Genetic Association between Reduced P300 Amplitude and the DRD2 Dopamine Receptor A1 Allele in Children at High Risk for Alcoholism

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**Background:** There is evidence that both reduction in P300 amplitude and the presence of the A1 allele are risk markers for alcoholism. We hypothesized that demonstration of a relationship between the marker and the trait in young children who had not begun to drink regularly would provide evidence for dopaminergic mediation of the reduction in P300 often seen among high-risk children. A previous association between the A1 and the P300 amplitude in screened controls supports the hypothesis that this association occurs in the general population.

**Methods:** Children were assessed using both visual and auditory paradigms to elicit event-related potentials (ERPs). The P300 component of the ERP was investigated with respect to the genetic variation of the Taq1A D2 receptor in these children.

**Results:** Genetic association between a marker locus (Taq1A RFLP near the D2 receptor locus) and the amplitude of P300 was found to be present in 58 high-risk children and their relatives (a total of 100 high-risk individuals).

**Conclusions:** A higher proportion of children from alcoholic families may exhibit lower P300 because more of these children carry the A1 allele than is seen in the normal population. Biol Psychiatry 1998;43: 40–51 © 1998 Society of Biological Psychiatry

**Key Words:** P300, DRD2, high-risk, alcoholism, biological markers, dopamine

**Introduction**

**P300 Amplitude Reduction: A Risk Factor for Alcohol Dependence or a Toxic Effect?**

There is long-standing and extensive literature discussing the cognitive impairment seen in chronic alcoholics, starting with the classic work of Victor et al (1971), who described the neuropathological changes seen on autopsy. These subcortical changes were correlated with both memory and attentional deficits. The notion that cognitive changes, as reflected in scores on neuropsychological tests administered to alcoholics, might be a pre-morbid condition associated with the etiology of alcohol dependence rather than simply the consequences of alcohol use was conceptually quite appealing (Goodwin and Hill 1975).

A similar question arises with respect to P300 amplitude reduction when observed in adult alcoholics. Was the P300 reduction present before the alcoholic individual began to drink? Thus, the P300 component of the event-related brain potential (ERP) has been evaluated as a possible biological risk marker for the development of alcoholism in numerous studies involving not only adult alcoholics (Pfefferbaum et al 1979) but also utilizing high-risk children (Begleiter et al 1984; Hill et al 1987) for over a decade now.

**Developmental Changes in P300**

The P300 is a scalp positive wave that occurs approximately 300 msec after an informative stimulus occurs (Sutton et al 1965). Because the P300 component of the ERP is an electrophysiological index of an individual's capacity to process stimulus information, both P300 amplitude and latency have been studied in samples thought to vary on some neurocognitive, behavioral, or maturational dimension. For example, P300 amplitude and latency vary with the developmental stages of childhood (Howard and Polich 1985). This variation appears to be due to differing rates of maturation of the auditory and visual modalities during childhood (Courchesne 1984; Hill and Steinhauser 1993a; Steinhauser and Hill 1993). The varying rates of maturation by modality may be due to the possibility that the neural generators of P300 could differ by modality (Johnson 1989a, 1989b). Also, there is a substantive literature demonstrating a relationship between increased latency
of P300 (Neshige et al 1988; Papanicolaou et al 1984; Polich 1989, 1991) and neuropathological states such as Alzheimer's disease, closed head injury, and other organic brain syndromes.

*Amplitude versus Latency*

In contrast to the increased latency seen in association with environmental exposures or organic disease states, decrements in P300 amplitude are more often associated with the presence of psychiatric disorders, as for example when schizophrenic patients are evaluated (Steinhauer and Zubin 1982; Steinhauer et al 1991; Pfefferbaum et al 1989), or when individuals without evidence of any psychiatric disorder carry an especially high loading for a psychiatric disorder, particularly alcoholism (Begleiter et al 1984; Hill et al 1987, 1995a; Hill and Steinhauer 1993a; Steinhauer et al 1987). Thus, it is possible that the amplitude of the P300 component may be an inherent characteristic of individuals before they develop psychiatric states that may be related to their vulnerability for incurring these disorders (Hill et al 1987; Hill 1994).

*Evidence for Heritability of P300*

Elucidating the factors responsible for variations in P300, particularly those that might be heritable, is of interest given the observed association between P300 and psychopathological conditions. There is a considerable body of evidence suggesting that brain neuroelectrical activity, whether background electroencephalograms (Vogel et al 1979; Young et al 1972; Propping et al 1980; Lykken et al 1974), averaged sensory evoked responses (Buchsbaum 1974; Rust 1975), or ERP's (Steinhauer et al 1987; Bock 1976; Surwillo 1980; O'Connor et al 1994; van Beijsterveldt 1996), is heritable.

