Prolongation of Brainstem Auditory-Evoked Responses in Autistic Probands and Their Unaffected Relatives

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Background: Brain function, as indexed by brain electrical activity, is heritable in humans, and it may be impaired in autism. Autism also has strong genetic determinants, and like all major psychiatric disorders, its complex clinical phenotype renders genetic studies difficult. Innovative strategies focused on alternative biological phenotypes are needed.

Methods: The early brain auditory-evoked response was assessed in 73 autistic probands and 251 relatives who were compared with 521 normal controls.

Results: We first confirmed in the autistic probands the presence of a slowing in nerve conduction in the auditory system as expressed by the prolongation of early brain auditory-evoked response under the form of I-III interpeak latencies (IPLs). Furthermore, we observed the same I-III IPL prolongation in the unaffected first degree relatives of the autistic probands compared with controls. Despite clear evidence of a coaggregation of autism and I-III IPL prolongation in families, the IPLs did not seem to be the sole liability factor for autism as suggested by the observation of 52% of families in which the autistic proband and relatives showed normal IPLs.

Conclusion: A prolongation of the early brain auditory-evoked response IPLs may be a marker for one of several deficits underlying autism and deserves further analysis as a potential alternative phenotype for the disorder.

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SUBJECTS AND METHODS

SUBJECTS

The sample consisted of 73 autistic probands (mean [SD] age, 7.3 [5.1] years; 57 males) referred over a period of 8 years to the Hôtel-Dieu du Sacré-Cœur de Jesus (HDSC), the university regional child psychiatric hospital providing inpatient and outpatient services to the whole Québec City metropolitan area, Canada. Since autism has an estimated prevalence between 0.02% to 0.04%, and since subjects aged younger than 16 years were targeted (population, \( \approx 200,000 \)), the expected number of autistic patients was about 80. Our present sample of 73 may be considered as reasonably representative of the population of autistic patients in our region.

Our best estimate diagnostic procedure has been previously described. Briefly, each subject had been diagnosed by 1 of 18 child psychiatrists at HDSC. Another psychiatrist, the principal investigator (J. T.), met all the patients and their parents to make a second best estimate diagnosis (DSM-III-R) according to all available clinical information. The longitudinal clinical observation in the present study extended for an average period of 4.0 years. When a disagreement occurred between the 2 diagnosticians, the case was not retained. The Autism Diagnostic Interview (ADI) was not part of our best estimate method but was used to estimate symptom severity.

Additional measures were obtained by a research psychologist who met with all the parents to complete 2 instruments. First, the Vineland Adaptive Behavior Scale (VABS) provides standardized measures of impairment for adaptive skills in socialization, communication, and daily living. The VABS is widely used and was standardized on a large population of children aged 2 to 18 years. It has high reliability and validity and is sensitive to the deficits observed in autistic patients. The mean (SD) total ADI score was 32.2 (8.6). We applied 2 exclusion criteria: absence of wave components and presence of known neurological or ears, nose, and throat diseases directly implicating the brainstem or auditory nerve.

The relatives of 39 of 73 autistic probands had their BAER assessed. The results of the ADI and VABS for these 39 probands were not different from the results for the 34 other probands. We assessed 251 relatives (114 males) of these 39 probands: 106 first, 83 second, and 62 third degree relatives. The mean (SD) age of the relatives was 35.5 (16.5) years.

Results of the ADI and VABS for these 39 probands were obtained.25 The controls were selected by advertisement and came from the same catchment area. They completed a structured medical questionnaire, and subjects with any medical conditions were excluded. This sample was stratified by age (0-7 years, \( n=54 \); 8-13 years, \( n=130 \); and 14 years and older, \( n=37 \)) and sex. The study was personally explained to the parents and relatives who signed a consent form.

BAER RECORDING

The procedure used in this study has already been reported. The recordings were made in a soundproof Faraday cage. We used unfiltered rarefaction clicks (100 \( \mu \)s in both right and left monaural stimulation (TDH-39 earphones; Telephonics, Farmingdale, NY) at a rate of 11.1 clicks per second for 2000 sweeps on the test and the retest. Prior to recording, the heating level of both ears was tested in every subject. We used sensation levels (SLs) instead of normalized hearing levels to maximize the comparability of the data across subjects and between ears for the same subject. We used a 70 dB SL intensity level along with 30 dB contralateral white noise masking. The time base was 10 ms for 512 points (dwell time, 19 \( \mu \)s). The filter bandwidth settings were 75 and 3000 Hz (filter slopes at 24 dB/octave). Cz was the recording site referenced to the ipsilateral mastoid.

In the group of autistic probands, whenever possible the hearing level of the child was determined, and a 70 dB SL intensity level along with 30 dB contralateral white noise masking was used. The BAER recording was a 2000 sweep average. The interpeak latencies (IPLs) were designated by a hyphen between 2 waves (I-III or III-V).

