Sensory Gating and Alpha-7 Nicotinic Receptor Gene Allelic Variants in Schizoaffective Disorder, Bipolar Type

Laura F. Martin,1,2* Sherry Leonard,1,2 Mei-Hua Hall,3 Jason R. Tregellas, 1
Robert Freedman,1,2 and Ann Olincy2

1Psychiatry, Denver Veterans Affairs Medical Center, Denver, Colorado
2Psychiatry, University of Colorado Health Sciences Center, Denver, Colorado
3Institute of Psychiatry, King’s College London, Strand, London

Objectives: Single nucleotide allelic variants in the promoter region of the chromosome 15 alpha-7 acetylcholine nicotinic receptor gene (CHRNA7) are associated with both schizophrenia and the P50 auditory evoked potential sensory gating deficit. The purpose of this study was to determine if CHRNA7 promoter allelic variants are also associated with abnormal P50 ratios in persons with schizoaffective disorder, bipolar type. Methods: P50 auditory evoked potentials were recorded in a paired stimulus paradigm in 17 subjects with schizoaffective disorder, bipolar type. The P50 test to conditioning ratio was used as the measure of sensory gating. Mutation screening of the CHRNA7 promoter region was performed on the subjects’ DNA samples. Comparisons to previously obtained data from persons with schizophrenia and controls were made. Results: Subjects with schizophrenia, regardless of allele status, had an abnormal mean P50 ratio. Subjects with schizoaffective disorder, bipolar type and a variant allele had an abnormal mean P50 ratio, whereas those schizoaffective subjects with the common alleles had a normal mean P50 ratio. Normal control subjects had a normal mean ratio, but controls with variant alleles had higher P50 ratios. Conclusions: In persons with bipolar type schizoaffective disorder, CHRNA7 promoter region allelic variants are linked to the capacity to inhibit the P50 auditory evoked potential and thus are associated with a type of illness genetically and biologically more similar to schizophrenia.

KEY WORDS: schizophrenia; bipolar disorder; chromosomes; human; pair 15; receptors; nicotinic; evoked potentials; auditory

This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/1552-4841/suppmat/index.html.

*Correspondence to: Laura F. Martin, M.D., Department of Psychiatry, University of Colorado Health Sciences Center, 4200 E. Ninth Avenue, C268-71, Denver, CO 80262.
E-mail: laura.martin@uchsc.edu

Received 19 May 2006; Accepted 17 October 2006

DOI 10.1002/ajmg.b.30470

© 2006 Wiley-Liss, Inc.
with schizoaffective disorder, bipolar type found no evidence for linkage at chromosome 15 [Hamshere et al., 2005].

No published studies have examined the relationship between the P50 ratio and CHRNA7 allelic variants in a schizoaffective population. Within our sample, we divided each of the subject groups into two subgroups. The first subgroup included those subjects with the most common alleles at each of the promoter region sites. The second subgroup included those subjects with less common or variant alleles occurring in the promoter region. We then sought to test the hypothesis that the mean P50 ratio in subjects with schizoaffective disorder, bipolar type could reflect 2 distinct groups of patients: those persons with the common CHRNA7 promoter region alleles and normal sensory gating, and those persons with one of the variant CHRNA7 promoter region alleles and impaired sensory gating.

MATERIALS AND METHODS

Subjects

Seventeen subjects with schizoaffective disorder, bipolar type (43.9 ± 9 years old and 42% male) were selected from a previous study of bipolar disorder [Olincy and Martin, 2005]. Subjects received a complete description of the study and then gave their written informed consent as required by the Colorado Multiple Institute Review Board. Cognitive capacity was informally assessed by asking the subjects to explain the risks of the study as well as to describe what would be expected of them during the study day. Diagnostic and symptom measures included the Structured Clinical Interview for DSM-IV [First et al., 1997] the Beck Depression Inventory [BDI; Beck et al., 1961], the Young Mania Rating Scale [YMRS; Young et al., 1978], and the Positive and Negative Symptom Scales [PANSS; Kay et al., 1987; Supplementary Table 1]. Groups were compared with 37 subjects with schizophrenia had a greater mean P50 ratio than those with schizoaffective disorder, bipolar type also had a mean P50 ratio.

Electrophysiological Recordings

The methodology for recording and analysis of the P50 auditory evoked potential is previously described [Olincy and Martin, 2005]. A conditioning testing paradigm presented auditory stimuli with an interpair interval of 0.5-sec and an interstimulus interval of 10-sec. Please see the supplementary materials for more details. Figure 1 in the supplementary materials demonstrates representative examples of the P50 evoked potentials for each of the diagnostic groups.