A number of studies have reported that when ERP waveforms of two individuals are compared, greater concordance is observed between first-degree relatives than unrelated individuals, with the greatest similarity observed in monozygotic (MZ) twins (Steinhauer et al 1987; Bock 1976; Surwillo 1980; O'Connor et al 1994; van Beijsterveldt 1996). In addition to the waveform similarity seen in MZ twins, P300 amplitude is highly correlated in twin pairs (between .5 and .9). A high degree of similarity in siblings has also been demonstrated, with correlations ranging between .61 and .82, depending on the task used to elicit the P300 (Steinhauer et al 1987). ERP data from a large family study of alcoholism has been analyzed using segregation analysis to determine possible modes of inheritance of the P300 component, with evidence presented for a major gene controlling the familial similarity in P300 amplitude (Aston and Hill 1990).

*P300 in Adult Alcoholics and High-Risk Children*

ERP differences, especially in the amplitude of the P300 component, have been reported for male alcoholics when compared with control subjects (Porjesz et al 1987a, 1987b; Pfefferbaum et al 1991), though differences have not been found in all studies (Pfefferbaum et al 1979; Hill et al 1987, 1995b; Lille et al 1987; Hermanutz et al 1981). On the other hand, adult female alcoholics show profound reductions in P300 amplitude in comparison to age-matched normal controls (Hill and Steinhauer 1993b).

Thus, it could be the case that the differences observed between high- and low-risk children for P300 amplitude may well normalize by adulthood for male in contrast to female subjects. At any rate, it is clear that a number of laboratories now have been able to document differences in P300 characteristics between high- and low-risk children (Begleiter et al 1984; Hill and Steinhauer 1993a; Steinhauer and Hill 1993; Hill et al 1990, 1995a; Whipple et al 1988; Berman et al 1993).

*The Dopaminergic System and Alcoholism*

The initial report of a population-based association between alcoholism and the dopamine D2 receptor locus (DRD2) (Blum et al 1990) continues to be challenged by a number of groups (Suarez et al 1994). Nevertheless, the fact that a number of independent investigations find evidence for a population-based association would appear to validate the need for further study dopaminergic allelic variation as it relates to alcoholism vulnerability. Recently, we have pointed out that the nature of the control group, whether screened for alcoholism and other psychiatric disorders or not, will determine whether significant population-based associations are found (Neiswanger et al 1995; Hill and Neiswanger 1997).

*Goal of the Present Study*

Previously we reported a population-based association for alcoholism and the Taq1 A1 allele (Neiswanger et al 1995). Because we have repeatedly seen reductions in P300 amplitude in minor children from these high-density alcoholism families, it was of interest to determine if dopaminergic mediation might explain this variation. Thus, we performed an association study within the high-risk group, contrasting the children who carry the A1 allele with those who do not.

*Methods and Materials*

Subjects

RECRUITMENT OF FAMILIES. A total of 58 high-risk children (33 girls and 25 boys) with a mean age of 11.31 ± 3.0 (SD) years
participated in the study. To reduce possible heterogeneity due to ethnic variation, only Caucasian children were studied. The children were considered high-risk because they came from families with multigenerational alcoholism. Children from 32 families were assessed. The A1 group of children consisted of 11 boys and 14 girls, whereas the A2 group contained 14 boys and 19 girls [A1 children were typed as either 11 (1 case), or 12; whereas A2 children were 22]. Among the 58 children were children who had siblings or cousins also in the data set. Therefore, the data analysis included 100 relatives in all, in order that linking parents and grandparents could be included in the data file. Inclusion of other members of the pedigree (linking parents and grandparents) from which the child arose provides a situation comparable to having performed the analysis on 58 children from 58 independent families as provided by an association program ASSOC (Statistical Algorithm for Genetic Epidemiology—SAGE) (S.A.G.E. 1994).

The high-risk families were part of two larger studies of alcoholism, one concerning families of female alcoholic probands (The Biological Risk Factors Family Study—BRFFS) and the other involving families of male alcoholic probands (Cognitive and Personality Factors Family Study—CPFFS). These studies originally were designed to include direct interview of all available first-degree relatives to determine both clinical status and variation on a number of neurobiological indicators of risk. These high-risk "target" families had been ascertained through a proband set comprised of two alcoholic sisters (BRFFS) or two alcoholic brothers (CPFFS), one of whom was in treatment for alcoholism at the time the family was ascertained. The presence or absence of alcoholism or other psychopathology was determined for the proband and all available first-degree relatives through face-to-face interviews (Diagnostic Interview Schedule—DIS), allowing for DSM-III and Feighner Research Diagnostic Criteria (Feighner et al 1972) to be applied. In some cases in which in-person interview information was not available, two consistent reports of the relatives' status was required to assign a diagnosis. Because our study design included obtaining family history information from all relatives interviewed, multiple reports on all members of the child's family, both first- and second-degree members, were available. Thus a valid family history diagnosis could be correctly assigned.

