Linkage analysis with chromosome 15q11–13 markers shows genomic imprinting in familial Angelman syndrome


Abstract
Angelman syndrome (AS) and Prader-Willi syndrome (PWS) have become the classical examples of genomic imprinting in man, as completely different phenotypes are generated by the absence of maternal (AS) or paternal (PWS) contributions to the q11–13 region of chromosome 15 as a result of deletion or uniparental disomy. Apparently, most patients are sporadic cases. The genetic mechanism underlying familial AS has remained enigmatic for a long time. Recently, evidence has been emerging suggesting autosomal dominant inheritance of a detectable or undetectable defect in a gene or genes at 15q11–13, subject to genomic imprinting. The present report describes an unusually large pedigree with segregation of AS through maternal inheritance and apparent asymptomatic transmission through several male ancestors. Deletion and paternal disomy at 15q11–13 were excluded. However, the genetic defect is still located in this region, as we obtained a maximum lod score of 5.4 to 40 for linkage to the GABA receptor locus GABRB3 and the anonymous DNA marker D15S10, which have been mapped within or adjacent to the AS critical region at 15q11–13. The size of the pedigree allowed calculation of an odds ratio in favour of genomic imprinting of 9.25 × 10^9. This family illustrates the necessity of extensive pedigree analysis when considering recurrence risks for relatives of AS patients, those without detectable deletion or disomy in particular. (J Med Genet 1993;30:853–7)

The main features of Angelman syndrome (AS) are severe mental retardation, absent speech, paroxysms of laughter, abnormal gait, seizures or EEG abnormalities, microcephaly, brachycephaly, macrostomia, and prognathism. 12 Diagnosis during the first year of life may be difficult because facial dysmorphism and seizures evolve with time. 23 The incidence of AS is estimated to be around 1 in 20,000.1 More than 50% of the patients have a cytogenetically visible deletion or rearrangement of chromosome 15q11–13 24 and 75 to 80% of the patients have molecular deletions. 25 The deletion always involves the maternally inherited chromosome 15. 26 A small percentage of non-deletion AS patients lack the maternal 15q11–13 region as a result of paternal disomy. 27,8 The same cytogenetically defined region of chromosome 15 is involved in Prader-Willi syndrome (PWS). 9 PWS is phenotypically very different from AS as PWS patients display infantile hypotonia, childhood hyperphagia and obesity, mental retardation, and hypogonadism. 10 In contrast to AS, in PWS the deletion of chromosome 15q11–13 is of paternal origin, 11 whereas uniparental disomies are of maternal origin. 12 The different parental origins of 15q deletions and disomies resulting in different phenotypes indicate that genes in this region show differential expression on maternal versus paternal chromosomes (genomic imprinting). The report of a family with a chromosomal translocation involving chromosome 15 leading to unbalanced translocations with deletions in 15q in the offspring supports the role of genomic imprinting in both syndromes, as identical unbalanced karyotypes in the offspring either produced AS or PWS, depending on the sex of the transmitting parent. 13

Recently, molecular analysis showed that the smallest regions of overlap (SRO) of the deletions in both syndromes are distinct, the SRO of AS residing between D15S11 and D15S104 and the SRO of PWS between D15S9 and D15S11. 15 This suggests that different genes are involved in the aetiology of both syndromes. It is conceivable that a defect in inhibitory neurotransmission mediated by the GABA_A receptor plays a role in the pathogenesis of AS. 16 Therefore, the GABA_A (γ-aminobutyric acid) β subunit receptor (GABRB3) gene, which has recently been localised to the SRO of AS, has been proposed as a candidate gene for AS. 16,17

The vast majority of patients with AS are apparently sporadic cases. Several familial AS patients have been reported and, unlike sporadic cases, detectable abnormalities of chromosome 15q are rare. 2 Since most of the familial AS patients are sibs, it has been hypothesised that AS may be inherited as an autosomal recessive trait. 18 However, Hamabe et al 14 reported a family in which three AS sibs did show a deletion of 15q, which they shared with their healthy mother and grandfather. Recently, Wagstaff et al 19 reported three unaffected sisters with affected offspring without...
detectable abnormalities involving 15q, most likely representing autosomal dominant inheritance and genomic imprinting at 15q. In this family a maximum lod score of 2.91 at θ = 0.00 with GABRA5(CA)-1 was obtained. Marker GABRA5(CA)-1 was reported to be localised distal to the SRO of AS.

