P300 and Genetic Risk for Schizophrenia

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Background: We assessed the suitability of event-related potential frontal and temporoparietal P300 changes as intermediate phenotypes in genetic studies of schizophrenia. We applied a principal component analysis approach based on the notion that P300 abnormalities in siblings of schizophrenic patients may involve a widespread network of relatively weak cortical generators and because an earlier, smaller study that used a topographic analysis of covariance model did not show that localized P300 changes predict risk for schizophrenia.

Methods: P300 changes in 66 schizophrenic patients, 115 healthy siblings of schizophrenic patients, and 89 unrelated controls were studied during a standard auditory oddball paradigm. Principal components were calculated across electrodes, revealing frontal and temporoparietal components for latency and amplitude, respectively. For the frontal and temporoparietal P300 amplitude and latency components, the intraclass correlations (ICCs) between sib-pairs (pairs of unaffected siblings and schizophrenic index patients) and the relative risk ratios (λ) were determined.

Results: Compared with controls, schizophrenic patients and their unaffected siblings showed significant reductions in the temporoparietal P300 amplitude component. Both groups were also characterized by a significantly higher frontal P300 amplitude component. Significant ICCs and increased relative risk ratios were found for the frontal (ICC(U) = 0.18; P = .04; λ = 3.4) and temporoparietal (ICC(U) = 0.24; P = .01; λ = 1.7) P300 amplitude components.

Conclusions: Temporoparietal P300 amplitude reduction and frontal P300 amplitude increase seem to be quantitative phenotypes associated with increased risk of schizophrenia. Both measures may be useful for increasing the statistical power of genetic studies of schizophrenia.

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Schizophrenia is considered a heritable disorder with a complex genetic architecture interacting with environmental factors. Family-based linkage studies with noncoding markers spanning the genome have identified a few minor susceptibility loci that have been difficult to replicate. Because of this genetic complexity, positional cloning of these loci may be a daunting task, requiring large study samples. The goal of reducing the genetic complexity is the rationale for studying biological traits as intermediate phenotypes. One example of biological traits is event-related potentials (ERPs). Event-related potentials tend to be relatively stable and heritable characteristics, and several studies have shown that the ERP P300, that is, its temporoparietal and frontal subcomponents, may be a useful electrophysiologic trait marker for schizophrenia.

The temporoparietal P300 (P3b) subcomponent has been thought to reflect the relative amount of resources allocated to processing an unpredicted stimulus. P3b amplitude increases with greater expenditures of attentional resources and greater demand on memory updating. On the other hand, the frontal P300 subcomponent is considered to embody the cortical orienting response, and the amplitude increases with perceptual discrimination difficulties.

Temporoparietal and frontal P300 subcomponents show medium to high test-retest reliability for amplitude and low to medium test-retest reliability for latency. Based on twin studies, heritability of P300 (P3b) measures was estimated to be 0.6 to 0.8, with latencies usually showing lower values than amplitudes. Heritability studies of the frontal P300 subcomponent suggest a heritability similar to that of the temporoparietal P3b subcomponent.
Of the present study population. Most of these earlier studies were relatively underpowered because of small sample sizes and did not consider the possibility that the P300 data might reflect dysfunction in a disturbed network of weak source generators. The present P300 analysis of schizophrenic patients and their siblings differs from earlier studies in that the larger sample size now available made it possible to calculate intra-class correlations (ICCs) between sib-pairs (pairs of unaffected siblings and schizophrenic index patients) and relative risk ratios (λ) for P300. In addition, in the present study we applied a principal component analysis (PCA) model instead of a topographic analysis of variance model.

Abbreviations: ALC, alcohol abuse; ANN, anorexia nervosa; BP, bipolar disorder; CLUSA, Cluster A personality disorder; CYC, cyclothymia; DYS, dysthymia; MD, major depressive disorder; PAN, panic disorder; PARA, paranoid schizophrenic subtype; PD, personality disorder; SUB, substance abuse; UNDIFF, undifferentiated, residual and disorganized schizophrenic subtype.

A few studies22-26 have described P3b latency prolongation in schizophrenic patients. However, most studies report P3b amplitude reduction, which is relatively independent of medication status, suggesting that P3b amplitude reduction may reflect a trait feature of schizophrenia.9,19,37,38 Topographic and source analysis studies10,24,30-48 have revealed that the P3b amplitude reduction in schizophrenia is most strongly expressed bilaterally over the temporoparietal areas, with some, but not all, studies showing a stronger left side amplitude reduction.