CHILD/adolescent ALCOHOL USE: K-SADS and AAS DETERMINATIONS. Drinking was determined by utilizing both self report data (Mayer and Filstead 1979) (Adolescent Alcohol Involvement Scale—AAS) and a clinician-administered interview format (Schedule for Affective Disorders and Schizophrenia for School-Aged Children—K-SADS) (Chambers et al 1985; Orvaschel et al 1982) designed to determine the presence of childhood psychiatric diagnoses. The interview format (K-SADS-P) covered the past year and included portions of the lifetime version of the instrument (K-SADS-E) that covered alcohol and drug use problems. Responses to questions from this interview were examined to establish convergent validity for the self-report data to be analyzed.

Utilizing information from both instruments, we determined that 11 children of the 58 had begun drinking, though minimally, at the time of the P300 assessment. Of the 11 children who drank, 3 had drunk only once or twice ever (average quantity of 1.0 drink), 6 drank less than 1 time per month (average of 2.2 drinks), and 2 drank more frequently than once a month but less often than once per week. Thus, the quantity and frequency was sufficiently small for the entire group of 11 children to conclude that any drinking that could have occurred would not have influenced the amplitude of the P300 measured.

PREGNANT DRINKING IN MOTHERS OF THESE OFFSPRING. Prenatal drinking in the mothers of the high-risk children was assessed using clinician-administered interviews. Information was sought concerning drinking during the first and subsequent trimesters of the pregnancy. This information was validated using other information gathered routinely for both parents, including current and lifetime drug and alcohol use. Where discrepancies arose between the mother's time line for lifetime drinking and self-reported drinking during pregnancy, further information was sought to resolve differences and improve the accuracy of the report. To compare the drinking of mothers of children in the A1 and A2 groups, intake by first trimester and overall was calculated for each mother and averages by group determined. Mothers of high-risk children who carried the A1 allele reported drinking an average of 1.66 drinks/day during the first trimester (0.75 overall). Mothers of high-risk children who did not carry the A1 allele drank 1.46 drinks/day during the first trimester (0.67 overall). Thus, we found no evidence that the mothers of children in the two groups differed in the amount of alcohol consumed during pregnancy.

SEVERITY OF ALCOHOLISM IN THE FAMILY. Two measures of severity were calculated: family density of alcoholism, and age of onset to begin drinking. For the severity measure, the affection status of all known first- and second-degree relatives was determined (first-degree relatives were given a score of 1, whereas second degree relatives .5). The number of affected relatives out of the total was calculated using a weighted average that gave greater weight to first-degree than second-degree relatives. Second, the age of onset for alcoholism using Feighner Criteria (Orvaschel et al 1982) was determined. Two methods were used to calculate the age of onset for alcoholism in parents. By convention, most studies report age at the first problem. Accordingly, this was calculated using the first problem area. The age at which three problem areas could be diagnosed was also determined. (For children without an alcoholic parent, the mean age of onset of the parent's affected siblings was used.) Using the first problem area, parents of the children with the A1 allele had an onset of 17.5 ± 3.9 (SD) years, whereas those without the allele had parents whose first alcohol problem averaged 19.8 ± 6.2 (SD) years, a nonsignificant difference. Using three problem areas, the mean age of onset of alcoholism for parents of the children carrying the A1 allele was 24.1 years, whereas the onset for the parents of children who did not carry the A1 allele was 25.6 years, also a nonsignificant difference. The ratio of affected to total relatives was determined for children from the A1 allele group to be .50, while the average
ratio for the children without the A1 allele was 0.47, also a nonsignificant difference.

**A1/A2 Groups**

All 58 children were genotyped for the Taq1 A1/A2 allele using standard restriction fragment length polymorphism (RFLP) techniques. To include linking parents and grandparents in this analysis, a total of 100 persons were genotyped. The Taq1A is approximately 12 kb 3' to the coding region of the D2 receptor gene on chromosome 11 (q22–23). The Taq1 A is in strong linkage disequilibrium Taq1 B RFLP in the first intron of the D2 gene (Hauge et al 1991).

