SHORT COMMUNICATION

Angelman syndrome caused by deletion: A genotype—phenotype correlation determined by breakpoint

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KEYWORDS
Angelman syndrome; Deletion; Breakpoint; Epilepsy

Summary
Objectives: Deletion of the chromosome 15q11-q13, the most common genetic mechanism associated with Angelman syndrome (AS), is highly associated with a severe phenotype. However, deletion is not a genetically homogeneous group as it is composed by two main groups: Class I with breakpoints at BP1 (proximal) and BP3 (distal) and Class II present breakpoints at BP2 (proximal) and BP3 (distal). In this study, we aimed to evaluate the impact of the breakpoint on the electroclinical profile.
Methods: We evaluated 16 patients with AS caused by 15q11-13 deletion (6 were Class I; 10 were Class II). We characterized epilepsy features by clinical history obtained from parents and caretakers with a pre-standard questionnaire. These data were corroborated by medical records, contact with previous physicians, and video-EEG monitoring. Suggestive EEG patterns for AS were classified according to the classical description of Boyd et al. (1988).
Results: AS patients with BP1—BP3 deletion had significantly more daily and disabling seizures than AS patients with BP1—BP2 deletion. They also presented a significant higher frequency of status epilepticus and epilepsy aggravated by fever. Need for polytherapy was significantly more frequent in BP1—BP3 patients. EEG features were similar in both groups.

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Introduction

Angelman syndrome (AS) is determined by four mechanisms: deletion (del15q11-13) (Kaplan et al., 1987), uniparental disomy (UPD) (Nicholls et al., 1992), imprinting defects (Buiting et al., 1995) and UBE3A mutations (Kishino et al., 1997), affecting the maternal chromosome 15. Most patients (75–80%) have del15q11-13, characterized by the loss of one part of the chromosome. These patients have a severer phenotype with worse physical growth, cognitive skills, albinism and ataxia (Lossie et al., 2001). Epilepsy in patients with del15q11-13 is also more severe compared to patients determined by other mechanisms (Minassian et al., 1998; Valente et al., 2005).

It is supposed that the presence and severity of some clinical features is determined by the deletion of genes in patients with del15q11-13, which are spared in other mechanisms. A cluster of GABAergic genes may play a major role in the severity of epilepsy of del15q11-13 patients, especially the GABA\(_A\) beta3 receptor (DeLorey et al., 1998).

Varela et al. (2004) described intra-group heterogeneity in patients with del15q11-13. Patients with larger deletions (BP1–BP3) showed speech impairment and severe development delay. The authors hypothesized that breakpoints might critically involve different genes, resulting in intra-group differences.

To test this hypothesis we studied the two main classes of deletion: Class I in which patients show breakpoints at BP1 (proximal) and BP3 (distal) and Class II that presents breakpoints at BP2 (proximal) and BP3 (distal) — these two classes represent 95% of AS patients with del15q11-13. In both groups, the cluster of GABAergic genes (\(\alpha_1\), \(\beta_3\) and \(\delta_3\)) is equally involved. Therefore we hypothesized that patients with del15q11-13 should present a homogeneous electroclinical profile as to the severity of epilepsy and EEG findings.

Methods

Patients

All patients were evaluated by a geneticist and a child neurologist. The inclusion criterion for this study was genetic confirmation of chromosome 15 deletion.

Methods

Genetic studies

DNA was extracted from peripheral blood leukocytes by standard procedures. Diagnosis was established by methylation pattern analysis of the PWS/AS region. DNA was modified by bisulfitete treatment, and the SNURF-SNRPN exon 1 amplified by PCR (Zeschnigk et al., 1997).

Conclusion: This study shows a significant correlation between the two deletion classes and AS clinical, but not the electrographic phenotype. Epilepsy is more severe and refractory to treatment in patients with larger deletions. Deletion is not a homogeneous group and knowledge on the breakpoint may have a clinical implication and represent an important factor in parental counseling.

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Electroclinical phenotype

Epilepsy characterization. Epilepsy was characterized by history and corroborated with medical files, revision of previous exams (EEGs and V-EEGs) and personal contact with referring physicians.

The following information was collected: (1) presence of epilepsy; (2) age of epilepsy onset; (3) seizure type at onset and during follow-up; (4) history of severe epilepsy; (5) status epilepticus (SE); (6) history of refractory epilepsy; and (7) occurrence of isolated seizures and/or febrile seizures.

Neurophysiological evaluation. All patients underwent prospective EEG and/or video-EEG (V-EEG) evaluations, with a minimum duration of 4 h. EEGs performed previously were considered if obtained with the same technical parameters used in our study.