P3b amplitude reductions also have been described in nonschizophrenic family members of schizophrenic patients, including discordant twins49 and healthy siblings.50-54 A few, but not all, studies36,32,55-59 also reported delayed P3b latencies in family members of schizophrenic patients. However, several studies57-59 did not replicate the finding of a decreased temporoparietal P300 amplitude reduction in family members of schizophrenic patients, including our own earlier study57 of a smaller subsample of the present study population. Most of these earlier studies were relatively underpowered because of small sample sizes and did not consider the possibility that the P300 data might reflect dysfunction in a disturbed network of weak source generators. The present P300 analysis of schizophrenic patients and their siblings differs from earlier studies in that the larger sample size now available made it possible to calculate intra-class correlations (ICCs) between sib-pairs (pairs of unaffected siblings and schizophrenic index patients) and relative risk ratios (λ) for P300. In addition, in the present study we applied a principal component analysis (PCA) model instead of a topographic analysis of variance model.

**METHODS**

**PARTICIPANTS**

Data were collected between July 1, 1998, and October 31, 2000, as part of the CBDB Sibling Study. The details of participant recruitment, evaluation, and potential ascertainment biases are discussed elsewhere.80 Briefly, participants had to be aged 18 to 60 years, have a premorbid IQ score greater than 70, and be able to give informed consent. Applicants with alcohol or drug abuse in the past 6 months, dependence in the past year, or more than a 5-year history of abuse or dependence were excluded. Preliminary diagnosis was established by a research psychiatric social worker through review of psychiatric records and interviews with family members. Schizophrenic outpatients and their siblings were recruited from local and national sources as described elsewhere.50 Control subjects were recruited through the National Institutes of Health Normal Volunteer Office or from the community and were screened according to the same criteria, with the additional requirement that they not have a first-degree relative with schizophrenia. Participants were interviewed and videotaped by a research psychiatrist (masked to control or sibling status) using the Structured Clinical Interview for DSM-IV (SCID and SCID-II)61 All available psychiatric records were reviewed. A second psychiatrist reviewed all Structured Clinical Interview for DSM-IV and family data; disagreements with respect to diagnosis were reviewed by a third psychiatrist, who was the “tie breaker” (M.F.E.). Psychiatric diagnosis was established on the basis of DSM-IV criteria. All procedures were approved by the National Institute of Mental Health institutional review board.

All index patients fulfilled DSM-IV criteria for schizophrenia. Patients with schizoaffective disorder (n = 9) and individuals with fewer than 30 artifact-free electroencephalographic (EEG) trials (n = 13) were excluded, as were schizophrenic patients with an additional Axis I diagnosis or any other neurological or medical diagnosis. Excluded individuals are not listed in Table 1. Siblings of schizophrenic patients were only included in group comparisons if they were considered unaffected, that is, that they had no history of psychotic illness, including schizophrenia, schizoaffective disorder, mood disorder with psychotic symptoms, or any other disorder that at some point was accompanied by psychotic symptoms. Accordingly, affected siblings (n = 16) were excluded and are not listed in Table 1. Fourteen unaffected siblings who fulfilled the criteria for any present “nonpsychotic” psychiatric disorder such as major depression or personality disorder were required to be clinically stable. Thirty-nine unaffected siblings had a history of a nonpsychotic psychiatric disorder but did not fulfill diagnostic criteria at the time of testing. These individuals were included because we were interested in trait characteristics independent of the illness-related state.

**AUDITORY ODDBALL TASK**

Participants were presented 250 tones of 2 different pitches by loudspeaker at an approximately 80-dB sound pressure level.
individuals (11 controls, 1 unaffected sibling, and 5 schizophrenic patients) who were matched for sex and age as far as possible. The analyses were performed using Statist. Group comparisons of the temporoparietal and frontal P300 amplitude and latency principal components were performed using MANCOVA and were followed by planned comparisons. These analyses were performed (1) under inclusion of all clinically unaffected siblings and schizophrenic index patients (multiple sibships) and (2) with only 1 randomly selected sibling from each family. Additional exploratory post hoc analyses were performed using the Tukey honest significance difference test. To test whether the observed effects were dependent on family membership, random-effects 2-way ANCOVAs (mixed model) were also performed, with family membership as the random factor under inclusion of those families with data available from at least 2 family members (unaffected siblings and schizophrenic patients).