**Genotyping Methods**

The children and their parents and grandparents provided consent to withdraw blood by venipuncture. To minimize stress to the children, all of the samples obtained from children were drawn at Children’s Hospital (Pittsburgh, PA) where technicians are highly skilled in drawing blood from children. DNA was extracted from both immortalized cell lines and peripheral blood using minor modifications of the salting out method (Miller et al 1988). Genomic DNA (10 μg) was restricted overnight using a threefold excess of Taq1 restriction endonuclease, according to the manufacturer’s specifications. Samples were size fractionated by electrophoresis on 1.0% agarose gels and transferred overnight by capillary action (Southern 1975) to MSN nylon transfer membranes (Magna NT; Micron Separations, Inc.). Filters were prehybridized 2–4 hours at 42°C in 50% formamide, 5% Denhardt’s solution, and 450 μg/mL sheared salmon sperm DNA. The Taq1A probe used in these analyses was the 1.7 kb BamHI fragment of HD2G1 provided by Dr. David Grandy (Vollum Institute, Oregon Health Sciences University). The probe was radiolabeled by random priming (Feinberg and Vogelstein 1983) to a specific activity of > 10⁶ cpm/μg with [α-32P]deoxyctydine triphosphate. Hybridization reactions were carried out at 42°C for 24–72 hours in 50% formamide, 6× standard saline citrate (SSC), 1× Denhardt’s solution, 10% dextran sulfate, 0.5% sodium dodecyl sulfate, and 800 μg/mL sheared salmon sperm DNA. Filters were washed extensively at a final stringency of 0.1× SSC at 65°C and exposed to Kodak XAR-5 film at ~70°C for 3–7 days.

**Event-Related Potentials**

**AUDITORY PROCEDURE.** Each child performed an auditory task during which ERPs were recorded. Prior to testing, subjects were given an audioscope screening test of 20 dB hearing level at frequencies of 500, 1000, 2000, and 4000 Hz. Results indicated that hearing was not impaired in any of the subjects.

The experiment consisted of a Choice Reaction Time task (RT), which has been employed previously (Hill and Steinhauser 1993a, 1993b; Steinhauser et al 1987). This task is a modified version of the typical oddball paradigm. For the task, the subjects sat in a sound-attenuated, darkened room and listened to “high” (1500 Hz) and “low” pitched (800 Hz) tones, presented every 3 sec through a speaker placed in front of the subject. Prior to testing, subjects were required to identify “high” and “low” tones to ensure pitch differentiation. Tones were 40 msec in duration with an abrupt (2 msec) rise and fall time, at an intensity of 70 dBA (Edmont-Wilson Sound Level Meter, Model 60-510). High and low tones were randomly generated by computer so that the overall probability of a high (infrequent) tone would be .25. All subjects were told at the onset of testing that 1) the first tone they would hear on each block of trials would be a low tone; 2) there would be fewer high tones than low tones; and 3) two high tones would never occur in a row. To be sure that the task was understood, each subject was asked which tone would be heard after a high tone. All subjects responded correctly that a low tone would follow. Because we have demonstrated that the amplitude of the P300 is dependent on the conditional probability of two tones occurring in succession (Steinhauer et al 1987), we calculate the conditional probability of a high tone following a low tone to be .33. (Note that because the subjects are informed that two high tones cannot occur in succession, this results in 25% of the stimuli, all low tones, being predictable. Of the remaining 75% of the tones, all unpredictable, infrequent high tones have a conditional probability of 25%/75% = .33.) The .33 probability condition was chosen to perform our association analysis as this is the probability condition producing the maximal P300 response (Steinhauer and Hill 1993; Hill et al 1995a).

Subjects were asked to perform two blocks of 80 trials each. Subjects pressed one button when a high tone was heard and another button when a low tone occurred, alternating with each subject as to whether the left button first corresponded to a high tone or a low tone. On the second RT block, the subject was required to do the opposite. Responses were automatically encoded to determine accuracy. Each error-free block resulted in a reward of $0.25; $0.10 was given for each block with one or two errors (three errors—no reward). Blocks with six or more errors were excluded from the analysis. All trials performed incorrectly were also discarded.

