Association study of polymorphisms in the alpha 7 nicotinic acetylcholine receptor subunit and catechol-o-methyl transferase genes with sensory gating in first-episode schizophrenia

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Abstract

The purpose of the current study was to explore the association of auditory P50 sensory gating (P50) and prepulse inhibition (PPI) of schizophrenia with polymorphisms in the CHRNA7 and COMT genes. One hundred and forty patients with schizophrenia participated in this study. They were administered the tests P50 and PPI. Moreover, three single nucleotide polymorphisms (SNPs) (rs2337980, rs1909884 and rs883473) in CHRNA7 and three SNPs (rs4680, rs737865 and rs165599) in COMT were selected to be genotyped by polyacrylamide gel microarray techniques. P50 index showed significant reduction in S2 amplitude between wild-type and mutation groups in the COMT rs4680. S1 amplitude of mutation group in the rs4480 was also lower compared to wild-type group. PPI index revealed a shorter pulse latency of mutation group in the rs4680. The suppression ratio of mutation group was lower in COMT rs165599. Negative findings were shown between comparisons in all the CHRNA7 SNPs. We find that P50 and PPI may be influenced by COMT rs4680 polymorphisms in schizophrenia; more excitingly, we find that P50 might be influenced by COMT rs737865 polymorphisms and PPI may be influenced by COMT rs165599 polymorphisms in schizophrenia, and their mutations are associated with the reduction of the risk of P50 or PPI defects in schizophrenia. Further studies with a larger number of subjects are needed to verify the present findings.

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1. Introduction

With the progress of genetic research, endophenotype has played an important role in enhancing our knowledge of the etiology, diagnosis, and treatment of schizophrenia. However, a potential endophenotype should be associated with the illness, heritable, state-independent, showing familial association and co-segregation (Braff et al., 2007; Gottesman and Gould, 2003). Substantial evidence has suggested that the neurophysiological markers—P50 sensory gating and prepulse inhibition (PPI) are both potential endophenotypes for schizophrenia (Mann et al., 2008). P50 sensory gating is an electrophysiological indicator which uses the auditory evoked potential to reflect suppression of brain (Clementz et al., 1997; Erwin et al., 1998). Because of its heritability and high stability, P50 has been studied widely as a promising endophenotype for schizophrenia (Myles-Worsley, 2002; Cadenhead et al., 2005). Bramon demonstrated that schizophrenia patients have deficits in P50 suppression and that the same gating defects also occurred in 50% of the unaffected first-degree relatives of the patients (Bramon et al., 2004). Twins studies have also shown that genetic factors play an important role in the occurrence of P50 suppression defects (Myles-Worsley et al., 1996; Hall et al., 2006; Young et al., 2001). Our prior study also found that P50 defects existed in first-episode schizophrenia patients and their unaffected first-degree relatives, indicating that P50 defects...
might be a potential biological genetic marker for schizophrenia (Wan et al., 2007).

Prepulse inhibition (PPI) of the startle reflex is another operational measurement used to evaluate the function of brain sensory gating (Mackeprang et al., 2002). Braff et al. (1978) showed that patients with schizophrenia demonstrated PPI deficits compared with healthy controls. This deficit was also present in the relatives of schizophrenic individuals (Cadenhead et al., 2001). These deficits were also suggested to be relatively stable before and after treatment throughout the course of the disease (Braff et al., 2005; Leumann et al., 2002; Ludewig et al., 2002; Weike et al., 2000). Our previous case-control research has found that first-episode schizophrenia had sensory gating (SG) deficits and showed PPI ratio decrease (Li et al., 2010). PPI seemed to be a sensitive intermediate endophenotypic marker for information processing and SG deficits in schizophrenics.

Although PPI and P50 sensory gating are thought to reflect similar or related constructs, the actual relationship between them is still unclear. Recent studies suggested that these paradigms might be assessing different neural mechanisms (Brenner et al., 2004; Oranje et al., 2006). For example, it has been suggested that PPI and P50 suppression are related only insofar as hippocampal circuitry is involved in both processes (Swerdlov et al., 2000). Schwarzkopf's study in healthy volunteers found a robust positive correlation between P50 amplitude and both measures of startle inhibition; habituation and PPI of startle were both highly correlated (positively) with P50 AEP amplitude (Schwarzkopf et al., 1993). Orange et al. (1999) found a significant positive correlation between PPI and P50 suppression mainly early in testing, when habituation of the startle reflex was taking place and a significant negative correlation was found between P50 suppression in the second half of testing and the habituation of the startle reflex in 31 healthy males, but another study of Orange et al. (2006) found no correlation between P50 and PPI in 20 men. So the relationship between these two paradigms is still worthy to be discovered, and the genetic mechanism of PPI and P50 suppression and the relationship between the two paradigms is yet to be fully clarified.