## ERP Recording

A more detailed description of ERP recording is given elsewhere. 

Figure 1. Structure of principal component analysis (PCA) of P300 amplitudes across electrodes showing factor loadings of P300 amplitudes at each electrode position on the temporoparietal and frontal P300 amplitude principal components and the associated eigenvalues and percentage of explained variance of each component. The PCAs did not include the 2 frontopolar electrodes Fp1 and Fp2 owing to their liability for artifact contamination. Using Scree-tests, an eigenvalue of 1 served as the minimum criteria for the determination of the number of factors. The PCAs were computed for the combined sample of schizophrenic patients, healthy siblings, and controls and for each group separately to obtain information about group differences of the factor structure. Because factor structure was similar between groups (data available on request), all subsequent analyses were performed based on the factor scores (weighted sum of standardized amplitude or latency values, weight=factor loading) from the combined sample (n=270), which is depicted in this figure.

Stimulus duration was 50 milliseconds, and rise and decay were each 5 milliseconds. The pseudorandomized interstimulus interval was 1.0 to 1.5 seconds with all stimuli presented in pseudorandomized order (target: 1500 Hz; probability, 20%; non-target: 1000 Hz; probability, 80%). Participants were asked to count the number of targets.

## ERP/EEG Analysis

Generation of ERP grand averages across the montage and subsequent topographic amplitude and latency analysis were performed using EEGSYS. P300 peak amplitudes against baseline and latencies were determined for the target condition of the ERPs by locating the peak within the specified time window for P300 (260-420 milliseconds after stimulus). Baseline was defined as the available 50 milliseconds before stimulus EEG.

## Statistical Analysis

Statistical analyses were performed using Statistica. Group comparisons of demographic and clinical data were performed using analysis of covariance (ANCOVA), the $x^2$ statistic, or the t test. All multivariate ANCOVAs (MANCOVAs) and ANCOVAs of neurophysiologic data were performed with the 2 factors diagnosis and sex; age and number of ERP trials were the covariates, and the P300 measures were the dependent variables if not otherwise indicated.

Descriptive MANCOVAs of P300 latencies and amplitudes were performed across electrode positions, with either P300 latencies or amplitudes at all 16 electrode positions as dependent variables (although these 16 variables are not completely independent of each other). These analyses were followed by post hoc honestly significance difference analyses for unequal participants per group (n) at selected electrode positions.

To maximize the ability to measure distributed and correlated small effects, for reasons of data reduction and to increase measurement stability, exploratory PCAs were performed. The PCAs (varimax rotation) were performed separately on P300 latencies and P300 amplitudes across electrode positions. Similar approaches have been applied in previous studies to analyze topographic P300 data from schizophrenic patients, siblings, and controls (Figure 1). Test-retest stability of principal components was calculated for principal components with ICC. This was achieved by using factor loadings from the PCA as weight factors. These weights were multiplied by the raw amplitude or latency values at each electrode position and were subsequently summed for each individual. The resulting individual values for the test and retest were then subjected to ICC calculations.

Group comparisons of the temporoparietal and frontal P300 amplitude and latency principal components were performed using MANCOVA and were followed by planned comparisons. These analyses were performed (1) under inclusion of all clinically unaffected siblings and schizophrenic index patients (multiple sibships) and (2) with only 1 randomly selected sibling from each family. Additional exploratory post hoc analyses were performed using the Tukey honest significance difference test. To test whether the observed effects were dependent on family membership, random-effects 2-way ANCOVAs (mixed model) were also performed, with family membership as the random factor under inclusion of those families with data available from at least 2 family members (unaffected siblings and schizophrenic patients).