RESULTS

INTERPEAK LATENCIES IN THE AUTISTIC PROBANDS

The typical BAER waveforms of an autistic and a control subject are illustrated in Figure 1. In the sample of probands (matched with controls; \( n=73 \) pairs), the mean of the differences in I-III IPL values was significantly greater than 0, meaning that autistic subjects had, on average, longer I-III IPLs than their matched controls (\( t_{72}=4.73, P<.001 \)). Indeed, the histogram of the differences in I-III IPL values in the sample of probands showed a distribution that was clearly shifted to the right, (ie, not centered at 0) (Figure 2A). The I-V IPL was also significantly prolonged in autistic subjects (\( t_{72}=4.82, P<.001 \)) but not the III-V IPLs (\( t_{72}=0.66, P=.51 \)), suggesting that the significant difference in I-V IPLs was mainly due to the I-III component.
hypothosis of independence in risks between subjects in an experimental group and controls, $\chi^2$ statistics on $2 \times 2$ tables were used. For the probands, an odds ratio was computed with the corresponding 95% confidence interval (CI), as it is done in typical case-control studies. For the relatives, risk ratios (RRs) were calculated because in such a family study, being a relative of a case patient is similar to being exposed to a risk factor; therefore, data can be treated as in a cohort study situation. 

Given that subjects within families are not independent of one another, the 95% CI computed for the RR for relatives may not be valid, though the test of independence (hence, the $P$ value) remains valid. Therefore, in addition to computing RRs and 95% CIs in relatives classified according to their degree of relatedness, the sample of relatives was also stratified into subsamples of independent observations: (1) the parents ($n=71$); (2) the youngest sibling per family ($n=19$); (3) the eldest sibling per family ($n=19$); (4) the youngest maternal and paternal second degree relatives ($n=30$); (5) the oldest maternal and paternal second degree relatives ($n=30$); (6) the youngest maternal and paternal third degree relatives ($n=15$); and (7) the oldest maternal and paternal third degree relatives ($n=15$). The reason for selecting the oldest or youngest person within a class of relatives was to obtain nonrandom subsamples of subjects and, thus, be able to easily replicate our results.

Familial resemblance of the I-III IPL percentile of family members was assessed using hierarchical regression, which allows for fixed and random effects. We fitted a 2-level model in which the first and second level consisted respectively of the family members and the families. A likelihood ratio test was carried out for testing the significance of the effect of families by comparing the 2-level model with the model omitting the families (level 2). This yielded a $\chi^2$ statistic with 1 df. Familial resemblance was assessed in 3 samples: (1) probands and first degree relatives ($n=138$ family members; 39 families); (2) probands and first plus second degree relatives ($n=221$ family members; 39 families); and (3) all family members ($n=283$ family members; 39 families).

The cumulative and relative frequency distributions of the I-III IPL percentile ranks in autistic probands were also clearly shifted to the right compared with controls (Figure 3). The odds of exceeding the 95th percentile in probands compared with controls were 5.64 (95% CI, 3.10-10.28; $P<.001$).

We thus confirmed a prolongation of I-III IPL in the 73 autistic probands vs controls. There was no significant correlation between the IPL in the probands and severity of the autistic characteristics on each of 3 ADI subscales and the VABS ($n=67$ for ADI-1, $t=0.04$, $P=.76$; for ADI-2, $t=0.01$, $P=.94$; for ADI-3, $t=0.21$, $P=.09$; for VABS, $t=0.05$, $P=.66$).

IPLs IN RELATIVES

The first degree relatives also presented significantly longer I-III IPLs than matched controls ($n=94$ pairs, $t_{83}=2.36$, $P=.02$; Figure 2B). No significant I-III IPL prolongation was found in second ($n=82$ pairs, $t_{20}=1.16$, $P=.25$) or third degree relatives ($n=41$ pairs, $t_{20}=0.81$, $P=.43$). There was no significant evidence of a prolongation in I-V IPLs in first, second, or third degree relatives (see note in Figure 2B).

