 0.56$). The Val/Val group and the Val/Met group did not significantly differ ($d = 0.22$). Analyses of polynomial contrasts across the 3 genotype groups revealed a significant quadratic trend ($P < .05$) but no significance for a linear trend ($P = .13$).

If Met/Met carrier were compared with a merged group of carriers of the Val allele (Val/Met + Val/Val), both groups significantly differ regarding PPI ($F_{1,67} = 5.4, P < .05, d = 0.71$). Separate analyses of covariance (ANCOVAs) of the 3 genotype groups introducing age, smoking status, medication status, chlorpromazine equivalents, gender, PANSS scores, and startle amplitude as covariates, displayed significance level was set at $P < .005$ (2 tailed in order to avoid accumulation of $\alpha$ error.

**Table 2.** Means and SE of Means (in Parentheses) of Startle Amplitude and Peak and Onset Latency of the Acoustic Startle Response of Schizophrenia Patients Grouped According to Their Catechol O-Methyltransferase (COMT) Val\textsuperscript{158}Met Genotype

<table>
<thead>
<tr>
<th>COMT Val\textsuperscript{158}Met Genotype</th>
<th>Met/Met</th>
<th>Val/Met</th>
<th>Val/Val</th>
<th>Total</th>
<th>$F$</th>
<th>df/df\textsubscript{err}</th>
<th>$P$</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13 (19.1%)</td>
<td>38 (55.9%)</td>
<td>17 (25.0%)</td>
<td>68 (100%)</td>
<td>0.79</td>
<td>2/65</td>
<td>.50</td>
<td>0.02</td>
</tr>
<tr>
<td>First block, amplitude of pulse-alone trials (arbitrary units)</td>
<td>324 (53.9)</td>
<td>251 (31.0)</td>
<td>281.3 (50.0)</td>
<td>272 (23.6)</td>
<td>0.28</td>
<td>2/65</td>
<td>.76</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean amplitude of pulse-alone trials (arbitrary units)</td>
<td>223 (43.0)</td>
<td>194 (26.8)</td>
<td>226 (48.5)</td>
<td>207 (20.7)</td>
<td>0.28</td>
<td>2/65</td>
<td>.76</td>
<td>0.01</td>
</tr>
<tr>
<td>Onset latency pulse-alone trials (ms)</td>
<td>37.7 (2.5)</td>
<td>39.9 (1.3)</td>
<td>40.2 (2.5)</td>
<td>39.5 (1.0)</td>
<td>0.38</td>
<td>2/65</td>
<td>.69</td>
<td>0.01</td>
</tr>
<tr>
<td>Onset latency prepulse-pulse trials (ms)</td>
<td>43.2 (5.5)</td>
<td>40.4 (1.7)</td>
<td>40.9 (3.1)</td>
<td>41.1 (1.6)</td>
<td>0.21</td>
<td>2/65</td>
<td>.81</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak latency pulse-alone trials (ms)</td>
<td>63.8 (1.4)</td>
<td>62.5 (0.9)</td>
<td>62.5 (1.0)</td>
<td>62.7 (0.6)</td>
<td>0.31</td>
<td>2/65</td>
<td>.74</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak latency prepulse-pulse trials (ms)</td>
<td>62.1 (4.9)</td>
<td>58.7 (1.4)</td>
<td>59.1 (1.7)</td>
<td>59.4 (1.3)</td>
<td>0.51</td>
<td>2/65</td>
<td>.60</td>
<td>0.02</td>
</tr>
<tr>
<td>Peak latency facilitation (pulse-alone – prepulse pulse trials, ms)</td>
<td>1.7 (4.6)</td>
<td>3.8 (1.1)</td>
<td>3.4 (1.2)</td>
<td>3.3 (1.1)</td>
<td>0.27</td>
<td>2/65</td>
<td>.77</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

The mean percent PPI of startle magnitude was calculated using the formula: \% PPI = 100 × (magnitude on PA trials – magnitude on prepulse (PP) trials)/magnitude on PA trials.\textsuperscript{23} To assess habituation of startle reactivity, PA trials were divided each in 6 blocks. Startle reactivity was assessed by the mean amplitude of the first block of PA trials and the mean amplitude of all PA trials. The data were analyzed by analysis of variance (ANOVA) with exception of frequency data. Frequency data were analyzed using \chi^2 tests. Based on significant main effects, Tukey Honest Significance Difference (Tukey-HSD) post-hoc comparisons were performed. Interrelationships between startle measurements and clinical or demographic data were tested using Pearson product-moment-correlation. The confirmatory statistical comparisons were carried out at a significance level set at $P < .05$ (2 tailed). Within the correlation analyses, the
significant main effects of the factors genotype ($F_{2,56} = 3.21$, $P < .05$, $\eta^2 = 0.10$) and the covariate age ($F_{1,56} = 3.93$, $P < .05$, $\eta^2 = .07$), Gender, PANSS total score, and chlorpromazine equivalents did not show a significant impact on PPI and also the interaction of gender and genotype was not significant.

For the analysis of the possible impact of COMT genotype on the habituation curves, a repeated-measurement ANOVA (3 groups x 6 blocks) of the 6 PA blocks was done. This analysis revealed a significant main effect of factor block, reflecting total habituation ($F_{5,61} = 17.33$, $P < .001$, $\eta^2 = 0.59$). The factor group was not significant ($F_{2,65} = 0.69; P = .51$, $\eta^2 = 0.02$). Finally, the interaction of the factors block and group was also not significant ($F_{10,122} = 1.313; P = .23$, $\eta^2 = 0.10$), indicating a similar progression of habituation in all groups (see figure 2).

