Genetic Liability to Schizophrenia Measured by P300 in Concordant and Discordant Monozygotic Twins

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Key Words
Schizophrenia · Electroencephalography · P300 · Twins · Genes · Environment

Abstract
Background: Differential effects of genes and environment can contribute to etiological heterogeneity in schizophrenia. Twins concordant and discordant for schizophrenia may differ in genetic predisposition to schizophrenia with concordant twins having a higher genetic liability and discordant twins having a lower genetic liability to schizophrenia. We aimed to investigate whether P300 amplitude (which has been postulated as a genetic marker for schizophrenia) reflected this heterogeneity. Sampling and Methods: We compared P300 amplitudes across 36 monozygotic twin pairs (6 concordant for schizophrenia/schizoaffective disorder, 11 discordant and 19 healthy control pairs) performing an auditory oddball task, using multiple regression (age, gender, birth order, premorbid IQ as covariates). We further looked at the correlation between the Brief Psychiatric Rating Scale (BPRS) and P300 amplitude in affected twins, and compared concordant and discordant affected twins on the Global Assessment Scale (GAS). Results: Multiple regression yielded a highly significant model (p = 0.004) though the explained variance was limited (21%). The main effect of the group on P300 amplitude was significant (p = 0.0001): affected concordant twins showed a significantly lower P300 amplitude as compared to affected discordant (p = 0.005, Cohen’s d = 1.08) and control twins (p = 0.000, d = 1.16). Discordant affected and unaffected twins did not differ significantly from each other or from control twins. P300 did not correlate significantly with the BPRS and the affected groups did not differ across the GAS. Conclusions: Our results provide evidence for etiological heterogeneity within schizophrenia pointing to different pathophysiological mechanisms that may underlie more and less genetically determined forms of schizophrenia. They also indicate that P300 correlates with a differing degree of genetic liability to schizophrenia independently of the psychopathological status and even in the presence of similar functional profiles.

S.B. and M.W. contributed equally to the manuscript.
Introduction

The question of whether schizophrenia is a heterogeneous disorder has been much debated during the last century ever since the concept of dementia praecox was put forward by Kraepelin. Various approaches have been employed to delineate subtypes of schizophrenia based on clinical symptoms, factor analysis, genetic markers and cognitive dysfunction [1, 2]; however, the jury is still out on the issue.

Schizophrenia is known to result from the combined influence of genetic and environmental factors [3]. Therefore, one important etiological distinction can be based on differential effects of genes and environment on an individual, leading to subpopulations with more or less genetic or environmental influences. In the past, such subpopulations have been defined as familial (genetic) and sporadic (environmental) subtypes and have been distinguished on the basis of the presence or absence of family members with schizophrenia [4]. However, this important approach has several limitations [1, 5]; a major one being that the number of family members affected by the illness is confounded by the overall family size, especially in small families. An alternative approach could be the comparison of monozygotic twins concordant and discordant for schizophrenia [6]. Monozygotic twins share 100% of their genes and differences in phenotypic traits can be attributed to differential exposure to environmental factors. Previous twin studies have shown substantial heritability for liability to schizophrenia, that is to say, the variance in liability to schizophrenia is explained to a large extent by genetic factors [7, 8]. Therefore, monozygotic twins discordant for schizophrenia might have a lower genetic liability to schizophrenia (with environmental factors playing a greater role in the development of schizophrenia) reducing the chances of both twins developing schizophrenia as compared to monozygotic twins concordant for schizophrenia who may have a higher genetic liability increasing the chances of both twins developing schizophrenia. In other words, monozygotic twins that are discordant for schizophrenia are on average more likely to express a more environmentally determined form of schizophrenia as compared to twins concordant for schizophrenia that are more likely to express a relatively predominant genetic form of schizophrenia. This approach has yielded mixed results in the past studies that have compared discordant and concordant twins on the clinical profile and familial incidence of schizophrenia [6]. However, comparison of markers (endophenotypes) that have recently been associated with genetic liability to schizophrenia between twins concordant and discordant for schizophrenia can help verify the validity of this approach and aid the identification of markers that can be used to distinguish predominantly genetic or environmental subtypes of schizophrenia within non-twin clinical populations. It can further throw light on different pathophysiological mechanisms that may be operating in the different subtypes.