In our stratified subsamples of independent observations, siblings had significantly longer I-III IPLs than controls ($n=19$ pairs, $t_{20}=2.48$, $P=.02$ for youngest siblings, $n=19$ pairs, $t_{20}=3.23$, $P=.004$ for eldest siblings). Parents however did not have significantly longer I-III IPLs than controls ($n=221$ pairs, $t_{219}=1.61$, $P=.11$ for parents; $n=33$ pairs, $t_{32}=0.60$, $P=.55$ for fathers; $n=38$ pairs, $t_{37}=1.69$, $P=.10$ for mothers). No evidence of an I-III IPL prolongation was found in second or third degree relatives ($n=30$ pairs, $t_{29}=0.84$, $P=.41$ for youngest second degree relative; $n=30$ pairs, $t_{29}=0.84$, $P=.41$ for oldest second degree relative; $n=15$ pairs, $t_{14}=1.03$, $P=.32$ for youngest third degree relative; $n=15$ pairs, $t_{14}=-1.62$, $P=.13$ for oldest third degree relative).
The cumulative frequency distribution of the I-III IPL percentile ranks in unaffected relatives lay between that of the autistic probands and the controls (Figure 3A). In first degree relatives, the risk of having an I-III IPL exceeding the 95th percentile was 2.1 times greater than in controls \( (P = .007; \text{Table}) \). The RRs were also significantly greater than one for parents \( (RR = 2.2, P = .015) \), youngest siblings \( (RR = 3.7, P = .002) \), and eldest siblings \( (RR = 3.7, P = .002) \), but they did not consistently reach significance level in second or third degree relatives (Table). Among the 11 parents who exceeded the 95th percentile, there were 5 fathers and 6 mothers. When we characterized the first degree relatives by the highest I-III IPL values from 2 ears, results remained similar \( (RR = 2.39; 95\% \text{ CI}, 1.08-5.27; \chi^2_1 = 5.54; P = .019) \).

**FAMILIAL RESEMBLANCE OF THE I-III IPL TRAIT**

According to the likelihood ratio test, the effect of families was significant in each of 3 overlapping samples of family members, meaning that the IPL trait showed significant familial resemblance \( (\chi^2_1 = 5.41, P = .02 \text{ with first degree relatives}; \chi^2_1 = 7.18, P = .007 \text{ with first and second degree relatives}) \).
second degree relatives; $\chi^2_e = 8.28$, $P = .04$ with all family members).

Examples of pedigrees showing familial resemblance are shown in Figure 4 in which I-III IPL percentiles are indicated for each family member. Families R2 and R5 are examples in which both the proband and several relatives tended to have high percentile ranks. Family RD is one in which percentile ranks tended to be low. Despite an evidence of familial resemblance, we observed a proportion of families in which the proband alone had a high I-III IPL value (family RE) and some in which a parent and a few other relatives showed a high IPL value but not the autistic child (family R4). Using the 95th percentile as a cutoff, we found that 30% of the autistic probands had at least 1 parent with prolonged IPL, and 48% of the families had either the proband or a parent or both with a prolonged IPL.

### Risk of Exceeding I-III Interpeak Latency 95th Percentile in Relatives of Autistic Probands Compared With Normal Controls According to Degree of Relatedness

<table>
<thead>
<tr>
<th>Degree of Relatedness</th>
<th>N</th>
<th>Relatives of Autistic Probands</th>
<th>Control§ (N = 521)</th>
<th>Risk Ratio</th>
<th>95% CI</th>
<th>$\chi^2 (df = 1)$</th>
<th>$P$</th>
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<tr>
<td>First degree relatives</td>
<td>106</td>
<td>15.1</td>
<td>7.1</td>
<td>2.1</td>
<td>1.18-3.84</td>
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<td>15.5</td>
<td>7.1</td>
<td>2.2</td>
<td>1.12-4.26</td>
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<td>.015</td>
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<tr>
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<td>19</td>
<td>26.3</td>
<td>7.1</td>
<td>3.7</td>
<td>1.46-9.40</td>
<td>9.4</td>
<td>.002</td>
</tr>
<tr>
<td>Eldest siblings</td>
<td>19</td>
<td>26.3</td>
<td>7.1</td>
<td>3.7</td>
<td>1.46-9.40</td>
<td>9.4</td>
<td>.002</td>
</tr>
<tr>
<td>Second degree relatives</td>
<td>83</td>
<td>14.5</td>
<td>7.1</td>
<td>2.0</td>
<td>1.06-3.91</td>
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<td>.03</td>
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<td>Youngest§</td>
<td>30</td>
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<td>1.4</td>
<td>0.43-4.59</td>
<td>0.4</td>
<td>.55</td>
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<td>Oldest§</td>
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<td>26.7</td>
<td>7.1</td>
<td>3.8</td>
<td>1.76-8.04</td>
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<td>9.7</td>
<td>7.1</td>
<td>1.4</td>
<td>0.59-3.21</td>
<td>0.5</td>
<td>.46</td>
</tr>
<tr>
<td>Youngest§</td>
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<td>7.1</td>
<td>0.9</td>
<td>0.13-6.89</td>
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<td>.95</td>
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<tr>
<td>Oldest§</td>
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<td>7.1</td>
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<td>NA</td>
<td>1.1</td>
<td>.28</td>
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<td>13.6</td>
<td>7.1</td>
<td>1.9</td>
<td>1.19-3.06</td>
<td>8.4</td>
<td>.004</td>
</tr>
</tbody>
</table>

*CI indicates confidence interval; NA, not applicable due to a null risk ratio.
†Each subject was assigned a percentile rank based on normative values.
‡Proportions in controls slightly exceed the expected 5% due to the presence of equal I-III interpeak latency values within age and sex category.
§In each family, up to 2 independent observations could be used: 1 maternal and 1 paternal relative.