Correlational analysis of startle parameter and patient characteristics within the total sample revealed that the mean amplitude of PA trials was negatively associated with the PANSS positive score ($r = -0.37$, $P = .002$) and that the mean amplitude of PA trials in the first block was inversely correlated with the PANSS positive score ($r = -0.38$, $P = .002$) and the PANSS total score ($r = -0.34$, $P = .005$). These findings indicate that low startling patients displayed more severe psychotic symptoms. PPI did not correlate with any clinical or demographic data.

Discussion

To our knowledge, this is the first study demonstrating that the COMT Val^{158}Met polymorphism affects sensorimotor gating in schizophrenia. The present preliminary data are largely in agreement with the recent study of Roussos et al.\textsuperscript{11} who found similar effects in a sample of male healthy human volunteers. However, these authors found a much stronger effect of the factor COMT genotype ($\eta^2 = 0.25$) compared with our results ($\eta^2 = 0.09$). This could be explained by the fact that Roussos et al.\textsuperscript{11} measured a highly homogeneous sample of only male university students, whereas we assessed a heterogeneous patient sample with mixed gender, a broad age distribution, and psychotropic medication. Such moderating effects may also explain why Montag et al.\textsuperscript{24} did not find an effect of COMT Val^{158}Met on PPI in their sample of healthy female university students. However, this study is not directly comparable to Roussos’ and our study because of different stimulus parameters used for prepulse and PA trials. For a detailed discussion of these differences see Roussos et al.\textsuperscript{11} Furthermore, Roussos et al.\textsuperscript{11} reported significant lower PPI levels within homozygous carriers of the Val allele compared with both the Val/Met group and the Met/Met carriers. In contrast, we only found elevated PPI levels in the Met/Met group, while the Val/Met group and the Val/Val group displayed comparable PPI-levels. The reasons for this may be due either to a smaller sample size, demographic sample differences, an influence of schizophrenia itself, or a medication effect. The last explanation maybe the most likely one because it has been shown that atypical antipsychotics—such as quetiapine or clozapine—increase PPI in subjects with low (but not high) baseline PPI levels.\textsuperscript{25,26} It is therefore conceivable that Val/Val patients (who presumably have the lowest PPI if untreated) received the maximum benefit from medication compared with the other 2 genotype groups.

COMT messenger RNA is much more expressed in the prefrontal cortex and the hippocampus than in the striatum, the ventral tegmental area, or the substantia nigra.\textsuperscript{8} Because all these structures take part in the CSPP circuit regulating PPI, it is most likely that the Val^{158}Met polymorphism influences PPI at the prefrontal or hippocampal level.\textsuperscript{5} However, carriers of the Met allele display a lesser COMT activity compared with Val allele carriers resulting in increased dopamine concentrations especially in the prefrontal cortex of the Met allele carriers.\textsuperscript{5} Thus, enhanced prefrontal dopamine concentrations may contribute to the elevated PPI in the Met/Met variants, which would be in line with the observations that reduced dopamine activity in the prefrontal cortex results in a disruption of PPI.\textsuperscript{27–29} However, the impact of COMT Val^{158}Met genotype on hippocampal dopamine transmission is less understood so far, and thus, the hippocampus may play also a role in the PPI alterations by COMT Val^{158}Met genotype.

Currently, other genetic variations have also been reported to have an impact on sensorimotor gating. So
it has been shown that the dopamine-D3 receptor Ser<sup>9</sup>Gly polymorphism modulates PPI in healthy humans and that a mutation of the Neuregulin-1 gene also affects PPI in healthy human volunteers as well as in schizophrenia patients. Additionally, we have recently demonstrated that the 5-HT<sub>2A</sub>R T102C/A-1438G polymorphisms have a strong impact on PPI in the same sample of schizophrenia patients presented here. In agreement with some animal data, these results in humans support the view that PPI is probably a polygenetic trait. Moreover, in an explorative analysis, COMT Val<sup>158</sup>Met and 5-HT<sub>2A</sub>R T102C/A-1438G genotype did not appear to interact regarding PPI in our patient sample ($F_{3,60} = 0.1, \, P = .94, \, \eta^2 = 0.01$). Nevertheless, because the power of the present study is too small, any conclusions with regard to epistatic effects on PPI should await replication in larger samples.

The present study has some limitations. First, we only use one prepulse-pulse trial condition (85 dB–120 milliseconds) that may have masked possible stimulus or stimulus by genotype-dependent effects on PPI. However, Roussos et al. used several prepulse-pulse conditions, but they could not show any interactions between prepulse intensity, ISI, and COMT genotype. Second, we did not examine a healthy control group. But, in agreement with previous literature, the total group of schizophrenia patients did show significantly decreased PPI levels when compared with equivalent groups of healthy controls ($n = 46, 56.9\%$, standard error of the mean $= 3.2$), who were assessed with the same startle paradigm in our previous work ($t_{112} = 3.06; \, P < .01$). Nevertheless, the present findings drawn only from schizophrenia patients replicate and extent the previous results that COMT Val<sup>158</sup>Met genotype affect PPI in healthy human volunteers. Third, we could not rule out medication effects. However, it is impossible to study behavioral genetics only in unmedicated schizophrenia patients because the expectable sample sizes would be insufficient.

In conclusion, the present results tentatively suggest that PPI of schizophrenia patients depends on COMT Val<sup>158</sup>Met polymorphism. In concert with previous human and animal findings showing that PPI is affected by multiple mutations, it is suggested that PPI (like schizophrenia) is modulated by polygenetic factors. Future studies with larger samples of schizophrenia patients and healthy controls are needed to explore the multiple single and epistatic effects of different gene mutations on PPI, which may provide a window into the polygenetic causation of schizophrenia.

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