One such robust candidate marker for schizophrenia is the P300 amplitude. P300 is a positive event-related potential, observed in the electroencephalogram (EEG) over centroparietal scalp sites around 300 ms after the presentation of a novel stimulus. Twin and family studies in healthy subjects point to a high level of heritability of the P300 amplitude [9–13]. Reduced P300 amplitude has been found associated with schizophrenia in various studies [14–17] and family studies have postulated reduced P300 amplitude as a marker for genetic liability to schizophrenia [18–20] as it is present in unaffected family members of schizophrenia patients. However, none of these family studies took into account the number of affected family members (an approach which itself is confounded by the absolute family size) and to our knowledge, no study has looked at P300 in groups with possible differing degree of genetic predisposition to schizophrenia.

Therefore, in order to examine whether P300 could be a useful marker in indicating differential genetic liability to schizophrenia, we compared P300 amplitude across a sample of monozygotic twins concordant and discordant for schizophrenia as well as healthy concordant monozygotic twins. So far, only two previous studies have employed a twin design to look at P300 amplitudes in schizophrenia [21, 22], but neither examined the differences between affected discordant and concordant twin pairs. Although Weisbrod et al. [22] employed a sample overlapping with the current study (which, however, includes additional twin pairs), they grouped the discordant and the concordant affected twins together and did not compare P300 amplitudes between the two groups. Hall et al. [21] also did not statistically compare the P300 amplitudes between the discordant and concordant groups and their comparison control group included both monozygotic and dizygotic twins. To our knowledge, the present study is the first to examine systematically P300 amplitudes between monozygotic twins concordant and discordant (both affected and unaffected) for schizophrenia. Given the reported association of P300 with genetic susceptibility to schizophrenia, we expected to find differences between these groups of twin pairs that may carry different genetic liabilities to schizophrenia.
Assessment Scale (GAS) functioning level of the patients was measured using the Global Inventory diagnosed by DNA microsatellite analysis had a history of neurological disorder or head injury. Zygosity was family history research diagnostic criteria. None of the subjects had no personal or family history of mental illness, based on the through newspaper advertisements in the Heidelberg area. They recruiting antidepressants at the time of the study. One discordant non-469 mg/day of chlorpromazine. In addition, 3 patients were taking neuroleptic treatment with a mean dose equivalent of patients (5 discordant affected and 8 concordant affected) were in table 1. At the time of the P300 recordings, 13 [26]
test, the Mehrfachwahl-Wortschatz-Test, Version B (MWT-B) intelligence) was measured using the German language vocabulary [22]. Affected twins were identified from the re-ords of different Psychiatric Departments. ICD-10 diagnoses were established in all subjects by the Schedules for Clinical Assessment in Neuropsychiatry [23]. Affected twins of the discordant pairs fulfilled the criteria for a diagnosis of the F2 (schizo-phenia, schizotypal and delusional disorders) category of ICD-10 whereas the co-twin did not. However, 2 of the co-twins fulfilled the criteria F32.10 for moderate depressive episode without so-matic symptoms and 1 fulfilled the criteria F31.7 for remitted bi-polar disorder. The actual psychopathological status was assessed using the Brief Psychiatric Rating Scale (BPRS) [24]. The overall functioning level of the patients was measured using the Global Assessment Scale (GAS) [25] and premorbid IQ (crystallized in-telligence) was measured using the German language vocabulary test, the Mehrfachwahl-Wortschatz-Test, Version B (MWT-B) [26]. Handedness was measured using the Edinburgh Handedness Inventory [27]. Clinical characteristics of the subject population are given in table 1. At the time of the P300 recordings, 13 patients (5 discordant affected and 8 concordant affected) were receiving neuroleptic treatment with a mean dose equivalent of 469 mg/day of chlorpromazine. In addition, 3 patients were taking antidepressants at the time of the study. One discordant non-schizophrenic twin was taking lithium. Controls were recruited through newspaper advertisements in the Heidelberg area. They had no personal or family history of mental illness, based on the family history research diagnostic criteria. None of the subjects had a history of neurological disorder or head injury. Zygosity was diagnosed by DNA microsatellite analysis [28]. After a complete