We confirmed in a large sample of autistic patients the presence of a slowing in nerve conduction in the auditory system as expressed by the prolongation of early BAERs and I-III IPLs. Moreover, we now observe the same I-III IPL prolongation in the unaffected first degree relatives of autistic subjects and a familial resemblance of the IPL trait within families. The IPL prolongation is not the first biological anomaly to be found in unaffected relatives of autistic probands. Previous reports have shown that these relatives have other biological peculiarities either in terms of immunopathologic indices such as decreased plasma concentration of the C4B protein or an increased frequency of the B44-SC30-DR4 haplotype, or in terms of biochemical indices such as hyperserotoninemia.

We made 5 observations suggesting a complex relationship between IPL and autism. First, even though a significant association between the IPL marker and autism was observed, the deficit expressed by the IPL prolongation may not account for all cases of autism. Indeed, there were roughly 50% of the families in which neither the proband nor the parents showed an IPL prolonged greater than the 95th percentile. This implies that the IPL prolongation as a marker of a neuropathologic process would not be necessary to develop autism and would not consequently be the sole liability factor for autism. Hence, etiologic heterogeneity and possibly genetic heterogeneity would be likely for autism. Second, none of the relatives with extreme IPL expressed the autistic phenotype. This implies that the IPL prolongation would not be sufficient to develop autism, suggesting reduced penetrance and consequently, the need for IPL to interact with other genetic or environmental factors to be expressed as autism. Third, a proportion of autistic probands did not display a prolongation whereas at least one of their parents did. This may be due to pleiotropy (ie, the phenomenon in which a genetic latent trait can be expressed in 2 independent phenotypes, as was proposed by Holzman et al., to model the relationship between abnormal eye tracking and schizophrenia). Fourth, some probands carried the IPL prolongation, whereas their parents did not. This could reflect an increase in trait severity across successive generations, suggesting anticipation. Fifth, our data did not suggest a sex-linked transmission given the absence of an excess of transmission from the maternal or paternal side.

As well exemplified for schizophrenia and P50 anomalies, the present observation of IPL slowing in autism and in relatives of subjects with autism justifies further investigations of early BAER prolongation as a potential alternative phenotype for autism. Such an alternative phenotype could increase the statistical power of genetic analyses in autistic pedigrees by increasing the number of affecteds. Besides, the observed complex relationship between autism and IPL suggests that autism might consist of a combination of neurophysiological deficits and that IPL prolongation could be a marker of one
of these. The IPL could then show a simpler genetic transmission than autism itself and, therefore, be the target of further biological and genetic investigation.

Because much remains to be known about the neural generators of BAER waveforms, it is difficult to define the link between BAER and autism. Brainstem auditory-evoked responses might however be related to 3 kinds of neurobiological parameters reportedly associated with autism: (1) myelination, (2) cerebellum neuroanatomy, and (3) serotonin. First, myelination can be a target because of its implication in nerve propagation. Using magnetic resonance imaging to assess myelination status in children with developmental delays, Harbord et al reported abnormally prolonged BAER in children with diminished myelination, suggesting an association between increased and abnormal myelination. Second, cerebellum degenerative or tumoral diseases were found to be related to waveform BAER amplitude and latency values. In this regard, it is difficult not to evoke the description by Courchesne et al of cerebellar lobules VI and VII hypoplasia in autistic patients. Third, hyperserotonemia is the best replicated biochemical abnormality in autistic patients and their relatives. In that respect, serotonin has been found to stop axon elongation for synapse formation of particular neurons.

Piven et al found in the parents of autistic probands the presence of a broad or lesser autistic phenotype (i.e., parental personality characteristics of aloofness, rigidity, hypersensitivity to criticism, speech, and language deficits). Only a proportion of families presented this lesser phenotype. We intend to evaluate such a lesser phenotype in unaffected relatives with the hypothesis that the relatives with extreme IPLs will be more likely to display the lesser clinical variant than those below the IPL median. If this hypothesis is confirmed, analyses of the segregation and/or cosegregation of the IPL and the autistic (or autistic-like) phenotypes in the pedigrees will be possible and will hopefully facilitate genetic linkage analysis of autism or the IPL endophenotype.

The present study has limitations. First, because of the small number of autistic subjects in a pedigree, it was impossible to look at the cosegregation of the illness with the electrophysiological deficit. Second, even though the present sample constitutes a relatively large family collection for autism, the numbers in the subsamples had limited power. Third, the present findings apply only to autism and not to other forms of pervasive developmental disorder that have been shown to be related to autism in family studies.
REFERENCES