Sensitivity between sib-pairs of selected phenotypes (ie, individual factor scores) was obtained by treating the electrophysiologic measures as quantitative variables and calculating ICC values. These analyses were performed (1) for 1 randomly selected sib-pair for family and (2) for sib-pairs (1 per family) that were matched for sex and age as far as possible. For the matched sib-pairs, we also performed repeated-measures MANCOVAs with subsequent planned comparisons and the Pearson product moment correlation coefficient $r$ to test the consistency of within-family slopes. Relative risk ($\lambda$)
calculations were performed for the obtained ERP phenotypes based on “qualitative phenotype” definitions analogous to those previously reported for spectroscopic, neuropsychologic, and resting EEG data. Accordingly, a cutoff value for abnormality (ie, for the qualitative phenotype) was arbitrarily delimited based on the arithmetic mean of control subjects and subtracting 1SD. Relative risk values (λ) were calculated for the phenotype definition of 1 SD under inclusion of 1 randomly selected unaffected sibling per family. A relative risk greater than 5.0 is considered high. A relative risk greater than 2.0 is considered a reasonable indication that a trait is familial and, except for the contribution of shared environmental factors, potentially heritable. A relative risk greater than 5.0 is considered high.

RESULTS

CLINICAL AND DEMOGRAPHIC DATA

No significant age differences were found between healthy controls and schizophrenic patients (t153 =1.2; P=.23) and between controls and unaffected siblings (t101 =1.6; P=.12) (Table 1). Sex differed significantly between controls and schizophrenic patients (χ²=15.4; P<.001) and between controls and siblings (χ²=5.45; P=.02). Controls had more years of education than schizophrenic patients (t116 =5.18; P<.001) but not than unaffected siblings (t168 =0.33; P=.74). Controls were less frequently smokers than schizophrenic patients (χ²=16.3; P<.001) but not siblings (χ²=0.6; P=.44). With respect to handedness as measured by the Edinburgh Scale, no significant differences were found between controls and schizophrenic patients (χ²=0.1; P=.80) and between controls and siblings (χ²=2.1; P=.15). Comparing the percentage of correctly counted targets, controls did not differ significantly from schizophrenic patients (t80 =0.53; P=.60) and siblings (t110 =−1.02; P=.31).

CONVENTIONAL P300 ANALYSIS

Figure 2 shows unadjusted grand averages of the raw ERP activity across the montage. Adjusted 2-way MANCOVA analysis with diagnosis (controls, siblings, and schizophrenic patients) and sex as factors and P300 peak amplitudes across electrodes as dependent variables revealed a main effect of diagnosis (Rao H11021 =2.98; P<.001) and sex (Rao H11021 =2.90; P<.001) and a significant interaction between diagnosis and sex (Rao H11021 =1.71; P=.01). The equivalent multivariate analysis of variance for P300 peak latencies did not show significant main effects of diagnosis (Rao H11021 =0.80; P=.77) or sex (Rao H11021 =0.68; P=.81). Results from additional post hoc group comparisons of P300 amplitudes at 2 frontal and 2 temporal electrode positions are given in Table 2. The numerical decrease of temporoparietal P300 amplitudes and the numerical increase of frontal P300 amplitudes is more obvious in the MANCOVA model than in the raw grand averages (Figure 2) after having adjusted for age, number of trials, and sex (2-way ANCOVA). However, in siblings, the P300 amplitude differences mostly do not achieve statistical significance. Overall, the findings at frontal and temporal electrode sites hardly differ from a previous study using a similar statistical approach of a subsample of the present cohort.

PRINCIPAL COMPONENT STRUCTURE AND TEST-RETEST STABILITY

The PCA of the P300 amplitudes across electrodes reveals a 2-factor structure in the combined sample comprising controls, siblings, and schizophrenic patients: a frontal P300 subcomponent and a temporoparietal P300 subcomponent (P3b) (Figure 1). The PCA of P300 latencies across electrode positions (data not shown) shows an almost identical 2-factor solution as found for the P300 amplitudes. The larger principal latency component is a temporoparietal P3 latency component, which includes temporal, parietal, and occipital electrode positions (eigenvalue, 6.1; variance, 43.5%). The smaller principal component is a frontal P300 latency component (eigenvalue, 2.2; variance, 16.0%).

Test-retest stability of the obtained principal components is low for the frontal latency component (ICC11004 =0.25; P=.07) and temporoparietal latency component (ICC11004 =−0.23; P=.82), moderate for the temporoparietal amplitude component (ICC11004 =0.61; P=.001), and high for the frontal amplitude component (ICC11004 =0.88; P<.001).