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics</th>
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<tr>
<td>Group</td>
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<tr>
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<tr>
<td>SCAN</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Gender, F/M</td>
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<tr>
<td>Handedness</td>
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<tr>
<td>Schooling, years</td>
</tr>
<tr>
<td>MWT-B (raw score)</td>
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<tr>
<td>BPRS</td>
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<tr>
<td>GAS</td>
</tr>
</tbody>
</table>

SCAN = Schedules for Clinical Assessment in Neuropsychiatry; R = right; L = left; AMBI = ambidextrous. Figures are means ± standard deviation, unless indicated otherwise.

**Subjects and Methods**

**Subjects**
Thirty-six monozygotic twin pairs entered the study – 6 concordant pairs with both twins affected by schizophrenia/schizoaffective disorder, 11 discordant pairs where only 1 twin was affected by schizophrenia/schizoaffective disorder and 19 concordant control pairs where both twins were healthy. Out of these, 5 discordant affected twin pairs, 8 discordant twin pairs and 9 concordant control pairs overlapped with the sample of the study of Weisbrod et al. [22]. Affected twins were identified from the records of different Psychiatric Departments. ICD-10 diagnoses were established in all subjects by the Schedules for Clinical Assessment in Neuropsychiatry [23]. Affected twins of the discordant pairs fulfilled the criteria for a diagnosis of the F2 (schizophrenia, schizotypal and delusional disorders) category of ICD-10 whereas the co-twin did not. However, 2 of the co-twins fulfilled the criteria F32.10 for moderate depressive episode without somatic symptoms and 1 fulfilled the criteria F31.7 for remitted bipolar disorder. The actual psychopathological status was assessed using the Brief Psychiatric Rating Scale (BPRS) [24]. The overall functioning level of the patients was measured using the Global Assessment Scale (GAS) [25] and premorbid IQ (crystallized intelligence) was measured using the German language vocabulary test, the Mehrfachwahl-Wortschatz-Test, Version B (MWT-B) [26]. Handedness was measured using the Edinburgh Handedness Inventory [27]. Clinical characteristics of the subject population are given in table 1. At the time of the P300 recordings, 13 patients (5 discordant affected and 8 concordant affected) were receiving neuroleptic treatment with a mean dose equivalent of 469 mg/day of chlorpromazine. In addition, 3 patients were taking antidepressants at the time of the study. One discordant non-schizophrenic twin was taking lithium. Controls were recruited through newspaper advertisements in the Heidelberg area. They had no personal or family history of mental illness, based on the family history research diagnostic criteria. None of the subjects had a history of neurological disorder or head injury. Zygosity was diagnosed by DNA microsatellite analysis [28]. After a complete description of the study, subjects provided written informed consent and were paid for participation.

**Task**
Auditory stimuli were presented by Etymotic Research ER-3 insert earphones. Thirty infrequent high-pitched tones were presented, pseudorandomly interspersed between 120 frequent low-pitched tones. Tones were 40 ms in duration, including 10 ms rise and fall time, with 97 dB SPL loudness. The interstimulus interval was 1 s. Subjects were instructed to close their eyes and count silently the number of the rare high-pitched tones. Disparity in pitch between the frequent and the infrequent tone was adjusted to the subject’s ability to discriminate tones. For this, subjects were presented with sequences of tones before the actual experiment. The tone sequences consisted of a 1,000-Hz tone and either a second 1,000-Hz tone (in 50% of the trials) or 1 of 3 different pitched tones. Tones were 40 ms in duration, including 10 ms rise and fall time, with 97 dB SPL loudness. The interstimulus interval was 1 s. Subjects were instructed to close their eyes and count silently the number of the rare high-pitched tones. Disparity in pitch between the frequent and the infrequent tone was adjusted to the subject’s ability to discriminate tones. For this, subjects were presented with sequences of tones before the actual experiment. The tone sequences consisted of a 1,000-Hz tone and either a second 1,000-Hz tone (in 50% of the trials) or 1 of 3 different (1,030 Hz, 1,050 Hz, and 1,100 Hz) tones. Subjects were asked to discriminate the target tone reliably.