The neurochemical basis of schizophrenia has been linked to dysfunction in a number of neurotransmitter systems, including the glutamatergic, dopaminergic, and serotonergic systems (for reviews see Laruelle et al., 2003; Geyer et al., 2001; Dean, 2003). However, to date, the exact mechanisms of their role in gating dysfunctions in schizophrenia have not been determined. Animal studies have shown that sensorimotor gating, as measured by PPI, can be modulated by a number of neurotransmitter systems including dopaminergic, glutamatergic, serotonergic, GABAergic, and cholinergic systems (for a review see Geyer et al., 2001). Ross et al. (2010) found that an early interaction between α7 nicotinic receptor density and choline availability might contribute to the development of schizophrenia-associated attentional deficits. Wildboer et al. (2009) found that the cholinergic system could mediate the ability to gate auditory stimuli. Several neurophysiological studies found that low α7 receptor expression or function abnormalities might be one of the mechanisms of sensory gating defect (Luntz-Leybman et al., 1992; Freedman, et al., 1994; Leonard et al., 1996; Stevens et al., 1996). Pharmacological studies found that nicotine could instantly improve P50 suppression defects of schizophrenia patients and their first-degree relatives (Bickford and Wear, 1995; Adler et al., 1992, 1993; Stevens et al., 1995). Patients with schizophrenia have high rates of smoking compared with normal subjects, even compared with patients with other mental illnesses (Hamer et al., 1995); this nicotine dependence reflects a self-treatment with sensory gating deficits (Goff et al., 1992). Animal studies have also found the CHRNA7 gene involved in the regulation of sensory gating inhibition (Luntz-Leybman et al., 1992; Couturier et al., 1990; Stevens et al., 1998). One study has suggested that the P50 suppression in schizophrenia had linkage disequilibrium with a promoter variant in the CHRNA7 gene, encoding the alpha 7 nicotinic acetylcholine receptor on chromosome 15q14 (Leonard et al., 2002). On the other hand, studies have also shown that single nucleotide polymorphisms (SNPs) in the promoter region of 5′ end in CHRNA7 gene were associated with defects of P50 suppression, but the specific SNPs were not consistent (Raux et al., 2002; Houy et al., 2004; Stephens et al., 2009). Some neurophysiological studies have also shown that low expression or abnormal functioning of nicotinic receptors, including the α7 receptor, was one of the mechanisms leading to P50 defect (Leonard et al., 1996; Stevens et al., 1996). So these studies have indicated that the CHRNA7 gene might have some linkage to SG in schizophrenia. Although animal models found that PPI might have some linkage with cholinergic systems, surprisingly no human study has been reported.

Catechol-o-methyltransferase has been intensely investigated in schizophrenia genetics as a functional candidate gene because of its active role in dopamine (DA) metabolism (Weinshilboum et al., 1999), and as a structural candidate gene because it sits within a chromosome 22q11 deletion that results in velocardiofacial syndrome (VCFS), a neurogenetic disorder that shows 30-fold increased risk for schizophrenia-like psychosis (Murphy, 2002). The catabolic action of COMT is particularly important in regulating DA levels in the pre-frontal cortex (PFC) (Lewis et al., 2001). The Val158Met SNP within COMT (rs4680) encodes a Valine → Methionine substitution at protein residue 158 of the membrane-bound COMT isoform that predominates in brain tissue. This substitution impacts COMT protein thermostability, such that the Val allele encodes a protein with approximately 40% greater enzymatic activity than the Met allele, as demonstrated in lymphocytes as well as postmortem human dorsolateral prefrontal cortex (dPFC) tissue (Chen et al., 2004). Increased DA catabolism imparted by the Val allele is thought to result in reduced synaptic DA availability, with consequences for PFC function that vary according to background dopaminergic tone (Turnbridge et al., 2006). So association between the catechol-O-methyl transferase (COMT) gene and PPI deficit in schizophrenia is focused on rs4680 and the results are controversial (Rousso et al., 2008; Montag et al., 2008; Quednow et al., 2010). Rousso et al. (2008) and Quednow et al. (2010) found that PPI levels were dependent on the COMT (Val158Met) gene polymorphism, with Val/Val individuals having the lowest PPI, Met/Met having the highest, and Val/Met having intermediate levels. These findings suggest that PPI of schizophrenia may also be adjusted by the COMT Val158Met genotype. On the contrary, findings by Montag et al. (2008) did not support the hypothesis that two of the most prominent dopaminergic candidate loci (DRD2 TaqIa and COMT Val158Met) affected PPI. On the other hand, there were several reports about the relationship between the COMT gene and P50. Lu et al. (2007) found that Valine homozygous individuals were more likely to have gating defects, supporting COMT as a genetic determinant of the P50 endophenotype, as well as a role for prefrontal dopamine in auditory filtering. However, Shiakh et al. (2011) found that there was no evidence for an association between COMT Val158Met and the P50 endophenotype. These findings suggest that the association of the COMT gene with SG in schizophrenia is still not fully understood.