Suggestive EEG patterns of AS were classified, according to those previously described (Boyd et al., 1988; Valente et al., 2003), as follows: delta pattern: runs of generalized, high amplitude, rhythmic delta activity, usually with frontal emphasis, sometimes associated with epileptiform discharges; theta pattern: high amplitude, 4–6 Hz activity, generalized or over posterior regions, occupying most of the tracing and; posterior discharges: spike and sharp waves mixed with high amplitude 3–4 Hz activity, over posterior regions, sometimes asymmetrical and typically triggered by passive eye closure (Fig. 1).

Interictal and ictal abnormalities, which could not be classified into the groups mentioned above, were also analyzed considering the same criteria (morphology, localization, occurrence, distribution and amplitude) and were then grouped according to their similarities.

Background activity was analyzed regarding frequency, amplitude, spatial distribution, inter-hemispheric symmetry and synchrony, during wakefulness, drowsiness and sleep.
Statistical analysis
Continuous data were described by minimum and maximum values, median, mean and standard deviations and then categorized by absolute and relative frequencies.

Comparisons between Classes I and II as to continuous variables were carried out with t Student test for independent samples or Mann—Whitney test in case distribution might supposedly not be normal. Such a supposition was verified by use of the normal probability graph and by Shapiro—Wilks and Kolmogorov—Smirnov tests.

Comparison of Classes I and II as to categorize variables were carried out by Pearson’s chi-square test or Fisher test.

Follow-up duration of patients until seizure control was compared between groups by the log rank test. Control free survival curves were estimated by the Kaplan—Meyer method.

This work was approved by the local Ethical Committee. All parents and legal guardians signed a legal consent in order to take part in this study.

Results

Genetic studies
We found five breakpoint regions in AS deletion patients: BP1, BP2, BP3, BP4, and BP5. A total of 18 patients presented informative results for proximal and distal breakpoints concomitantly: 6 (33.3%) belonging to Class I (BP1—BP3), 10 (55.5%) to Class II (BP2—BP3). In the Class I patients, a S541/S542 deletion was found, indicating that BP1 was the proximal deletion breakpoint. Twelve (57.1%) were heterozygous at S541/S542, indicating that BP2 was the proximal breakpoint.

One patient with BP2—BP4 and another with BP2—BP5 breakpoint were excluded from this analysis.

Electroclinical phenotype—genotype correlation

Tables 1 and 2 show the electroclinical phenotype in Classes I and II.

AS patients with BP1—BP3 deletion had more daily (p 0.0357), disabling seizures (p 0.0350), a higher frequency of SE (p 0.0357) and recurrent seizures during febrile events (p 0.0357) compared to patients with BP1—BP2 deletion. Need for polytherapy was significantly more frequent in BP1—BP3 patients (p 0.0350).

Suggestive EEG patterns occurred in both groups, with prolonged and frequent runs. Background abnormalities, interictal and ictal epileptiform discharges were equally represented.

Discussion

AS patients, determined by del15q11-q13, have a high prevalence of epilepsy with early onset, often severe and refractory (Valente et al., 2006). The question raised is whether patients with deletion are a homogeneous group considering epilepsy and EEG. The importance of this question lies on the fact that patients with deletion represent the vast majority of AS patients. Therefore, the understanding of this group would bring important answers for counseling and for better understanding of the possible genes implicated in the severity of AS.

Our study showed that that Class I patients present severer epilepsy with a worse outcome and need of polytherapy. Some aspects of epilepsy seem to be similar in both groups, such as the presence of generalized epilepsy. As to EEG features, both groups had similar findings. Therefore, corroborating our previous findings, proper characterization of epilepsy and EEG in AS may be used as an important diagnostic tool (Valente et al., 2003, 2006), which is relevant for the diagnosis, especially in early ages when phenotype is not well-delineated.
We believe that it is important to emphasize that in our series, electroclinical phenotype was determined with a longitudinal study of these patients that ranged from 3 to 10 years. In addition, epilepsy characterization was done based on the patients’ history besides the current evaluation in order to avoid age-related electroclinical changes that may influence results when one analyses one specific moment and not the patient’s history.

To our knowledge, this is the first study showing that epilepsy has a severity-related expression determined by the breakpoint, suggesting that larger deletions may determine severer epilepsy.