**EEG Data**
Evoked potentials were recorded from 20 sintered Ag/Ag-Cl electrodes positioned according to the international 10-20 system. Linked mastoids were used as reference and FPz as ground. Vertical EOG was recorded with supra- and infraorbital electrodes. Electrodes on the external canthi recorded horizontal EOG. Electrical EOG was recorded with supra- and infraorbital electrodes. Linked mastoids were used as reference and FPz as ground. Vertical EOG was recorded with supra- and infraorbital electrodes. Electrodes on the external canthi recorded horizontal EOG. Electrode impedance was maintained below 5 kΩ for all recordings. EEG was continuously recorded (low-pass filter 70 Hz, A/D rate 400 Hz) and processed off-line. Continuous EEG was segmented in 1,200-ms epochs and a low-pass filter of 16 Hz (24 dB/octave) was applied. Correction for ocular artifacts was performed using regression-based weighting coefficients [29]. Individual epochs
were inspected visually by an experienced person and segments containing artifacts were excluded from further analysis. At least 16 artifact-free epochs entered the averages of each subject and a prestimulus interval of 200 ms was used for baseline correction. Event-related potentials were averaged for the infrequent targets for each subject and P300 was measured as the mean voltage between 300 and 500 ms after stimulus at the Pz electrode.

### Statistical Analysis

Statistical analysis was performed using STATA 10 (STATA Corp., College Station, Tex., USA). The 'survey' option in STATA allows for nonindependent observations and controls for the similarity between twins of a pair by calculating a robust sandwich estimator to estimate standard errors. The relatedness between twins of a pair (cluster correlation) violates the independence observation assumption in the analysis of variance, and the sandwich estimator corrects for this bias and provides robust estimates of 95% confidence intervals, standard errors and p values [30, 31].

A multiple regression model was tested which included P300 amplitude as the dependent variable and group (concordant affected, discordant affected, discordant unaffected, and concordant healthy) as the predictor variable and age, gender, birth order and premorbid IQ (MWT-B raw score) as covariates. Two twin pairs (1 discordant pair, 1 healthy control pair) were excluded from the regression analysis because of missing data for birth order, so finally 10 discordant and 18 concordant healthy pairs entered this analysis along with 6 concordant affected pairs. The variable ‘group’, which is a 4-level categorical variable, was coded into 3 dummy variables (each reflecting the presence or absence of a particular group with the healthy concordant group as the reference group) and the regression tested the significance of individual dummy variables with reference to the healthy concordant group. Comparisons with other groups as reference were also made using the same regression. Since there were 4 groups and therefore, 6 comparisons were made, the corrected α level was calculated using the Bonferroni correction for multiple comparisons. Effect sizes (Cohen’s d) for individual group comparisons were also calculated [32]. In order to test for the collective effect of all groups, the Wald test was used. To examine if the obtained results were due to the effect of medication, the same model was run after excluding the patients taking medication at the time of testing. The groups were also compared with respect to the healthy concordant group on the number of errors using regression.

To further examine whether the group differences that may be seen in P300 reflected trait differences in genetic liability or were correlated with the clinical state of the patients, we looked at the correlation between P300 and BPRS scores in the affected twins (both concordant and discordant affected). The BPRS evaluates the psychopathological status of the patients [24] and therefore, a nonsignificant correlation of P300 with the BPRS score would imply a nondependence of P300 amplitude on the clinical state of the patients and add reliability to the use of P300 as a trait marker.

In addition, we examined if the concordant and discordant affected twins showed any differences in their overall functioning level by comparing the two groups on GAS scores using a t test. The GAS measures the overall functioning of patients including social, occupational and psychological functioning and has been associated with future prognosis [25]. Hence, a significant difference would imply that the liability differences in the two affected groups are manifested at a functional and prognostic level.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>t statistic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant affected</td>
<td>-4.70</td>
<td>1.1</td>
<td>-4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Discordant unaffected</td>
<td>-1.82</td>
<td>1.4</td>
<td>-1.3</td>
<td>0.20</td>
</tr>
<tr>
<td>Discordant affected</td>
<td>-2.39</td>
<td>1.5</td>
<td>-1.6</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Covariates</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.07</td>
<td>0.05</td>
<td>-1.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Gender</td>
<td>0.11</td>
<td>1.2</td>
<td>0.1</td>
<td>0.90</td>
</tr>
<tr>
<td>Birth order</td>
<td>-1.61</td>
<td>0.9</td>
<td>-1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>0.08</td>
<td>0.1</td>
<td>0.8</td>
<td>0.40</td>
</tr>
<tr>
<td>Constant</td>
<td>11.85</td>
<td>3.8</td>
<td>3.1</td>
<td>0.004</td>
</tr>
</tbody>
</table>