In summary, discrepancies in the previous results, as well as lack of data on the relationships between the CHRNA7 or COMT genes and the two paradigms (P50 and PPI) of SG in schizophrenia provided impetus for the current study. The purpose of the current study was to examine the relationships between the two endophenotypes (P50 and PPI) and the two candidate genes (the CHRNA7 or COMT genes) of schizophrenia. In particular, for the CHRNA7 gene, the three SNPs (rs2337980, rs1909884 and rs883473) were chosen to be genotyped, for our preliminary study.
of CHRNA7 gene with schizophrenia identified that the haplotypes between rs2337980 and rs909884 as well as the haplotypes among rs2337980, rs1909884 and rs883473 may have significant association with schizophrenia (Peng et al., 2007, 2008); for COMT gene, rs4680, rs737865 and rs165599 were selected to be genotyped, because rs4680 has been suggested to influence enzyme activity (Jacobsen et al., 2012, Bhakta et al., 2012; Lanni et al., 2012), while rs737865 and rs165599 might influence clinical channel (http://www.ncbi.nlm.nih.gov/projects/snp); the three variants (rs737865, rs4680 and rs165599) (Shifman et al., 2002) or a haplotype including the three variants were associated with schizophrenia in a large sample of schizophrenic patients (Bray et al., 2003). It is speculated that the two genes might have certain association with the two endophenotypes of SG in schizophrenia.

2. Methods

2.1. Patients

One hundred and forty in-patients with schizophrenia were recruited from the Mental Health Center of Shantou University. None of them had previously contacted psychiatric services. Patients were administered the Structured Clinical Interview for DSM-IV by trained psychiatrists, and all of them met the DSM-IV criteria for schizophrenia or schizophreniform disorder. Those with the latter diagnosis were asked to abstain from smoking for at least 30 min prior to testing, were seated in a comfortable examination chair and instructed to relax with their eyes fixed on a point on the wall in front of them. The testing took place in a quiet, bright room that was not electrically shielded. Eye movements were recorded via electro-oculography (EOG) with Ag/AgCl disc electrodes placed at the outer canthus and below the right eye. Recordings were obtained with a disc electrode affixed to the vertex (CZ site) and referenced to left temporal apoppsy. All of the electrode resistances were less than 5 KΩ. To control background noise during stimulus presentation, 70–80 dB (A) broadband white noise was presented continuously throughout the session. The stimuli were generated by means of computer-driven, 90–dB pulses of 0.1 ms in duration produced by a signal generator, and a data acquisition system was used to record the ERP wave forms. The 32 pairs of auditory clicks were presented every 10 s, with a 500-ms inter-click interval. The ERP responses were amplified and band-pass filtered with a 0.1–300 Hz analog filter and no 50–60 Hz notch filter, at a sampling rate of 512 Hz, for a total of 1000 ms (100 ms before to 400 ms after the stimuli with a 500-ms gap between stimulus 1 and stimulus 2). After acquisition of the data, those trials that contained artifacts (± 50 µV wave amplitude deflection) were not included in the waveform averaging. Data were analyzed offline: band-pass filtered (10–50 Hz, 128 dB/octave roll-off). The P50 testing results were analyzed by two independent neurophysiologists, both of whom were evoked potential specialists and remained blinded to the patient’s diagnosis and treatment. When a significant difference was found between the findings of the two examiners, the study was reanalyzed by both, and a consensus value was obtained. In brief, measurement indices were developed to observe the conditioning latencies (S1L) and the testing latencies (S2L); the conditioning amplitudes (S1A) and the testing amplitudes (S2A); the amplitude difference (S1A–S2A), the gating ratio (S1A/S2A), and P50 suppression ratio [(100 × (S2A–S1A))/S1A]; the assessment standard of latency difference between S1L and S2L was ± 10 ms. If the difference was greater than 10 ms, S2A was determined to be zero (complete inhibition of response) to prevent excessive deviation of data distribution, the minimum value of the P50 suppression ratio was corrected as –100% (Myles-Worsley, 2002).