We present a family of exceptional size as it spans five generations with AS occurring in several sibships. Maternal inheritance was apparent in each case. Asymptomatic transmission through several male ancestors could be inferred from the pedigree. Cytogenetic analysis was performed to study chromosomal rearrangements in the patients and their mothers. Molecular and statistical analyses were applied to investigate subtle deletions or uniparental disomy involving 15q, linkage between AS and markers on 15q, and to determine the mode of inheritance.

Materials and methods

PATIENTS

The pedigree of a five generation Caucasian family with eight AS patients is shown in fig 1. Seven of the patients showed typical features of AS, including severe mental retardation, absent speech, and frequent laughter and smiling (table 1). Before this family presented to us, the only patient in generation III (fig 1) had died at the age of 53 years. Her medical records and photographs confirmed that she was also affected with AS. Patient V.3 was seen at the age of 10 months and all other patients and family members investigated were older than 10 years. Seven patients had EEG abnormalities, while two patients, IV.10 and III.x, had seizures. Strabismus was seen in patients V.1, V.3, IV.6, and III.x. Fig 2A and B show the development of the facial dysmorphism in patient IV.10. Fig 2C shows patient V.1 at the age of 8 years. There was no other family history of congenital malformations, developmental delay, or epilepsy. Consanguineous marriages were not recorded.

CYTOGENETIC ANALYSIS

Chromosomal analysis on lymphocytes of patients V.1, V.3, IV.6, IV.7, IV.15, and IV.16 was performed using standard techniques. High resolution GTG banding was applied to study one of the patients (IV.10) in more detail. In order to enable detection of a familial translocation of chromosome 15 in its unbalanced form, the patients’ mothers, IV.2, III.8, III.10, and III.13, were also karyotyped.

DNA ANALYSIS

Blood samples for DNA analysis were collected from 38 subjects, as indicated in fig 1, representing three generations and including all available nuclear families with affected offspring. DNA from leucocytes was isolated according to Miller et al. Dinucleotide (CA) repeat markers at GABRB3 and D15S10 were analysed using radiolabelled PCR conditions essentially as previously described. RFLP

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**Table 1 Clinical symptoms in the eight AS patients.**

<table>
<thead>
<tr>
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<th>V.1</th>
<th>V.3</th>
<th>IV.6</th>
<th>III.x</th>
<th>IV.7</th>
<th>IV.10</th>
<th>IV.15</th>
<th>IV.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental retardation</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Absent speech</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Paroxysms of laughter</td>
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<tr>
<td>Abnormal gait</td>
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<tr>
<td>Seizures/EEG abnormalities</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Microcephaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Brachycephaly</td>
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<tr>
<td>Macrotomia</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Prognathism</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

+ = present, - = absent, ND = no data available.
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Results

CYTOGENETIC AND DNA ANALYSIS
Chromosome analysis showed normal karyotypes in all seven investigated patients and their mothers, with no cyogenetically visible deletions or translocations involving chromosome 15q.

DNA marker analysis showed heterozygosity at GABRB3 in all patients and at D15S10 (CA repeat) in two patients (fig 1). Heterozygosity was also observed at D15S11 in the DNA of four patients when analysing the Styl and RsaI polymorphisms detected by IR4-3R (not shown). The hybridisation patterns obtained by Southern blotting using other RFLP markers from chromosome 15q11-13 were all in agreement with the presence of two alleles in the patients' DNA. Hence, we were unable to detect a deletion in the chromosomal region we investigated at the molecular level. Moreover, the heterozygous patterns at GABRB3 were of unequivocal biparental origin in five of the patients (fig 1). The maternal allele could also be distinguished with certainty in the four patients who were heterozygous at D15S11 (not shown). Assuming that one genetic defect segregates in this family this rules out paternal disomy at these loci as the cause of AS in this family.