PRINCIPAL COMPONENT GROUP COMPARISONS

Group comparisons based on the principal component values are depicted in Figure 3. The reader should recall that the factor scores (P300 amplitude principal component values) can be negative because these values were standardized. Overall MANCOVA of schizophrenic patients, siblings, and controls for the temporoparietal and frontal P300 amplitude principal components resulted in a significant main effect of diagnosis (Rao H11003 =3.94; P=.004) (Figure 3) and sex (Rao H11021 =11.16; P<.001). No significant diagnosis × sex interaction was seen (Rao H11010 =0.86; P=.49). The significance level increased when including the control group and randomly selecting only 2 individuals from each family (1 index patient and 1 clinically unaffected sibling): we observed a significant main effect of diagnosis (Rao H11003 =3.84; P=.005), a significant main effect of sex (Rao H11021 =9.04; P<.001), and, again, no significant interaction (Rao H11010 =0.86; P=.49). Finally, the MANCOVA of schizophrenic patients with diagnostic subtype (paranoid, undifferentiated) and sex as independent factors showed a significant effect of diagnosis (Rao H11021 =5.24; P=.008) and sex (Rao H11021 =6.23; P=.004) but no significant interaction (Rao H11010 =2.03; P=.14). An equivalent MANCOVA of siblings with the 2 subgroups (nonpsychotic, psychiatric lifetime diagnosis [DSM-IV Axis I and II present vs not present]) revealed no significant main effect of subgroup (Rao H11003 =1.80; P=.17) or sex (Rao H11021 =0.68; P=.51) and no interaction (Rao H11010 =1.05; P=.33). The MANCOVA of patients with neuroleptic drug treatment vs no medication showed no significant main effects of medication use (Rao H11003 =0.36; P=.70) or sex (Rao H11021 =2.03; P=.14) and no interaction (Rao H11010 =1.05; P=.86). Likewise, schizo-
Figure 2. Oddball event-related potential group grand averages (targets). In schizophrenic patients, strongly reduced P300 (P3b) amplitudes are seen in the temporoparietal area, whereas in unaffected siblings, only slightly reduced P3b amplitudes are observed (strongest in the right posterior temporal region). In siblings, a double-peak configuration is seen, with an earlier peak between 250 and 300 milliseconds in the centroparietal region, which is less obvious in controls and absent in schizophrenic patients. In the frontal regions (F3 and F4) of schizophrenic patients and siblings, the slope of the P300 amplitude is shifted toward more positive values, and the P300 is generally less well expressed, as in the temporoparietal region. In schizophrenic patients, amplitudes at electrode positions Fp1 and Fp2 are possibly contaminated by eye movement artifacts, whereas there is no evidence of such contamination in siblings.

### Table 2. Group Comparisons of Event-Related Potential P300 Amplitudes*

<table>
<thead>
<tr>
<th>Electrode Position</th>
<th>Controls (n = 89) vs Siblings (n = 115)</th>
<th>Controls (n = 89) vs Schizophrenic Patients (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude, Mean (SD), µV</td>
<td>P Value</td>
</tr>
<tr>
<td>F3</td>
<td>5.1 (4.5) vs 6.4 (7.2)</td>
<td>.23</td>
</tr>
<tr>
<td>F4</td>
<td>5.0 (4.1) vs 6.4 (6.2)</td>
<td>.16</td>
</tr>
<tr>
<td>T5</td>
<td>8.4 (4.9) vs 8.1 (4.7)</td>
<td>.64</td>
</tr>
<tr>
<td>T6</td>
<td>8.3 (4.4) vs 7.1 (3.7)</td>
<td>.009</td>
</tr>
</tbody>
</table>

*This post hoc honest significant difference analysis for unequal participants per group (n) is based on a previous multivariate analysis of covariance.
phrenic patients with and without a family history of schizophrenia did not show significantly different P300 amplitude principal component values (Rao $R_{5,41}^2=1.72$; $P=.19$), although a statistical trend interaction with sex was observed (Rao $R_{5,41}^2=2.96$; $P=.06$).