2.2. Genotyping

Peripheral venous blood (6 ml) was collected from patients, and genomic DNA was extracted using a genomic DNA extraction kit (Shanghai Bio-Engineering Co., Ltd.). DNA purity was detected by a UV spectrophotometer. Primers were designed by Primer5 10 software and the SNPBLAST Network Service System. After optimizing PCR reaction conditions, the reverse primers were re-synthesized and the 5’ ends were modified with acryl amide groups. Then each sample was subjected to PCR with the common forward primers and the modified reverse in turn. The corresponding locus of each sample was genotyped using polyacrylamide gel chip technology, as previously described (Xiao et al., 2006, 2007). Finally, genotyping results were sampled with DNA sequencing.

Table 1

The demographic and clinical characteristics of the sample.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SE)</td>
<td>24.6 ± 6.9</td>
</tr>
<tr>
<td>Gender (percentage)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>65.71</td>
</tr>
<tr>
<td>Female</td>
<td>34.29</td>
</tr>
<tr>
<td>Education (years)</td>
<td>9.34 ± 2.92</td>
</tr>
<tr>
<td>Age at onset</td>
<td>23.16 ± 6.9</td>
</tr>
<tr>
<td>Illness duration (months)</td>
<td>15.93 ± 19.01</td>
</tr>
<tr>
<td>PANS rating (mean ± SE)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18.24 ± 4.81</td>
</tr>
<tr>
<td>Negative</td>
<td>15.74 ± 4.70</td>
</tr>
<tr>
<td>Total</td>
<td>76.76 ± 13.65</td>
</tr>
</tbody>
</table>

2.3. P50 testing

The P50 testing method has been described in detail elsewhere (Hong et al., 2009): the electrophysiological examinations were performed using a signal generator and the data acquisition system of a fully functional Italian-made digital 40-channel EBNeuro Sirius BB BE. Sensory gating was evaluated by recording the P50 response of the auditory-evoked response in a paired-stimulus or conditioning-testing paradigm. All of the patients were asked to abstain from smoking for at least 30 min prior to testing, were seated in a comfortable examination chair and instructed to relax with their eyes fixed on a point on the wall in front of them. The testing took place in a quiet, bright room that was not electrically shielded. Eye movements were recorded via electro-oculography (EOG) with Ag/AgCl disc electrodes placed at the outer canthus and below the right eye. Recordings were obtained with a disc electrode affixed to the vertex (CZ site) and referenced to left temporal apoppsy. All of the electrode resistances were less than 5 KΩ. To control background noise during stimulus presentation, 70–80 dB (A) broadband white noise was presented continuously throughout the session. The stimuli were generated by means of computer-driven, 90–dB pulses of 0.1 ms in duration produced by a signal generator, and a data acquisition system was used to record the ERP wave forms. The 32 pairs of auditory clicks were presented every 10 s, with a 500-ms inter-click interval. The ERP responses were amplified and band-pass filtered with a 0.1–300 Hz analog filter and no 50–60 Hz notch filter, at a sampling rate of 512 Hz, for a total of 1000 ms (100 ms before to 400 ms after the stimuli with a 500-ms gap between stimulus 1 and stimulus 2). After acquisition of the data, those trials that contained artifacts (± 50 µV wave amplitude deflection) were not included in the waveform averaging. Data were analyzed offline: band-pass filtered (10–50 Hz, 128 dB/octave roll-off). The P50 testing results were analyzed by two independent neurophysiologists, both of whom were evoked potential specialists and remained blinded to the patient’s diagnosis and treatment. When a significant difference was found between the findings of the two examiners, the study was reanalyzed by both, and a consensus value was obtained. In brief, measurement indices were developed to observe the conditioning latencies (S1L) and the testing latencies (S2L); the conditioning amplitudes (S1A) and the testing amplitudes (S2A); the amplitude difference (S1A–S2A), the gating ratio (S1A/S2A), and P50 suppression ratio [(100 × (S2A–S1A))/S1A]; the assessment standard of latency difference between S1L and S2L was ± 10 ms. If the difference was greater than 10 ms, S2A was determined to be zero (complete inhibition of response) to prevent excessive deviation of data distribution, the minimum value of the P50 suppression ratio was corrected as –100% (Myles-Worsley, 2002).