**VISUAL PROCEDURE.** The visual event-related potential task employed was patterned after the procedure utilized by Begleiter et al (1984). Stimuli were generated by an Atari 130 computer for a 33 msec duration, with intertrial interval varying randomly between 2.25 and 4 sec. Stimuli were displayed on a Magnavox RGB Monitor 80, placed 132 cm from the subject, subtending a visual angle of 3.8°. The monitor was set to the default green mode, resembling the oscilloscope display used by Begleiter et al (1984). Five stimuli were randomly presented. One view, the nontarget stimulus, was a simple circle to which the subject was instructed not to respond (blank condition). There were four possible aerial views of target stimuli, a representation of a head with a nose and only one ear. The subject was instructed to press the button that corresponded to the depicted ear. The easy condition occurred when the nose was oriented upward and the ear (right or left) was on the same side as the button depressed. In the hard condition, the nose was oriented downward and the subject was required to spatially rotate the head to respond correctly. Thus, in the hard condition, the ear was depicted on the opposite side of the head as the button pressed. Because previous
Table 1. P300 Amplitude and Latency in Children from High-Risk Families (Means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>A1 (n = 25)</th>
<th>A2 (n = 33)</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$p$</th>
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<tbody>
<tr>
<td><strong>Choice reaction task</strong></td>
<td></td>
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</tr>
<tr>
<td>P300 amplitude (µV)</td>
<td>15.88 (8.2)</td>
<td>21.09 (9.6)</td>
<td>5.21</td>
<td>1</td>
<td>.0224</td>
</tr>
<tr>
<td>P300 latency (ms)</td>
<td>385.28 (73.1)</td>
<td>371.88 (67.2)</td>
<td>1.11</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td># Correct</td>
<td>77.40 (2.8)</td>
<td>77.40 (2.7)</td>
<td>ns</td>
<td></td>
<td></td>
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<tr>
<td><strong>Visual task</strong></td>
<td></td>
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<tr>
<td>P300 amplitude (µV)</td>
<td>27.27 (9.3)</td>
<td>34.86 (10.6)</td>
<td>10.93</td>
<td>1</td>
<td>.0009</td>
</tr>
<tr>
<td>P300 latency (ms)</td>
<td>519.04 (93.0)</td>
<td>511.03 (76.1)</td>
<td>0.65</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td># Correct</td>
<td>222.40 (24.9)</td>
<td>221.61 (21.4)</td>
<td>ns</td>
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</tbody>
</table>

Chi-square values were based on the log likelihood estimates from two models: one with and one without the marker obtained from the SAGE program ASSOC. Analysis of the data by $t$ tests also showed significant differences in amplitude for the A1 and A2 groups in both the auditory and visual modalities. For the auditory modality, $t = 2.18$, df = 56, $p = .03$. For the visual modality, the means of the two groups were also significantly different with $t = 2.83$, df = 56, $p = .006$.

reports (Begleiter et al 1984; Hill and Steinbauer 1993a) utilizing this paradigm have not found statistical differences between responses in the hard and easy conditions, we chose to analyze the hard condition only with respect to the A1/A2 alleles.

A standard set of instructions was read to each child. If additional clarification was needed, directions were amplified by the experimenter. The child was first shown a picture of each of the stimuli and asked to make the correct response to each (target) stimulus. Next, each stimulus was presented on the video monitor at a slow pace using a long exposure duration (3200 msec). Once the child was performing correctly (usually less than 10 trials), the visual display duration was decreased to the 33 msec exposure time required for the main experiment for several additional practice trials. (The children were encouraged to respond quickly, but more importantly, to respond accurately.) Two blocks of 120 trials were presented to the subjects. Of the 240 total trials, 160 were blank (nontargets), 40 were easy condition targets (20 right, 20 left), and 40 were hard condition targets (20 right, 20 left).

**Electrophysiological Recording and Peak Detection**

ERPs were recorded using SensorMedic Ag/AgCl electrodes placed at midline frontal, vertex, parietal, and occipital locations (Fz, Cz, Pz, Oz) as well as left and right parietal sites (P3, P4). All active electrodes were referred to linked ears, with a forehead ground. Eye movement and blink artifacts were recorded by an additional electrode located under the left eye, which also was referred to linked ears. All data were monitored online by an oscilloscope, and all trials affected by eye artifact (exceeding approximately 50 µV) were coded for exclusion. Data were digitized by a PDP 11/23 computer for 1200 msec at 125 Hz, beginning 200 msec prior to stimulus onset, and stored on magnetic media. Artifact-free trials for each task were averaged for each condition and electrode.

In our laboratory, ERP components (N100, P200, N250, P300) are typically identified using an interactive computer algorithm that chooses the maximal amplitude for a given component (at Cz for N100, P200, and N250; at Pz for P300) within a predefined latency window (N100: 80–136 msec; P200: 136–240 msec; N250: 200–320 msec; P300: 264–424 msec). For the present analysis, our focus was solely on P300 amplitude and latency at Pz (the typical maximal response site for P300), because this component has been most strongly associated with alcoholism risk to date.