**Temporalparietal P300 Amplitude**

Planned comparisons of the temporalparietal P300 amplitude principal components between groups under inclusion of all participants within the framework of the previous overall MANCOVA are shown in Figure 3. Similar results were obtained when randomly selecting only 1 sibling from each family, with siblings showing significantly lower temporalparietal P300 principal component amplitude mean values than controls ($−0.02$ vs $0.24$; $P=.01$) and higher mean values than schizophrenic patients ($−0.02$ vs $−0.29$; $P=.02$). The temporalparietal P300 amplitude principal component values also differed significantly by sex, with males showing lower mean amplitudes in the whole group ($−0.20$ vs $0.18$; $P<.001$), which was also seen when randomly selecting only 2 family members ($−0.23$ vs $0.18$; $P<.001$). Random-effects 2-way ANCOVA results were consistent with the results of the previous analyses. A significant effect was found for diagnosis with respect to the dependent variable temporalparietal P300 amplitude component ($F_{1,28,3}=19.77$; $P<.001$), without a significant effect of family membership ($F_{9,2,1}=3.54$; $P=.24$).

**Frontal P300 Amplitude**

Figure 3 also shows planned comparisons of frontal P300 amplitude principal component values within the framework of the previous overall MANCOVA under inclusion of all participants. When only 1 unaffected sibling and schizophrenic patient was randomly selected from each family, similar results were obtained. Siblings showed higher frontal P300 amplitude principal component mean values than controls ($0.18$ vs $−0.23$; $P=.004$) but did not significantly differ from schizophrenic patients ($P=.60$). No significant sex differences in frontal P300 amplitude principal component mean values were seen between males and females in the whole group ($−0.03$ vs $−0.005$; $P=.85$) or in the group with no more than 2 participants per family ($0.07$ vs $−0.05$; $P=.32$). The additional random-effects 2-way ANCOVA results were again consistent with the results of the previous analyses. No significant effect was seen for the frontal P300 principal component ($F_{1,40}=0.00$; $P=.95$), again without a significant effect of family membership ($F_{9,12,0}=1.44$; $P=.24$).

**GENETIC MODELING**

Relative risk values ($\lambda$) of unaffected siblings (1 randomly selected unaffected sibling per family) are presented for the principal component values given in Table 3. Separate relative risk calculations for males (7 of 29 siblings and 10 of 49 controls with the phenotype) and females (10 of 51 siblings and 2 of 40 controls with the phenotype) revealed an increased risk in females ($\lambda=3.92$) but not males ($\lambda=1.18$) with respect to the temporalparietal P300 amplitude principal component. With regard to the frontal P300 amplitude principal component, increased risk values were found in males ($\lambda=4.39$, 95% confidence interval, 1.7-11.1) and females ($\lambda=2.94$, 95% confidence interval, 1.1-8.2).

Sib-pair ICC$_{U}$ values (1 randomly selected sib-pair per family) are also presented in Table 3. Equivalent ICC analyses for P300 amplitude values at selected electrode positions (F3, F4, T5, and T6) were not significant (data not shown). Repeated-measures MANCOVAs, ICC calculations, and Pearson $r$ values were calculated for sib-pairs, that is, 1 pair per family, with matching of

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**Table 3. Relative Risk ($\lambda$) and Sib-Pair Intraclass Correlation (ICC$_{U}$) Values**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Schizophrenic Patients, No. (%) (n = 66)</th>
<th>Siblings, No. (%)</th>
<th>Controls, No. (%)</th>
<th>Concordant Pairs, No.</th>
<th>Discordant Pairs, No.</th>
<th>Relative Risk $\lambda$ (95% CI)</th>
<th>ICC$_{U}$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3b amplitude</td>
<td>42 (58)</td>
<td>14 (19)</td>
<td>12 (14)</td>
<td>12</td>
<td>32</td>
<td>4.39 (0.9-7.3)</td>
<td>0.024</td>
<td>.01</td>
</tr>
<tr>
<td>Frontal amplitude</td>
<td>20 (27)</td>
<td>24 (33)</td>
<td>9 (10)</td>
<td>11</td>
<td>21</td>
<td>3.41 (1.7-6.8)</td>
<td>0.177</td>
<td>.04</td>
</tr>
<tr>
<td>P3b latency</td>
<td>11 (15)</td>
<td>10 (14)</td>
<td>20 (22)</td>
<td>2</td>
<td>17</td>
<td>1.00 (0.6-1.6)</td>
<td>0.019</td>
<td>.39</td>
</tr>
<tr>
<td>Frontal latency</td>
<td>20 (29)</td>
<td>18 (25)</td>
<td>11 (12)</td>
<td>3</td>
<td>32</td>
<td>1.50 (0.7-3.1)</td>
<td>-0.227</td>
<td>.97</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

*Relative risk values for unaffected siblings (1 randomly selected per family, n = 80) and ICC$_{U}$ values for 73 available sib-pairs (multiple assignations [n = 16] of 1 index patient to several siblings within 1 family; also see the “Results” section).