2.4. PPI testing

The methodology for PPI of startle response measurement followed that of Parwani et al. (2000) and Duncan et al. (2001), and has been described in detail elsewhere (Li et al., 2010). The electrophysiological examinations were performed using a signal generator and the data acquisition system of a fully functional Italian-made digital 40-channel EBNeuro Sirius BB BE. Each patient’s right eye was propped and two disc electrodes (Ag–AgCl; impedance less than 5 KΩ) were positioned over the orbicularis oculi muscle, one directly below the pupil and the second approximately 1 cm above the right paropia. A third electrode (Ag–AgCl; impedance less than 5 KΩ) was placed behind the right eye over the mastoid to serve as a ground. Patients were seated comfortably in a chair and asked to gaze straight ahead at a point on a wall facing the patient. They were told that they might think of whatever they wished and did not have to attend specifically to the acoustic stimuli. All acoustic stimuli were delivered binaurally through headphones. The startle session began with a three minute acclimation period consisting of 70 dB(A) broadband noise, which continued as the background noise throughout the session. The pulse-alone stimulus was a 116 dB(A), 40 ms duration burst of 750 Hz pure tone with a near instantaneous rise time. The prepulse stimuli were 86 dB(A) 20 ms bursts of pure tone, also at 750 Hz, presented 120 ms prior to the startle stimulus. The startle session consisted of six startle stimuli consisting of three pulse-alone trials plus three trials of pulse with prepulse at each prepulse interval 120 ms. Each session began with an initial pulse-alone trial. Inter-trial intervals were 10–20 s (average 15 s) occurring in randomized order. The eyelblink component of the acoustic startle response was measured via EMG of the right orbicularis oculi muscle. EMG activity was filtered (1–500 Hz), amplified, digitized by using a signal generator and the data acquisition system of a fully functional Italian-made digital 40-channel EBNeuro Sirius BB BE. Digital signals were smoothed by an averaging paradigm that calculates a rolling average of the digital signals. In brief, measurement indices were developed to observe amplitude of pulse-alone (PA) trials; amplitude of prepulse and pulse (PPA) trials; peak latencies (PL); prepulse peak latencies (PPL); prepulse inhibition (PPI) (calculated as PPI = (PAA–PPA)/PA × 100, and its value ranged from –100% to 100%, which calculated by the correction of a much larger deviation of the data distribution). The peak latency was the latency of the maximum amplitude after the start of startle reflex; the peak latencies observed in this study were within 150 ms after the start of startle stimuli, using the criteria of Postma et al. (2006).
2.5. Statistical analysis

The patients were compared with the index of sensory gating after being divided into wild-type group (wild genotype) and the variant group (heterozygous genotype+mutant genotype) according to the genotypes of the six SNPs. For statistical analysis convenience, homozygous mutants were combined with heterozygous genotypes because the number of each mutant genotype was too small. The goodness-of-fit chi-square test was used to analyze whether the genotype frequency distribution met the Hardy–Weinberg equilibrium. P50 and PPI data were tested for normal distribution, the independent sample t test was used to analyze normal distribution parameters, and the Mann–Whitney U test was used to analyze skewed distribution parameters. These analyses were performed using SPSS software (SPSS 13.0 for Microsoft Windows). Unphased software was used to analyze tests within the group of the genotype frequencies of the SNPs.

3. Results

3.1. Hardy–Weinberg equilibrium

The genotype frequencies for all the patients showed no significant deviation from the distributions expected according to Hardy–Weinberg equilibrium. The genotype frequencies for each SNP and the results of the Hardy–Weinberg test were displayed in Table 2.

3.2. CHRNA7 and COMT genes and P50 of schizophrenia

Two patients could not complete the P50 test, resulting in 138 cases of P50 testing. Comparison of all P50 parameters between the variant group and wild-type group for each CHRNA7 SNP showed no significant difference (P > 0.05). Patients were then divided into two groups according to a P50 index of S2/S1 > 0.5 and a P50 index of S2/S1 < 0.45, known as P50 normal group and P50 abnormal group; six patients of 0.45 ≤ S2/S1 ≤ 0.5 were excluded, according to Houy’s standard (Houy et al., 2004). No difference was found in the comparison of allele frequencies and distribution of each SNP in either group (P > 0.05) (Table 3).

Comparison of all P50 parameters between the variant group and wild-type group of each SNP of the COMT gene showed that the S2A variant of rs4680 was statistically different (F = 2.196, t = 3.112, P = 0.002) (Fig. 1, Table 4). S1A of rs737865 was statistically different (F = 3.226, t = 2.234, P = 0.027) (Fig. 2, Table 4); other parameters and all the indicators of rs165599 showed no statistical difference (P > 0.05).

3.3. CHRNA7 and COMT genes and PPI of schizophrenia

There were 93 patients out of 140 patients who completed the PPI test; five patients displayed either a startle reflex amplitude ≤ 5 μV or no response after a single strong stimulation, and 88 cases had complete data. The PPI indicators of the patients were also divided into wild-type group and variant group (heterozygous + genotype) of each SNP to compare the difference of these indices.