For the visual task, the P300 latency window was extended if necessary. Components were judged to be outside the latency range based on consensual agreement between two raters blind to each subject’s family history. The computer algorithm used for determining peaks within the latency windows was temporarily overridden in such cases to allow for selection of latencies outside the range. This is of particular importance, since component latencies are typically longer in children than in adults, and are decreased for older children. Peak amplitude was computed as the deviation from the median voltage during the 200 msec prestimulus baseline, using the same time point for all electrode sites. Latency and peak to baseline amplitude data were automatically extracted and stored in ASCII files for subsequent analysis.

**Method of Genetic Analysis**

Associations between the A1 allele of the DRD2 locus and either amplitude or latency of the P300 component of the ERP were determined using the trait-marker program ASSOC (SAGE version 2.2) developed by George and Elston (1987). The analysis was performed using age and gender as covariates. This method utilizes a log likelihood estimate in which two models are compared: one with and one without the marker in the model. Two times the difference in the log likelihoods is then evaluated under a chi-square distribution with one degree of freedom. Results of the SAGE analyses are presented in Table 1 as chi-squares with associated $p$ values. The children were matched for age, education, and IQ (Table 2).

Table 2. Relationship of Demographic Measures to Taq1 A DRD2 Alleles in Children from High-Risk Families (Means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>A1 (n = 25)</th>
<th>A2 (n = 33)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>11.16 (3.2)</td>
<td>11.42 (2.9)</td>
</tr>
<tr>
<td>Education (grade level)</td>
<td>5.80 (3.1)</td>
<td>5.85 (2.9)</td>
</tr>
<tr>
<td>IQ*</td>
<td>120.47 (16.0)</td>
<td>109.67 (13.5)</td>
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*IQ measures were available for 19 A1 children and 27 A2 children.
Results

P300 Amplitude for the High-Risk Children with and without A1

In Figure 1, the grand mean ERP response for the boys and girls with and without A1 alleles may be seen for both our auditory and visual paradigms. Data are presented for Fz, Cz, Pz, and electro-oculogram (EOG). As may be seen, the amplitude of P300 is reduced in children carrying the A1 allele. On average these represent reductions in P300 amplitude of approximately 5 and 7 µV in the auditory and visual paradigms, respectively. These are substantial differences, similar in size to those commonly reported for differences between high and low-risk control children.

As is typically observed for this paradigm, the P300 component is maximal over the parietal region (Pz electrode). Therefore, the association analyses were performed only with respect to this electrode. Analyses were based on
data for 58 children and 42 available relatives using age and gender as covariates. As may be seen in Table 1, significant associations were found between the presence or absence of the A1 allele and both the auditory and visual P300 amplitudes recorded for these children.

Differences between the groups were not the result of poorer performance of the children carrying the A1 allele. The reduction of P300 amplitude seen in this group occurred in the presence of equal behavioral performance of the tasks. As may be noted in Table 1, the average P300 latency in the auditory RT task and the visual task were equivalent for the A1 and A2 groups. Behaviorally, the groups were equivalent with respect to the number of correct responses. This confirms previous work from this laboratory indicating that high-risk children/adolescents tend to produce smaller P300 amplitudes than control children with equal behavioral performance (Hill and Steinhauser 1993a; Hill et al 1995a).

Discussion

The trait/marker association method described by George and Elston (1987) made it possible to test for a genetic association in a sample of 58 children/adolescents, some of whom were related (siblings and cousins). Use of the method allows for testing associations within pedigrees. Further, when the goal is to relate a quantitative trait (e.g., P300 amplitude) and a genetic marker (A1/A2) genotypes, the necessity of a nonalcoholic control group is not required, as it would be if the goal were to relate a disease (alcoholism) to allelic variation. The method has recently been elaborated by Nick et al (1995), providing estimates concerning the size of the sample needed to make statistically valid inferences using the nonindependent data from pedigrees. They have suggested that only 50 individuals are needed when relative correlations are close to zero, but 100–200 individuals may be needed to test associations where correlations between relatives are close to .5. In the present data set, 58 children were the focus of the investigation, though a total of 100 individuals were needed to include the linking parents and grandparents for children who were nonindependent (siblings and cousins). The sample size utilized would appear to be adequate for statistically valid inferences to be made.