†A concordant pair is an index patient and a sibbing with the electrophysiologic phenotype.

‡A discordant pair is an index patient with the electrophysiologic phenotype and a sibling without the electrophysiologic phenotype.
sib-pairs for age and sex as much as possible (a separate analysis for male- or female-only sib-pairs was not possible because of small numbers). Repeated-measures MANCOVAs with frontal and temporoparietal P300 amplitude principal components as dependent variables resulted in significant effects of diagnosis (Rao $R^2_{6,48}=7.35; P=0.002$) and sex (Rao $R^2_{6,96}=3.26; P=0.006$), without any evidence of interaction (Rao $R^2_{6,96}=0.43; P=0.86$). Subsequent planned comparisons of matched siblings and index patients were consistent with the previously presented larger group comparisons (Figure 3), revealing a significant difference in temporoparietal P300 amplitude between schizophrenic patients and their related siblings ($P<0.001$) and no significant difference in frontal P300 amplitude between the 2 groups ($P=0.74$). When performing the same ICC calculations as depicted in Table 3 for the matched sib-pairs (Figure 4), ICCU values increased for the temporoparietal and frontal P300 amplitude components. For the temporoparietal P300 amplitude component, the Pearson $r$ value was high ($r=0.531; P<0.001$), with a parallel slope for most sib-pairs (Figure 4). The Pearson $r$ value for the frontal P300 amplitude component was considerably lower, with trend significance ($r=0.282; P=0.07$). The lower correlation of the frontal component is reflected by the relatively steep slopes in those sib-pairs with the highest values, although, for the most part, schizophrenic patients with high frontal amplitude are related to unaffected siblings who also have relatively high amplitude values and vice versa.

The present study was performed to further explore the suitability of the ERP P300 as an intermediate phenotype for genetic studies of schizophrenia. To our knowledge, this is the first study to report similarity (ICCU) and relative risk (λ) estimates of ERP P300 measures in clinically unaffected siblings of schizophrenic patients. This allows inferences about the association of P300 abnormalities and the genetic risk for schizophrenia, especially because electrophysiologic variables seem to be little affected by shared environment.7

The detection of statistically significant group differences between siblings and controls in the present and a previous study57 of a subsample of this investigation was unsuccessful when applying an analysis of variance model that only takes into account local information from single electrodes as dependent variables. Of note, any demonstration of group differences may have been difficult because of a possible ascertainment bias toward relatively healthy siblings.47,60 Therefore, we decided to use a PCA, which combines the weighted, correlated effects across the montage in a data-driven way and thus might offer greater statistical power. In this context, it is important to mention that intracortical investigations of P300 and source analyses of scalp-recorded data clearly indicate that frontal and temporoparietal P300 is composed of multiple, cortically widespread, and relatively weak
cortical generators.\textsuperscript{10,41,48,75} Also, source analyses of scalp-recorded P300 have provided some evidence that frontal and temporoparietal P300 changes in schizophrenic patients involve an extended cortical “P300 network” rather than only affecting isolated P300 generators, for example, at the temporoparietal junction.\textsuperscript{46} Previous, equivalent PCAs of P300 have resulted in stable and neurophysiologically plausible frontal and temporoparietal components,\textsuperscript{20,59} which provides validity to this particular approach. The obtained simple 2-factor structure in the present study is, to a large extent, in accordance with these previous factor analyses.

Four potential phenotypes were analyzed: the temporoparietal and frontal P300 amplitude and latency principal components. Largely in accordance with previous literature,\textsuperscript{17-26} the 2 latency components showed relatively low test-retest stability, whereas stability was moderate to high for the 2 amplitude components.