Comparison of PPI indices between the wild-type group and the variant group of the three SNPs in the CHRNA7 gene showed no statistical difference (P > 0.05), indicating that the PPI and the CHRNA7 gene have no association.

Comparison of the PPI index for the COMT gene SNPs revealed a difference in PL of rs4680 between the wild-type and mutation groups (F = 5.929, t = 2.145, P = 0.035), and the former was longer than the latter. No statistical significance was found for the other indexes (P > 0.05) (Fig. 3, Table 5). Statistical difference was presented in the suppression ratio between the two groups of rs165599 (F = 1.959, t = 2.487, P = 0.015) (Fig. 4, Table 5), where the former was lower than the latter, and other rs165599 indicators showed negative results. No significance was found for rs737865 (P > 0.05). These results indicated the PPI had some association with the COMT gene.

3.4. P50 and PPI of schizophrenia

According to Houy et al. (2004), a ratio of S1/S2 < 0.45 should be treated as normal, while a S1/S2 > 0.5 should be treated as abnormal. We applied the same standard to the PPA/PA ratio. By this standard, patients were divided to three groups: an SG normal group, an SG doubled abnormal group and an SG single abnormal group. The genotype distribution in the three groups for each SNP (because the number of homozygous mutations was too small, the heterozygous and homozygous mutant genotypes were combined), revealed that the differences of the genotype distribution of the six sites were not statistically significant (P > 0.05). The results showed no linkage in P50 and PPI.

4. Discussion

The purpose of the current study was to find the relationships between the two paradigms (P50 and PPI) of SG and the genes (the CHRNA7 or COMT genes) in the same schizophrenia. We speculated that the CHRNA7 or COMT gene polymorphisms would have association with the P50 or PPI in schizophrenia, and we did find that the COMT gene polymorphisms were associated with the P50 or PPI in schizophrenia, but we did not find any associations between the CHRNA7 gene and P50 or PPI in schizophrenia. This result indicates that the COMT gene is involved in the regulation of sensory gating of schizophrenia.

4.1. The CHRNA7 gene and P50 or PPI

Previous positive reports about a relationship of CHRNA7 and schizophrenia and P50 focused on the promoter region of the CHRNA7 gene. Leonard et al. (2002) studied 195 schizophrenia patients and 165 controls, found that a mutation at –86 bp in the
Table 4
Comparison of the variations of P50 (mean ± S.D.) between wild-type group and mutation group of the three SNPs in COMT gene among the cases.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotypes</th>
<th>Cases</th>
<th>S1L (mS) ± S.D.</th>
<th>S2L (mS) ± S.D.</th>
<th>S1A (uV) ± S.D.</th>
<th>S2A (uV) ± S.D.</th>
<th>S1A–S2A</th>
<th>S2A/S1A</th>
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<tbody>
<tr>
<td>i</td>
<td>GG</td>
<td>75</td>
<td>57.03 ± 11.09</td>
<td>57.08 ± 11.53</td>
<td>2.52 ± 1.57</td>
<td>2.28 ± 1.49</td>
<td>0.25 ± 1.56</td>
<td>1.02 ± 0.60</td>
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<tr>
<td></td>
<td>GA+AA</td>
<td>63</td>
<td>55.44 ± 11.69</td>
<td>54.46 ± 13.28</td>
<td>2.02 ± 1.49</td>
<td>1.54 ± 1.24</td>
<td>0.48 ± 1.41</td>
<td>0.81 ± 0.75</td>
</tr>
<tr>
<td>ii</td>
<td>TT</td>
<td>81</td>
<td>57.53 ± 11.41</td>
<td>56.79 ± 12.69</td>
<td>2.63 ± 1.67</td>
<td>2.00 ± 1.34</td>
<td>0.64 ± 1.44</td>
<td>0.90 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>TC+CC</td>
<td>57</td>
<td>54.56 ± 11.14</td>
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<td>2.31 ± 1.49</td>
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<td>0.38 ± 1.53</td>
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<tr>
<td>iii</td>
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<td>31</td>
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<td>58.09 ± 13.14</td>
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<td></td>
<td>AG+GG</td>
<td>107</td>
<td>55.53 ± 11.45</td>
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<td>2.31 ± 1.49</td>
<td>1.93 ± 1.43</td>
<td>0.38 ± 1.53</td>
<td>0.90 ± 0.70</td>
</tr>
</tbody>
</table>

* P < 0.05.
** P < 0.01.