Because the amplitude of P300 was so much lower in the children with the A1 allele, the issue arises as to whether possible confounding effects could have occurred. For example, if children/adolescents who carry the A1 allele have a significantly earlier onset to drink with more alcohol being consumed, lower P300 could be a consequence of exposure to alcohol rather than a manifestation of genotypic differences. In the present study, we controlled for the effects of confounding variables for all individuals utilized in the analysis. This included assessment of current and past health status, as well as exposure to alcohol and drugs. No differences between groups were seen in alcohol or drug consumption. Additionally, no group differences by gender or age were seen.

It should be noted that no association between P300 latency and presence of the A1 allele was found. This would not appear to be surprising in view of possibly greater impact of environmental variance (e.g., toxic exposure) on the production of P300 latency and the lesser heritability reported for P300 latency than amplitude. A previous report by Noble et al (1994a), in which two groups of children of alcoholics and children of social drinking controls were compared, demonstrated an association between P300 latency but a nonsignificant association between P300 amplitude and parental alcoholism status. The P300 was generated by a visual paradigm different than the one used in the present study; however, it should be noted that results presented for children of their “active alcoholic” parents, who were more severe alcoholics than their “recovered alcoholic” group, revealed a reduction, though nonsignificant, in P300 amplitude among the A1 compared to A2 individuals. Thus, Noble and colleagues found indications of reduction in amplitude among the more severe group of alcoholics, who were most similar to the alcoholics selected for study in the present report.

The present results suggest that the amplitude of both auditory and visual P300 is determined, at least in part, by dopaminergic activity. This conclusion is supported by the association found in this study between carrying the A1 allele and P300 amplitude in children and adolescents. These youngsters had not yet begun to drink large quantities of alcohol, though they were clearly at risk for developing alcohol problems due to their high familial loading for alcoholism. The strength of this association is even more remarkable because all were high-risk children. We know from previous studies that not all children presumed to be at risk because of their family history will display abnormal P300. In our own studies of high-risk children, we have found approximately one third of boys and one fifth of girls displaying reduced P300. We would argue that those children who do display reduced P300 might be those who display the less common DRD2 allele. The present data appear to support this conclusion, with P300 being significantly reduced in the children carrying the A1 allele. Additionally, we have supporting data from an association analysis performed on 28 older adult controls, screened for alcoholism and other major psychopathology, in which the amplitude of the P300 was associated with allelic variation of the Taq1 A1/A2 (Hill, unpublished data). Thus, the association between the marker and trait persists in many samples studied, and
therefore suggests that the association is present in the general population.

What might be the mechanism whereby this reduction in P300 amplitude, commonly seen in high-risk children, could occur? Evidence for neurotransmitter modulation of P300 appears to be well documented. Pharmacological manipulations have been shown to alter the amplitude of P300 amplitude; however, there is currently evidence for the involvement of each of a number of neurotransmitter systems. These include noradrenergic (Callaway 1991), serotonergic (Ehlers 1988), cholinergic (Pineda et al 1991; Meador et al 1989), and dopaminergic (Rosler et al 1985; Hansenne et al 1995; Takeshita and Ogura 1994) systems. Therefore, the present results should not be construed as indicating an exclusivity of the dopaminergic system.

Numerous studies, however, offer evidence for involvement of the dopaminergic system in the production of the P300 component of the ERP. First, P300 latency is prolonged in Parkinson patients who suffer degeneration of cortical and subcortical dopaminergic fibers (Hansch et al 1982; Stanzione et al 1991). This prolonged latency can be reversed by the administration of L-Dopa (Stanzione et al 1991; Starkstein et al 1989). Additionally, there is evidence that P300 amplitude is altered by pharmacologic manipulation. Takeshita and Ogura (1994) have found that sulpiride, a dopamine antagonist, increases P300 in low-P300 subjects while decreasing P300 in high-P300 subjects. Hansenne et al (1995) found that growth hormone response to apomorphine in depressed patients was correlated significantly with P300 amplitude.

Although it is clear that pharmacological manipulation of dopamine can alter the amplitude and latency of P300, it is important to evaluate the evidence that alternative alleles of the DRD2 Taq1 system can bring about changes in dopaminergic activity. Noble et al (1991) have reported a relationship between Kd (binding affinity) and Bmax (number of binding sites) in alcoholic subjects who carried the A1 allele in comparison with subjects who did not. Even if this finding cannot ultimately be replicated, the importance of the A1 allele to dopaminergic function is quite plausible. Areas outside of the coding region of the gene could have major functional significance, as demonstrated by the recent genome-wide search for diabetes susceptibility genes in which several regions were identified as influencing susceptibility, though only one gene had a major effect (Davies et al 1994). Although no mutation has been found in the coding region of the DRD2 gene (Gejman et al 1994) to date, there have been no systematic searches for mutations outside of the coding region.