Genotyping

DNA was isolated from blood, collected by venipuncture on the day of testing, as described [Miller et al., 1988; Leonard et al., 2002]. A fragment of 270 bp, containing sequence of the proximal promoter of the CHRNA7 gene, was generated from each DNA by PCR, utilizing 500 ng DNA, and 0.6 µM each of the following primer sets: forward primer, 5’ AGTACCCTCCCGCTCACACCTCG 3’; reverse primer, 5’ ATGTTGATGCCG-GAGCTGCAG 3’. The PCR reactions also included 0.2 mM dNTPs, DNA polymerase and 5X buffer from the GC-Rich PCR kit (Roche Applied Sciences, Indianapolis, IN), adjusted to 0.2 mM final MgCl2. The PCR program was: 95°C, 3 min; 38 cycles of 95°C, 30 sec; 56°C, 30 sec; 72°C, 1 min, then a 4°C hold. The PCR fragments were screened for variant alleles utilizing a Transgenicome WAVE™ denaturing high performance liquid chromatography (DHPLC) system. The methodology for this instrument is described in [Leonard et al., 2002, Gault et al., 2003]. Fragments showing a pattern different from the common allele by DHPLC were sequenced for identification of the specific mutation (see Fig. 2 in the supplementary materials for the variant allele locations). Patterns similar to the common allele were mixed with a common allele sample to ensure that any homozygotic mutations were not missed.

Statistical Analyses

Chi-square and Fisher’s Exact Tests were used to evaluate differences in frequencies of allelic variants and categorical clinical variables between groups. t-tests were used to evaluate differences in electrophysiological and clinical variables between subjects with and without the common alleles. If the variances of the two populations were not equal, a separate t-test for means rather than a pooled variance t-test was used, thereby decreasing the degrees of freedom. Cohen’s d was calculated to estimate effect sizes (abbreviated as “es”). Pearson’s correlations were used to assess the relationship between clinical variables and electrophysiological measures.

RESULTS

Eleven of the 37 subjects with schizophrenia (30%), 6 of the 17 subjects with schizoaffective disorder (35%) and 41 of 149 normal control subjects (28%) had a variant CHRNA7 promoter region allele (χ² = 0.48, df = 2, P = 0.79; Supplementary Table 2).

The mean P50 ratio differed among subject groups (F = 3.09, df = 2,000, P < 0.01). Post hoc analyses revealed that the subjects with schizophrenia had a greater mean P50 ratio than both the subjects with schizoaffective, bipolar type disorder (103.9 ± 64.6 vs. 50.5 ± 41.1; P < 0.001; es 0.93) and the normal control subjects (21.8 ± 25.5; P < 0.001; es 2.26). Subjects with schizoaffective disorder, bipolar type also had a mean P50 ratio.

### TABLE I. Physiologic Variables

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Conditioning wave latency</th>
<th>Conditioning wave amplitude</th>
<th>Test wave amplitude</th>
<th>P50 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, common allele N = 108</td>
<td>62.0 ± 6.8 (42.0–83.0)</td>
<td>3.1 ± 1.7 (0.3–9.1)</td>
<td>0.6 ± 0.7 (0.0–4.0)</td>
<td>0.17 ± 0.15* (0.00–0.63)</td>
</tr>
<tr>
<td>Control, variant allele N = 41</td>
<td>63.1 ± 7.5 (46.0–78.0)</td>
<td>2.8 ± 1.3 (0.7–5.5)</td>
<td>0.8 ± 0.7 (0.0–2.0)</td>
<td>0.34 ± 0.39 (0.00–1.91)</td>
</tr>
<tr>
<td>SZAD, bipolar common allele N = 11</td>
<td>63.1 ± 8.5 (47.0–75.0)</td>
<td>1.9 ± 0.5 (1.2–2.7)</td>
<td>0.6 ± 0.5* (0.0–2.0)</td>
<td>0.30 ± 0.22* (0.00–0.61)</td>
</tr>
<tr>
<td>SZAD, bipolar variant allele N = 6</td>
<td>64.5 ± 11.2 (57.0–87.0)</td>
<td>2.7 ± 1.8 (0.8–5.7)</td>
<td>1.9 ± 0.7 (1.0–3.0)</td>
<td>0.88 ± 0.43 (0.39–1.53)</td>
</tr>
<tr>
<td>Schizophrenia common allele N = 26</td>
<td>62.5 ± 7.7 (45.0–81.0)</td>
<td>2.1 ± 0.9 (0.7–4.2)</td>
<td>1.8 ± 0.9 (1.0–4.0)</td>
<td>1.01 ± 0.63 (0.27–2.75)</td>
</tr>
<tr>
<td>Schizophrenia variant allele N = 11</td>
<td>62.7 ± 10.0 (38.0–72.0)</td>
<td>2.2 ± 1.5 (0.8–5.6)</td>
<td>2.1 ± 1.5 (0.0–5.0)</td>
<td>1.11 ± 0.72 (0.00–2.31)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation, range.