The main effect of group (ascertained by Wald test) after controlling for the effect of covariates was F(3, 31) = 9.5, p = 0.0001.

### Results

#### Performance across Groups

A comparison of the number of errors across the 4 groups yielded a trend for the main effect of group [F(3, 33) = 2.5, p = 0.08] with concordant affected twins making significantly more errors than healthy controls (p = 0.01), while both discordant groups did not differ significantly from the controls.

#### P300 Difference across Groups Reflecting Different Levels of Genetic Liability

Multiple regression which included P300 amplitude as the dependent variable and group as the predictor variable (age, gender, birth order and premorbid IQ as covariates) yielded a highly significant model [F(7, 27) = 4.0, p = 0.004], though the explained variance was limited (R$^2$ = 0.21). Regression coefficients and the constant for the model are given in table 2. The overall effect of group after controlling for the covariates still turned out to be highly significant [F(3, 31) = 9.5, p = 0.0001]. Among the covariates, only age (p = 0.1) and birth order (p = 0.09) showed a trend for significance; t statistic, p values and effect sizes for the comparison of different groups after controlling for the effect of covariates are reported in table 3 along with the P300 mean values and standard deviations for all groups. Figure 1 shows the event-related waveform at the Pz electrode for the 4 groups and figure 2
illustrates the distribution of P300 amplitude for all groups. Concordant affected twins had the lowest P300 amplitudes, which were significantly lower than those of the discordant affected twins and the control twins and showed a trend for being lower than those of the discordant unaffected twins. Discordant affected and unaffected twins did not differ significantly from each other, nor did they differ significantly from the control twins, although unaffected discordant twins showed a trend for having lower P300 amplitudes than the control twins. However, for the corrected α level (0.008), the trend for a difference between discordant unaffected and concordant affected, and the trend for a difference between discordant unaffected and control twins disappeared (Table 3).

**Effect of Medication**

The regression model after excluding the patients taking medication yielded a significant main effect of group \(F(3, 27) = 4.7, p = 0.008\) with a statistical trend for a difference in the P300 amplitude between concordant and discordant affected twins \(p = 0.09\).
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State-Dependent Influences on P300 Amplitude in the Affected Twins

The BPRS and P300 amplitude did not show a significant correlation in the affected twins (standardized $\beta = -0.16$, $p = 0.4$) indicating that the group differences in P300 were mostly independent of the psychotic symptomatology and rather reflected trait differences. Mean BPRS scores are given in table 1.

Differences in the Functional Profiles between the Concordant and Discordant Affected Groups

The t test revealed that there were no significant differences in the GAS scores between the concordant and discordant affected groups [$t(16) = 0.7$, $p = 0.5$] indicating that the two groups had a similar overall level of functioning/disease severity. Mean GAS scores are given in table 1.

Discussion

This study compared P300 amplitudes across a sample of monozygotic twins concordant and discordant for schizophrenia as well as concordant healthy control twins and found significant differences between the groups. Twins concordant for schizophrenia had significantly lower P300 amplitudes as compared to the affected discordant twins as well as control pairs. Discordant affected and unaffected twins did not differ significantly from each other or from the control twins. Our results provide evidence for the assumption of a differing degree of genetic predisposition to schizophrenia in concordant and discordant twins and indicate that P300 amplitude correlates with genetic liability to schizophrenia, pointing to the etiological heterogeneity within schizophrenia with different mechanisms (genetic and/or environmental) that may operate in different subgroups to bring about the broad symptomatology observed in schizophrenia.