These results may be important to our understanding of genetic mediation of alcoholism for a number of reasons. First, data from a number of laboratories have now shown that nonalcoholic children/adolescents with minimal drinking histories who come from alcoholic families display reduced P300 amplitude compared to controls (Begleiter et al 1984; Hill and Steinbauer 1993a; Steinbauer and Hill 1993; Hill et al 1990; Whipple et al 1991). Second, though the association and/or linkage of the alcoholic phenotype to allelic variation in DRD2 is highly controversial, there is a sufficiently large body of data showing associations (Neiswanger et al 1995; Parsian et al 1991; Pato et al 1993) to warrant further consideration of this hypothesis. Moreover, the arguments offered against there being a population-based association have typically invoked concerns about possible sampling error and/or ethnic variation between alcoholic and control samples (Gelernter et al 1993). It should be noted, however, that studies showing significant differences between alcoholics and controls (Blum et al 1990; Neiswanger et al 1995; Parsian et al 1991) appear to frequently have one important common characteristic; all have used screened controls. Screening has either included removing alcoholism from the control group (Blum et al 1990) or removing both alcoholism and other psychiatric diagnoses (Neiswanger et al 1995).

Although the association and linkage study results in alcoholics may be viewed as controversial, there is ample evidence from the animal literature to suggest that dopamine plays an important role in the appetite for alcohol. In general, enhancement of dopamine transmission in the nucleus accumbens increases ethanol-reinforced responding, whereas decreasing transmission decreases ethanol responding (Hodge et al 1992, 1994). These findings, along with observations that administration of dopamine receptor antagonists reduce both lever pressing for alcohol and home cage intake in rats (Pfeffer and Sanbon 1985, 1986, 1988), suggest the importance of dopaminergic activity in the acute rewarding effects of alcohol. Although the gamma-aminobutyric (GABA)A receptor complex, along with opioid peptides, appear to operate in concert with dopamine in the ethanol “reward” circuit (midbrain–forebrain–extrapyramidal circuit), dopamine clearly has an important role (Koob 1992).

Possibly the alteration in the rewarding effects of alcohol might be part of a generalized reduction or enhancement of reward related to inherited variation in D2 receptors, as suggested by Noble et al (1994b). This would appear to be unlikely, based on what is currently known regarding the only partially overlapping distribution of dopaminergic fibers and “reward fibers” seen upon mapping of animal brains utilizing electrical stimulation techniques (Wise and Rompre 1989). As these authors point out, dopamine is not the only reward transmitter, and dopaminergic neurons are not the final common path for all rewards. Nevertheless, activation of the dopamine system appears to alter general arousal as reflected in
locomotor activity, a role that may be necessary in the performance of motivated behavior.

Considerations of the role of dopamine in motivated behavior may explain the observed relationship between P300 amplitude and allelic variation in the D2 receptor among individuals. The P300 amplitude has been shown to vary in response to the individuals’ evaluation of a stimulus. For example, in the auditory modality, the evaluation consists of asking the subject to make a discrimination regarding whether or not he/she heard a “target” tone. The stimulus evaluation includes making a decision regarding the significance of the stimulus. As originally elaborated on by Tueting et al (1971), the significance of the stimulus is in part determined by the context in which it occurs, such that the probability of the stimulus (tone) being heard alters the amplitude of P300 elicited in both adults (Polich 1987) and in children (Ladish and Polich 1989). It is very likely that this aspect of information processing could have arisen through the selective advantage of organisms who could more accurately detect patterns and therefore probabilities of both rewarding and aversive events in their environment. If so, it might well be important that the dopaminergic system, having such extensive responsibility for motoric functioning as it does, would need to be tied to P300 generators indexing the significance of environmental events. Dopaminergic mediation of P300 generation could then be tied to the dopaminergically innervated motor pathways necessary for flight from danger.

What then can we say about the relationship to alcoholism risk? Possibly P300 amplitude is a marker for later development of alcoholism, as suggested by preliminary data from two clinical follow-up studies conducted at 4 years (Berman et al 1993) and 8 years (Hill et al 1995c), with repeated ERP assessments in the Hill and colleagues study. This reduced amplitude might be related to alteration in dopamine receptor number or affinity, as suggested by the present results indicating lower P300 in children carrying the A1 allele. Given the higher rates of A1 observed among adult alcoholics seen in our laboratory, we have to conclude that dopaminergic receptor activity, undoubtedly in conjunction with other major neurotransmitters, plays a role in the development of alcoholism.

References


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