*common allele group P50 ratio < variant allele group (t44 = −2.69, P = 0.01).

(1)common allele group test wave amplitude < variant allele group (t14,1 = −3.07, P = 0.02).

(2)common allele group P50 ratio < variant allele group (t15 = −3.69, P = 0.002).

SZAD, schizoaffective.

CHRNA7 gene.
greater than the normal control subjects ($P = 0.008$; es 1.05). Group differences emerged for the conditioning wave amplitude ($F = 6.6$, $df = 2,200$, $P = 0.002$) with the schizophrenia group having a smaller mean conditioning wave amplitude than the normal control group ($2.2 \pm 1.1$ mV vs. $3.0 \pm 1.6$ mV; $P = 0.004$; es 0.6) but not the schizoaffective, bipolar type group ($2.2 \pm 1.1$ mV; $P = 0.969$; es 0.09). The schizoaffective group trended toward a difference from the normal control group ($P = 0.099$; es 0.52). The test wave amplitudes also differed among groups ($F = 37.7$, $df = 2,200$, $P < 0.001$). The schizophrenia group had a larger mean test wave amplitude than both the schizoaffective, bipolar type (1.9 $\pm$ 1.1 mV vs. 1.1 $\pm$ 0.8, $P = 0.001$; es 0.8) and normal control groups (0.7 $\pm$ 0.7 mV, $P < 0.001$; es 1.52). The schizoaffective group trended toward a difference from the normal control group ($P = 0.125$; es 0.57).

Subjects with schizophrenia and with a variant allele had a mean P50 ratio indistinguishable from those subjects with schizophrenia and with the common alleles (Table I; Fig. 1; es 0.16). In contrast, subjects with schizoaffective disorder, bipolar type who carry a variant allele had a significantly larger mean P50 ratio than those subjects with schizoaffective disorder and with the common alleles (es 2.01). Control subjects with a variant allele had a higher mean P50 ratio when compared to those control subjects with the common alleles (es 0.71). Additional P50 wave characteristics including conditioning wave latency, conditioning wave amplitude and test wave amplitude did not differ between those subjects with schizophrenia and with a variant or common allele (Table I; es 0.02, 0.09, and 0.28, respectively). Subjects with schizoaffective disorder and a variant allele had a significantly smaller mean test wave amplitude than those subjects with schizoaffective disorder and with the common alleles (es 2.01). Control subjects with a variant allele had a higher mean P50 ratio when compared to those control subjects with the common alleles (es 0.29). There were no differences in conditioning wave latency or amplitude between these two normal groups (es 0.14 and 0.19, respectively).

Clinical symptoms on the day of testing within the schizoaffective, bipolar type group were not correlated with any of the electrophysiological measures. Furthermore, they did not differ between those subjects with variant or common CHRNA7 promoter region alleles. The number of subjects taking a mood stabilizer, antidepressant or antipsychotic medication also did not differ between those subjects with the variant or common alleles. Finally, within the normal, schizoaffective and schizophrenic groups, smoking status was no different between these subjects with the variant or common alleles.

**DISCUSSION**

Persons with schizoaffective disorder, bipolar type and allelic variants in the CHRNA7 promoter region have an abnormally high mean P50 ratio, similar to persons with schizophrenia. Those subjects with the common CHRNA7 promoter alleles have a normal mean P50 ratio, similar to controls. This genetic finding sheds some light on the difficulties inherent in biological studies of schizoaffective disorder. Despite the ascertainment of one specific type of schizoaffective disorder (DSM-IV bipolar type), an initial biological measurement resulted in an interestingly impaired group [Olincy and Martin, 2005]. The current study, however, was able to parse out this still heterogeneous group into two distinct subgroups based on allelic variants in the alpha-7 nicotinic receptor gene promoter region. The impaired gene promoter region was more common in the schizoaffective group than in the normal control group ($P = 0.004$; es 1.05). The test wave amplitudes are significantly different ($F = 37.7$, $df = 2,200$, $P < 0.001$) between these subgroups (0.7 $\pm$ 0.7 mV, $P < 0.001$; es 1.52). The schizoaffective group trended toward a difference from the normal control group ($P = 0.125$; es 0.57).