The pattern of P300 amplitude across groups did not change (except for a loss of power) after removing the medicated patients from the analysis. The result that P300 amplitude did not correlate with BPRS scores in the affected twins further verified that the group differences in P300 amplitude were not due to the psychopathological status of the patients and rather reflected underlying liability. GAS scores did not differ between the concordant and discordant affected twins indicating that P300 amplitude distinguished groups with a differing extent of genetic liability even in the presence of similar functional profiles. However, as the variance in P300 amplitude explained by the regression model was limited, it showed that the division of the two subtypes into the concordant and the discordant groups may not be very precise but the results still held true on average.

The pattern of the P300 amplitude across groups seen in our sample – concordant affected having the lowest P300 amplitudes followed by discordant twins and followed by concordant healthy twins – is consistent with the reverse order of genetic liability to schizophrenia in these groups. Even though the trend for a difference between concordant affected and discordant unaffected and between discordant unaffected and discordant healthy twins disappeared after the Bonferroni correction, given that only 6 comparisons were made, the correction for multiple comparisons may have been over-conservative [33] and therefore, the significance of the uncorrected $p$ values for these comparisons cannot be completely discarded. Also, other group differences (lower P300 amplitudes in discordant affected as compared to discordant affected and healthy control twins) which

<table>
<thead>
<tr>
<th>Reference group</th>
<th>P300 amplitudes (mean ± SD, $\mu$V)</th>
<th>Discordant affected</th>
<th>Discordant unaffected</th>
<th>Concordant healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant affected</td>
<td>4.8 ± 3.0</td>
<td>2.9</td>
<td>0.005</td>
<td>1.08</td>
</tr>
<tr>
<td>Discordant affected</td>
<td>6.7 ± 4.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Discordant unaffected</td>
<td>8.0 ± 4.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Concordant healthy</td>
<td>9.8 ± 5.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</table>

The t values were generated by the regression model by comparison of P300 means across different groups after controlling for the effects of covariates, which is equivalent to performing an ANCOVA. The uncorrected $\alpha$ level was 0.05 and the Bonferroni corrected $\alpha$ level was 0.008.
remained significant even after the Bonferroni correction implied that the interpretation of the P300 amplitude as an index of differing degree of genetic liability to schizophrenia still held true.

Considering the two studies which have looked at P300 amplitudes in twin samples [21, 22], our results, wherever comparable, are mostly consistent with these studies. Weisbrod et al. [22], who employed a sample partially overlapping with the current study, found lower P300 amplitudes in discordant unaffected as compared to the control twins. We obtained a trend in the same direction for the uncorrected \( \alpha \) level, which indicated that the discordant group had P300 values somewhat intermediate between concordant affected and control twins (even though the difference did not reach significance) and is consistent with our model in that the discordant group may carry some genetic liability to schizophrenia albeit to a lesser extent than the concordant affected group. They also reported nonsignificant within-pair differences in the discordant pairs which are in line with our results. However, the difference between their affected group and healthy controls is not directly comparable to our results as they grouped the discordant and the concordant affected twins together and did not compare the differences between these two groups. Hall et al. [21] also found significantly lower P300 amplitudes in the discordant (affected and unaffected) and concordant affected twins as compared to control twins but did not statistically compare P300 amplitudes between discordant and concordant affected groups. However, in their raw data, concordant affected and discordant twins showed overall similar P300 amplitudes (as opposed to lower P300 amplitudes in concordant affected than discordant twins in the current study) and further investigations will be required to clarify this inconsistency.

In the past, the association of P300 amplitude with genetic liability to schizophrenia has been mostly investigated in family studies and although many studies found a positive association [18–20], some studies failed to reproduce this association [18, 34]. Our results may explain this inconsistency as families investigated in these studies were ascertained by identifying the patients and did not take into consideration the number of ill family members and overall family size and therefore, may represent a spectrum of genetic liabilities to schizophrenia with some families containing more and some less genetically determined forms of schizophrenia. In our approach, the recruitment procedure might have the opposite effect of enriching the more genetic forms in concordant affected twins and less genetic forms in discordant affected twins.