The mechanisms implied in the severity of AS remain unknown. However, some previous hypotheses seem unlikely. The hypothesis that GABAergic genes (DeLorey et al., 1998; Handforth et al., 2005; Dan and Boyd, 2003) are directly correlated with the severity must be questioned, based on the fact that these genes are equally expressed in Class I and Class II. We believe that GABA\textsubscript{\textalpha} genes are probably related to the presence of epilepsy, as well as to the expression of many aspects of epilepsy and EEG in these patients (Dan and Boyd, 2003).

Four genes (NIPA1, NIPA2, CYFIP1, GCP5) were mapped to the region between breakpoints BP1 and BP2, but their function is still unknown (Chai et al., 2003). The work of Bittel et al. (2005), suggest that one single mechanism cannot explain such complex disorder, but interconnected mechanisms could produce changes in gene expression, explaining phenotypic differences among genetic subtypes of AS and in the same subtype.

Our study showed that although patients with deletion are treated as a homogeneous group, epilepsy was more severe in patients with larger deletions. It must be remembered that deletion is determined by different breakpoints involving different genes. It is not surprising that these patients have phenotypic differences, demonstrating an intra-group phenotype–genotype correlation. The present study was able to establish a correlation between

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**Table 1** Epilepsy phenotype in patients with AS determined by distinct breakpoints.

<table>
<thead>
<tr>
<th>Seizure types</th>
<th>BP2—BP3 (Class II)</th>
<th>BP1—BP3 (Class I)</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical absences</td>
<td>08, 80%</td>
<td>06, 100%</td>
<td>14</td>
<td>0.5000</td>
</tr>
<tr>
<td>Myoclonic seizures</td>
<td>07, 70%</td>
<td>04, 66.7%</td>
<td>11</td>
<td>1.0000</td>
</tr>
<tr>
<td>GTC</td>
<td>05, 50%</td>
<td>05, 83.3%</td>
<td>10</td>
<td>0.3069</td>
</tr>
<tr>
<td>CPS</td>
<td>02, 20%</td>
<td>01, 16.7%</td>
<td>03</td>
<td>1.0000</td>
</tr>
<tr>
<td>SPS (motor seizures)</td>
<td>03, 30%</td>
<td>03, 50%</td>
<td>06</td>
<td>0.6066</td>
</tr>
<tr>
<td>Daily seizures\textsuperscript{+}</td>
<td>01, 10%</td>
<td>04, 66.7%</td>
<td>05</td>
<td>0.0357</td>
</tr>
<tr>
<td>Disabling seizures</td>
<td>2, 20%</td>
<td>5, 83.3%</td>
<td>7</td>
<td>0.0350</td>
</tr>
<tr>
<td>Diversity of seizure types</td>
<td>4, 40%</td>
<td>5, 83.3%</td>
<td>9</td>
<td>0.1451</td>
</tr>
<tr>
<td>Polytherapy</td>
<td>2, 20%</td>
<td>5, 83.3%</td>
<td>7</td>
<td>0.0350</td>
</tr>
<tr>
<td>Seizures aggravated by fever</td>
<td>06, 60%</td>
<td>05, 83.3%</td>
<td>11</td>
<td>0.5879</td>
</tr>
<tr>
<td>Status Epilepticus during febrile events</td>
<td>01, 10%</td>
<td>04, 66.7%</td>
<td>05</td>
<td>0.0357</td>
</tr>
<tr>
<td>Repetitive events of seizures aggravated by fever</td>
<td>05, 50%</td>
<td>04, 66.7%</td>
<td>09</td>
<td>0.6329</td>
</tr>
<tr>
<td>Status epilepticus</td>
<td>09, 90%</td>
<td>05, 83.3%</td>
<td>14</td>
<td>0.6329</td>
</tr>
<tr>
<td>Repetitive status epilepticus</td>
<td>4, 40%</td>
<td>5, 83.3%</td>
<td>09</td>
<td>0.1451</td>
</tr>
<tr>
<td>Long-duration status epilepticus</td>
<td>3, 30%</td>
<td>1, 16.7%</td>
<td>4</td>
<td>1.0000</td>
</tr>
<tr>
<td>Seizure control</td>
<td>7, 70%</td>
<td>3, 50%</td>
<td>10</td>
<td>0.6066</td>
</tr>
</tbody>
</table>

\textsuperscript{+} Predominance of daily seizures; CPS, complex partial seizures; SPS, simple partial seizures.

\( p \leq 0.05 \).
the breakpoint and the electroclinical phenotype in the two major classes of deletion. Therefore, the breakpoint may be a predictive factor for epilepsy severity, representing an important factor for parental counseling.

References