LINKAGE ANALYSIS
We noticed that the same allele, numbered 3 in fig 1, at GABRB3 was found in all seven patients investigated, their mothers, and the only living grandparent (III.1), whereas this allele was not observed in unaffected sibs of patients (fig 1). Next, we analysed whether the genetic defect in this family showed linkage to GABRB3 and D15S10.

Table 2 summarises the lod scores obtained at different 0, loci, and models. No recombinations between AS and either D15S10 or the GABRB3 locus were observed. A maximum
multipoint lod score of 5.40 was obtained at \(0 = 0.00\) between \(AS\) and \(GABRB3\) under the assumption of genomic imprinting. The alternative model assumed incomplete (50%) penetrance instead of genomic imprinting and yielded a substantially lower lod score. The ratio between the maximum likelihoods of the two models determines the odds for genomic imprinting. Assuming complete linkage of the \(AS\) gene(s) with \(D15S10\) and \(GABRB3\), an odds ratio in favour of imprinting of \(9.25 \times 10^3\) was calculated.

### Discussion

The \(AS\) patients in the family reported here showed no evidence for a deletion or other chromosomal abnormality involving 15q11–13. Paternal disomy of chromosome 15 was excluded as the cause of \(AS\) in this family. The maximum lod score of 5.40 for linkage with \(GABRB3\) and \(D15S10\) provides strong evidence for a dominant mutation in 15q11–13. For the first time the role of genomic imprinting in familial \(AS\) could be established by statistical analysis, as an odds ratio of \(9.25 \times 10^3\) in favour of genomic imprinting versus reduced penetrance was achieved. The pattern of imprinting in this family was such that no father passed on the disease, but only the females II.2, III.4, 8, 10, 13, and IV.2 (fig 1). The data indicate that the mutation causing \(AS\) in the eight family members originates from the male ancestor in generation I (fig 1). Apparently, the mutation was transmitted through as many as three generations by male carriers without any phenotypic expression. This fact is of great importance in view of the risk assessments for even distant female relatives, who may have a 50% risk of affected offspring depending on their position in the pedigree (fig 1).

The \(AS\) families reported by Hamabe et al. and Wagstaff et al. are in agreement with the concept of a dominant mutation in one or more genes on 15q11–13 subject to imprinting as the cause of familial \(AS\). Two other \(AS\) families have been reported that were investigated with DNA markers. In one case the affected sibs inherited the same maternal 15q11–13 region. The affected sibs in the other family inherited at least partly different maternal regions of 15q11–13, but a possible recombination event between the SRO of \(AS\) and the tested markers made the latter result inconclusive.

We conclude that the present molecular data from familial \(AS\) patients are either in favour of a dominant genetic defect on 15q11–13 with phenotypic expression depending on imprinting or do not contradict this hypothesis.

Further molecular genetic analysis of non-deletion \(AS\) patients will be essential to determine the gene(s) involved in the pathogenesis of \(AS\). Our data do not contradict a possible role of the \(GABRB3\) gene in the pathogenesis of \(AS\) as no recombinations were detected between \(AS\) and this locus in the entire family.

The consistent lack of PWS in the offspring of the male carriers of an \(AS\) mutation adds to the evidence that distinct defects are involved in the aetiologies of \(AS\) and PWS. In our family non-penetrance as an explanation for the lack of PWS offspring was highly unlikely as multiple transmissions of the mutation occurred through male meioses without phenotypic expression of PWS.

The influence of genomic imprinting on the expression of mutation has been described in a growing number of human genetic diseases. Hereditary paranglioma is another example where the role of genomic imprinting in the phenotypic expression in an extended family has been shown to be absolute.

The lack of implication of genomic imprinting in familial \(AS\) may complicate genetic counselling. In familial \(AS\) cases, an X linked mode of inheritance may falsely be deduced when the syndrome diagnosis is overlooked. On the other hand, imprinting may obscure the hereditary nature of the defect owing to the lack of phenotypic expression in offspring of male carriers. If a genetic defect at 15q11–13 and consequently genomic imprinting are a universal phenomenon in familial \(AS\), unaffected sibs of \(AS\) patients would have no increased risk for \(AS\) in their offspring. Risk assessments for more distant female relatives of \(AS\) patients, particularly those without detectable genetic defects, would necessarily involve an extensive search of the pedigree for \(AS\).

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