Subjects with schizophrenia and with a variant allele had a mean P50 ratio indistinguishable from those subjects with schizophrenia and with the common alleles (Table I; Fig. 1; es 0.16). In contrast, subjects with schizoaffective disorder, bipolar type who carry a variant allele had a significantly larger mean P50 ratio than those subjects with schizoaffective disorder and with the common alleles (es 2.01). Control subjects with a variant allele had a higher mean P50 ratio when compared to those control subjects with the common alleles (es 0.71). Additional P50 wave characteristics including conditioning wave latency, conditioning wave amplitude and test wave amplitude did not differ between those subjects with schizophrenia and with a variant or common allele (Table I; es 0.02, 0.09, and 0.28, respectively). Subjects with schizoaffective disorder and a variant allele had a significantly smaller mean test wave amplitude than those subjects with schizoaffective disorder and with the common alleles (es 2.01). Control subjects with a variant allele had a higher mean P50 ratio when compared to those control subjects with the common alleles (es 0.29). There were no differences in conditioning wave latency or amplitude between these two normal groups (es 0.14 and 0.19, respectively).

Clinical symptoms on the day of testing within the schizoaffective, bipolar type group were not correlated with any of the electrophysiological measures. Furthermore, they did not differ between those subjects with variant or common CHRNA7 promoter region alleles. The number of subjects taking a mood stabilizer, antidepressant or antipsychotic medication also did not differ between those subjects with the variant or common alleles. Finally, within the normal, schizoaffective and schizophrenic groups, smoking status was no different between these subjects with the variant or common alleles.

Persons with schizoaffective disorder, bipolar type and allelic variants in the CHRNA7 promoter region have an abnormally high mean P50 ratio, similar to persons with schizophrenia. Those subjects with the common CHRNA7 promoter alleles have a normal mean P50 ratio, similar to controls. This genetic finding sheds some light on the difficulties inherent in biological studies of schizoaffective disorder. Despite the ascertainment of one specific type of schizoaffective disorder (DSM-IV bipolar type), an initial biological measurement resulted in an interestingly impaired group [Olincy and Martin, 2005]. The current study, however, was able to parse out this still heterogeneous group into two distinct subgroups based on allelic variants in the alpha-7 nicotinic receptor gene promoter region. The impaired gene promoter region was more common in the schizoaffective group than in the normal control group ($P = 0.004$; es 1.05). The test wave amplitudes are significantly different ($F = 37.7$, $df = 2,200$, $P < 0.001$) between these subgroups (0.7 $\pm$ 0.7 mV, $P < 0.001$; es 1.52). The schizoaffective group trended toward a difference from the normal control group ($P = 0.125$; es 0.57).

Subjects with schizophrenia and with a variant allele had a mean P50 ratio indistinguishable from those subjects with schizophrenia and with the common alleles (Table I; Fig. 1; es 0.16). In contrast, subjects with schizoaffective disorder, bipolar type who carry a variant allele had a significantly larger mean P50 ratio than those subjects with schizoaffective disorder and with the common alleles (es 2.01). Control subjects with a variant allele had a higher mean P50 ratio when compared to those control subjects with the common alleles (es 0.71). Additional P50 wave characteristics including conditioning wave latency, conditioning wave amplitude and test wave amplitude did not differ between those subjects with schizophrenia and with a variant or common allele (Table I; es 0.02, 0.09, and 0.28, respectively). Subjects with schizoaffective disorder and a variant allele had a significantly smaller mean test wave amplitude than those subjects with schizoaffective disorder and with the common alleles (es 2.01). Control subjects with a variant allele had a higher mean P50 ratio when compared to those control subjects with the common alleles (es 0.29). There were no differences in conditioning wave latency or amplitude between these two normal groups (es 0.14 and 0.19, respectively).

Clinical symptoms on the day of testing within the schizoaffective, bipolar type group were not correlated with any of the electrophysiological measures. Furthermore, they did not differ between those subjects with variant or common
ACKNOWLEDGMENTS

This work was supported by the Veterans Affairs Medical Research Service and MH 38321. The authors would like to thank Jamey Ellis for his technical assistance in creating Supplementary Figure 1.

REFERENCES