\textbf{TEMPOROPARIETAL P300 (P3b) AMPLITUDE}

Based on earlier studies,\textsuperscript{47,49-59} it was predicted that a reduction in the temporoparietal P300 amplitude is associated with increased risk of schizophrenia. The present study is in agreement with this prediction. Relative risk for reduced P3b amplitude is increased in clinically unaffected siblings, and group comparisons revealed that schizophrenic patients have lower P3b amplitudes than controls, with their siblings showing intermediate values. The random-effects analysis suggests that this finding can be generalized and is not explained by family membership. The similarity of P3b amplitudes between sib-pairs seems to be in the low to moderate range, which provides indirect evidence that P3b amplitude is heritable. The P3b amplitude distribution across groups suggests that reduced P3b amplitude is a quantitative rather than a qualitative risk factor, although a more conclusive answer to this question would have also required analysis of control sib-pairs with no schizophrenic relative in their family. Schizophrenic patients generally have lower amplitudes than their related sibling; however, many clinically affected-unaffected sib-pairs still have P300 amplitudes that extend well into the range of controls. Therefore, and because the relative risk (\(\lambda\)) value for temporoparietal P300 reduction in siblings is relatively low, this phenotype may have some value for increasing statistical power in large genetic studies but is obviously of limited value with respect to the prediction of the individual risk for schizophrenic illness. Also, sex-specific differences need to be taken into account.

\textbf{FRONTAL P300 AMPLITUDE}

Previous studies of frontal P300 amplitudes have found unchanged or slightly lower frontal P300 amplitudes in schizophrenic patients and siblings.\textsuperscript{*} However, in the present study, increased amplitudes were found in both groups. When exclusively regarding schizophrenic patients, the possibility of artifacts as an explanation cannot be discarded because an increased rate of eye move-ments is a frequent observation in schizophrenic patients and because the P300 amplitudes are highest over the frontopolar electrodes. In siblings, however, P300 amplitude differences are strongest over the frontal and not frontopolar electrodes, suggesting that the increased P300 amplitude is generated in the frontal cortex. An important observation in this context is that the ICCs between P300 amplitude principal components of clinically affected-unaffected sib-pairs are significant, which suggests that the increased P300 amplitude in schizophrenic patients is at least in part also cortically generated. Another possible explanation for the difference between our results and those of some earlier studies of frontal P300 may involve our method for measuring frontoparietal P300. In the present study, an automatic peak detection procedure was used instead of manual peak detection, which usually has been used in previous and mostly smaller studies. This technical difference might be important because although the temporoparietal subcomponent of the P300 is a true ERP with a clear peak in the ERP tracings and cortical oscillations in selective frequency bands with phase locking and polarity reversal, the poorly expressed frontal P300 subcomponent can be considered to be a correlate of cortically generated, neuronal noise with some embedded signal-like activity.\textsuperscript{14,16,48,75} When peak detection of frontal P300 is performed manually, it is in many cases impossible to be objective about the real peak. When using automatic peak detection, the signal peak also can be missed because of the noisy structure of this subcomponent. Accordingly, it is conceivable that we have monitored with our approach an increase in cortically generated noise in patients and clinically unaffected siblings.

Another possibility that could explain the frontal P300 amplitude difference between studies has to do with statistical power to detect differences. Moreover, and in contrast to most earlier studies, we used a PCA, which is sensitive in the detection of relatively small but correlated activity changes. With respect to schizophrenic patients, it also might be relevant that most earlier studies of frontal P300 amplitude studied inpatients, whereas our study only included clinically stable outpatients. This could be important because it was shown that a frontal amplitude increase correlates with symptom improvement.\textsuperscript{19,26,36}

Siblings of patients with high frontal P300 amplitudes also have relatively high amplitudes, and our findings show that familiality plays a role because relative risk is increased in the moderate range and ICCs are significant. As with the temporoparietal P300, the amplitude distribution suggests a quantitative rather than a qualitative trait. However, the steep slopes of frontal P300 amplitudes between sib-pairs within the upper portion of the amplitude factor score range suggest that as-yet-unidentified neurophysiologic mechanisms that might not directly be trait dependent also may play a role. One possible explanation could be that the high amplitude in some siblings and schizophrenic patients reflects a higher engagement of the frontal cortex to compensate for a cortical deficit either within the frontal cortex or elsewhere, for example, in the temporal lobe. The plausibility of this explanation would lie in the neurophysiologic observation that the orientation-related, frontal P300 am-

\*References 23, 24, 36, 39, 40, 43, 45-48, 58, 76-78.
plibility component assists in probing widespread cortical areas for rapid evaluation of the biological significance of stimuli. 14,16

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