Table 5
Comparison of the variations of PPI (mean ± S.D.) between wild-type group and mutation group of the three SNPs in COMT gene among the cases.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotypes</th>
<th>Cases</th>
<th>PL (mS) ± S.D.</th>
<th>PPL (mS) ± S.D.</th>
<th>PA (uV) ± S.D.</th>
<th>PPA (uV) ± S.D.</th>
<th>(1–PPA/PA)%</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>GG</td>
<td>47</td>
<td>91.45 ± 10.75*</td>
<td>95.68 ± 12.47</td>
<td>81.61 ± 68.62</td>
<td>32.50 ± 31.80</td>
<td>49.08 ± 39.27</td>
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<tr>
<td></td>
<td>GA+AA</td>
<td>41</td>
<td>85.13 ± 16.59*</td>
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<td>81.85 ± 75.46</td>
<td>51.56 ± 67.22</td>
<td>35.79 ± 51.93</td>
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<tr>
<td>ii</td>
<td>TT</td>
<td>50</td>
<td>88.13 ± 13.94</td>
<td>93.64 ± 13.52</td>
<td>83.27 ± 79.10</td>
<td>40.71 ± 59.99</td>
<td>46.50 ± 46.36</td>
</tr>
<tr>
<td></td>
<td>TC+CC</td>
<td>38</td>
<td>88.99 ± 14.39</td>
<td>96.24 ± 10.19</td>
<td>79.69 ± 60.97</td>
<td>42.25 ± 39.87</td>
<td>38.13 ± 45.28</td>
</tr>
<tr>
<td>iii</td>
<td>AA</td>
<td>21</td>
<td>87.50 ± 16.68</td>
<td>95.06 ± 15.52</td>
<td>69.40 ± 63.92</td>
<td>49.58 ± 75.81</td>
<td>21.81 ± 52.35*</td>
</tr>
<tr>
<td></td>
<td>AG+GG</td>
<td>67</td>
<td>88.82 ± 13.26</td>
<td>94.67 ± 11.18</td>
<td>85.58 ± 73.70</td>
<td>38.81 ± 42.36</td>
<td>49.49 ± 41.85*</td>
</tr>
</tbody>
</table>

* P < 0.05.

i—rs4680; ii—rs737865 and iii—rs165599.
promoter region was associated with schizophrenia in Caucasians, and that, with the P50 sensory gating ratio increased in the control group, the promoter region polymorphism mutation frequency was significantly higher, indicating a strong relationship between the presence of a promoter variant and decreased sensory processing. Houy et al. (2004) investigated 111 schizophrenia patients and 85 controls, found a significant association between the −194 C allele of CHRNA7 gene and a T/C ratio < 0.45 in a French Caucasian population, thus demonstrating a protective effect of this variant for the sensory gating deficit, and Stephens’s (2009) family-based study about 329 African-Americans (47 nuclear families) and Caucasian-Non Hispanic patients (73 nuclear families) and case-control study of about 612 African-American and Caucasian-Non Hispanic schizophrenics found that the rs3087454 SNP, located at position 1831 bp in the upstream regulatory region of CHRNA7, was significantly associated with schizophrenia in the case-control samples after multiple-testing correction; two proximal promoter SNPs (SNP –86 bp, SNP –191 bp) were nominally associated with higher P50 ratios in control subjects in African-Americans and non-Caucasian Spanishes. But the current study did not find that polymorphisms of three SNPs in CHRNA7 gene were associated with the P50 gating deficit. We compared the genotype distributions of the P50 ratio S2/S1 > 0.5 and S2/S1 < 0.45 and also found negative results. This negative result may be due to statistical deviation by small sample size of this study, but also might be related to the different polymorphic sites from other studies; the three SNPs we chose are located in the 2, 4 intron, and the previous studies were concentrated in the promoter region. In addition, previous studies are from the French-Caucasian, African-American, Spanish and other white groups, while the current sample is from yellow groups; this group difference suggests that CHRNA7 gene polymorphism could differentially regulate P50 gating inhibition in different ethnic schizophrenia groups, further indicating that genetic heterogeneity might play a role in different ethnic backgrounds.

Although animal models found that PPI might have some linkage with cholestatic systems, this study found that the three SNPs in the CHRNA7 gene had no significant association with the PPI deficits of schizophrenia, but this result could not exclude the possibility that PPI dysfunction in schizophrenia resulted from polymorphisms in the CHRNA7 gene because of the limitation of the sample and only three SNPs.