Our results also fit in well with the results of studies that have looked at schizophrenia classified according to Leonhard’s [35] nosology, a system which classifies psychoses into various etiologically distinct classes. A twin study that used Leonhard’s classification to compare concordance rates in monozygotic and dizygotic twins for each class of psychoses found much lower genetic load for cycloid psychoses and systematic schizophrenias as compared to unsystematic schizophrenia [36]. Another study found no reduction in P300 amplitudes in patients with cycloid psychoses as compared to healthy controls [37]. These results indicated that P300 amplitudes were spared in psychoses with lower genetic loadings and our results are consistent with this indication. A more rigorous validation of this assertion would call for a further study where twin pairs concordant and discordant for schizophrenia are classified according to Leonhard’s system and compared for P300 amplitudes.

Given that we obtained a significant main effect of group on P300 amplitude even after excluding the medicated patients and that there was a trend for a difference in P300 amplitude between concordant and discordant affected twins, this indicated that the pattern of obtained results was not likely influenced significantly by medication effects. That the latter result was only a trend could be explained by a significant decrease in power after excluding medicated twins from the model. Moreover, previous literature has reported that temporoparietal P300 amplitude is not significantly normalized by antipsychotic medication [38, 39].

A lack of correlation between BPRS and P300 amplitude in affected twins further verified the validity of P300 amplitude as a trait marker for schizophrenia. Many studies have shown P300 amplitude, especially the temporoparietal P300b subcomponent, to be independent of the clinical state and reflect trait liability to schizophrenia [16, 40, 41]. However, some state-dependent influences have also been reported [42, 43]. Although in the present study, P300 measured at the Pz electrode corresponds to P300b and our results are consistent with the independence of P300 amplitude from symptomatology, given that the concordant ill twins showed numerically higher BPRS mean scores as compared to discordant ill twins (table 1), some state effects cannot be completely ruled out.

Similarly, although we found no statistically significant differences between concordant and discordant affected twins in the GAS scores, the concordant affected twins had a numerically lower mean score than the discordant affected twins (table 1). Therefore, our results do
not imply that there cannot be any differences manifested at a clinical and functional level in the different subgroups, but another scale or a study with more power may tap these differences better.

One limitation of the study could be that the concordant affected group made more errors than the healthy control group while the discordant groups did not differ from the control group. A similar pattern obtained for the P300 amplitude across groups (lowest P300 amplitude in concordant affected group) could have been confounded by the number of incorrect trials (which could not be controlled for because it was a passive task and the subjects only reported the results at the end of the block). However, previous literature has shown that P300 amplitude on the preceding trials predicts error propensity on the subsequent trials [44] and a likely relation between P300 and errors should not affect the utility of P300 as a marker of genetic liability to schizophrenia.

Our results point towards etiological heterogeneity in schizophrenia indexed by P300 amplitude based on differential influences of genetic and environmental factors. Since P300 is associated with cognitive efficiency [45], lower P300 amplitudes may imply greater cognitive deficits in patients with more genetically determined forms of schizophrenia which in turn may imply a different underlying pathophysiology as compared to patients with more environmentally determined forms of schizophrenia. This interpretation is consistent with studies that have reported a greater degree of cognitive impairments in patients with a family history of schizophrenia compared to patients without a family history [46, 47]. Future studies should examine concordance together with familial incidence in schizophrenia and take into account a range of cognitive measures along with P300 to be able to better address this issue.

One point to be noted is that during the recordings, the described auditory oddball task was presented along with a simpler task (using 1,000 Hz as frequent and 1,500 Hz as infrequent stimuli). However, since we obtained a similar pattern of results in both the easy and difficult tasks for the current comparisons, we report in this paper the results only from the difficult task. Another point to be noted is that one discordant nonschizophrenic twin diagnosed with remitted bipolar disorder was included in the analysis and since there are postulated genetic overlaps between schizophrenia and bipolar disorder [48], inclusion of this twin pair may be deemed problematic. However, once again, the pattern of our results did not change even after excluding the bipolar twin from the analysis and therefore, we retained it in the present study.

To conclude, the evidence presented in this study has important implications towards identifying etiologically distinct forms of schizophrenia based on differential influences of genes and environment, and provides preliminary indications towards different pathophysiological mechanisms that may underlie the different subtypes.

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References


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Psychopathology 2011;44:398–406
16 Blackwood D: P300, a state and a trait marker in schizophrenia. Lancet 2000;355:771–772.