4.2. The COMT gene and P50 or PPI

High S2 amplitude reflects a weakened ability of the brain to exclude irrelevant stimulus, so lower S2 amplitude of the COMT rs4680 mutated genotype shows that the wild-type group has weaker ability to exclude irrelevant stimulus than the variant group difference. This result suggests that the G > A mutation in rs4680 reduces the risk of P50 deficits in schizophrenia, i.e. that this mutation is a protective mutation. This is consistent with a prior study showing that Val homozygous individuals had the most abnormal P50 response (Lu et al., 2007). The present findings suggest that prefrontal cortex may exert a great effect on P50 gating in schizophrenia, possibly as compensation for local gating deficits in the primary auditory cortices (PAC) (Huang et al., 2003). These results contrast a recent large sample study in which although patients with schizophrenia and their unaffected relatives had P50 defects, the Val158Met in COMT was not associated with P50 gating (Shaikh et al., 2011). So further research is needed to verify the present findings. The next step is to resolve whether the trend of a greater COMT effect existed on P50 defect in schizophrenia and investigate the specific role of dopamine in the prefrontal cortex playing on sensory gating in a larger sample.

S1 amplitude is the conditioning stimulus, reflecting synchronization of the response to external stimuli, including the biological information that plays a key role in regulating suppression of S2. The majority of patients with schizophrenia have attention disorder early in the disease, focusing on their own internal experience and ignoring the outside world stimulation, resulting in a decreased synchronous response to outside stimuli. Higher S1A of the variant group suggests that the rs737865 variation reduces P50 defects. Therefore the T > C mutation may also reflect a protective mutation to the schizophrenia onset risk. It is the first time to find this, but follow-up studies are required to verify the present results.

The relationship between COMT gene and PPI of schizophrenia focused on rs4680; Roussos et al. (2008) found that PPI levels were associated with Val158Met polymorphism of COMT gene, Val/Val individuals had the lowest PPI, Met/Met carriers had the highest, and Val/Met were intermediate, suggesting that PPI is regulated by the dopamine neurotransmission of prefrontal cortex and its inhibition ratio depends on Val158Met polymorphism. Quednow et al. (2009) first found that the PPI inhibition ratio of male Met158Met individuals was higher than other genotypes in healthy people, and thus drew a conclusion that the effects of the COMT Val158Met genotype on PPI might be gender-related. Recent study also found that Val158Met of genotypes in COMT were related to inhibition ratios of PPI in schizophrenia, and PPI inhibition ratios of Met/Met allele carriers were higher (Quednow et al., 2010). Though this study did not find the same results as above, our findings are consistent with a prior report that the Val158Met polymorphism of rs4680 did not affect PPI inhibition ratio (Montag et al., 2008). But our study found that for another PPI index the peak latency of the wild-type group was longer than that of the variant group. The length of latency is generally believed to reflect the information processing speed; the extended latency prompts a delay of information processing on the stimulus. Thus, a shorter delay in information processing provides another explanation of the G > A mutation being a protective mutation.
We also found that the prepulse inhibition ratios were higher in individuals with the COMT of rs165599 variant. A greater prepulse inhibition ratio shows a stronger filtering function to unrelated external stimuli. Thus, the rs165599 A > G mutation may be a protective mutation. It is possible that PPI in a more generic sense is an endophenotypic marker for schizophrenia, but the present results cannot prove this; further studies with a larger number of patients are needed to verify the present findings.

4.3. Conclusion

In short, from our study, we found no correlation between the three SNPs in the CHRNA7 gene and both P50 and PPI in schizophrenia, but mutations of three COMT SNPs correlated with P50 gating and PPI in schizophrenia. These mutations increased the sensory gating function in patients with schizophrenia. The results are consistent with our preliminary case-control study results. Our haplotype analysis on 8 SNPs in COMT gene found that the (G)–GAA (rs4680)–rs165599–rs2075507–rs6269) and AAC–(G)(rs2075507–rs6269–rs4633–(rs6267) haplotypes may be protective in schizophrenia. Though this so-called protective effect seems like the patients have good sensory gating function, we believe that it is an indication of a more cognitive defect leading to poor sensitivity to external stimuli, rather than improving sensory gating function, and so it is necessary to study the sensory gating function associated with symptoms and cognitive functions in the future. However, this study was limited by the lack of healthy controls, small sample size, and only three SNPs were tested in COMT gene. Using the genomic and other research methods (e.g. gene exome sequencing) to detect more pathogenic mutations in the COMT and CHRNA7 genes will help us to understand more information of the COMT and CHRNA7 genes in the modulation of P50 or PPI in schizophrenia.

Role of funding source

These funding agents had no further role in the study design; in the collection, analysis and interpretation of the data; in the writing of the manuscript; and in the decision to submit the paper for publication.

Acknowledgments

This study was supported partially by a grant from the National “Tenth Five-year” Science and Technology Program (2002BA711A08), the National Science Fund China (81088001), the Knowledge Innovation Project of the Chinese Academy of Sciences (KSCX2-EW-J-8), and the National Basic Research Programme (973 Programme No. 2007CB512305).

References

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