

ORIGINAL ARTICLES

Molecular mechanisms in Angelman syndrome: a survey of 93 patients

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Abstract

Angelman syndrome (AS) results from a lack of maternal contribution from chromosome 15q11-13, arising from de novo deletion in most cases or rarely from uniparental disomy. These families are associated with a low recurrence risk. However, in a minority of families, more than one child is affected. No deletion has been found in these families,^{1,2} except one.³ The mode of inheritance in these families is autosomal dominant modified by imprinting. Sporadic cases, with no observable deletion, therefore pose a counselling dilemma as there could be a recurrence risk as high as 50%. We present a series of 93 AS patients, showing the relative contribution of these different genetic mechanisms.

Eighty-one AS patients were sporadic cases while 12 cases came from six families. Sixty cases had deletions in 15q11-13 detected by a set of highly polymorphic (CA)_n repeats markers and conventional RFLPs. Ten sporadic cases plus all 12 familial cases had no detectable deletion. In addition, two cases of de novo deletions occurred in a chromosome 15 carrying a pericentric inversion. In one of these the AS child had a cousin with Prader-Willi syndrome (PWS) arising from a de novo deletion in an inv(15) inherited from his father. One case arose from a maternal balanced t(9;15)(p24;q15) translocation. There were three cases of uniparental disomy. Five patients were monoallelic for all loci across the minimal AS critical region, but the presence of a deletion cannot be confirmed.

In familial cases, all affected sibs inherited the same maternal chromosome 15 markers for the region 15q11-13. Two cases were observed with a de novo deletion starting close to the locus D15S11 (IR4-2R), providing evidence for the development of classical AS with smaller deletions.

Cytogenetic analysis proved limited in its ability to detect deletions, detecting only 42 out of 60 cases. However, cytogenetic analysis is still essential to detect chromosomal abnormalities other than deletions such as inversions and balanced

translocations since both have an increased risk for deletions. A staged diagnostic strategy based on the use of highly informative (CA)_n repeat markers is proposed.

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Angelman syndrome (AS) is a condition characterised by developmental delay, ataxia, absence of speech, fits, spontaneous laughter, and hypopigmentation.⁴ The incidence is estimated to be 1 in 20 000.⁵ In many cases it is associated with a cytogenetic deletion at 15q11-13.⁶⁻⁹ A similar cytogenetic deletion is also found in patients with Prader-Willi syndrome (PWS),^{8,10} a condition which is phenotypically distinct from AS. Children with PWS are characterised by developmental delay, hypotonia, hyperphagia, obesity, and hypogonadism.

The observations that the deleted chromosome 15 in AS is always maternal in origin^{11,12} but paternal in PWS¹⁰ suggest that this region of the human genome is subject to genetic imprinting. Consistent with this, both AS and PWS offspring have been described within a family that carries a balanced translocation between chromosomes 15 and 22.¹³ The sex of the parent transmitting the translocated chromosome determined whether the child developed AS or PWS. Reports of uniparental disomy (UPD) in both AS and PWS further strengthen the pathogenetic role of genetic imprinting in both conditions. Uniparental paternal disomy of chromosome 15 leads to AS¹⁴ while uniparental maternal disomy of chromosome 15 results in PWS.¹⁵

Although most cases of AS are sporadic and appear to have arisen as a result of spontaneous de novo deletions,¹⁶ it can occur in more than one sib within the same family. Wagstaff *et al*,¹⁷ described an extended pedigree of four AS children born to three sisters. No deletions at 15q11-13 were found but genetic analyses showed that the affected children had all inherited the same maternal chromosome 15 derived from the maternal grandfather. Meijers-Heijboer *et al*¹ have recently reported a large pedigree spanning five generations in which eight AS patients in six different sibships were born. The pattern of transmission in these families is consistent with an autosomal dominant mode of inheritance, but because of imprinting, the

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AS phenotype only develops when the abnormal chromosome 15 passes through the female germline. Since the mothers who were carrying and transmitting the abnormal chromosomes were phenotypically normal, these reports suggest that the AS and PWS critical regions are distinct. Hamabe *et al*³ described a family with three affected children all of whom had inherited the same maternal deletion spanning D15S10(3–21) from their maternal grandfather. This remains the only reported example of familial AS in which a small deletion has been found but serves to define a minimal AS critical region spanning D15S113(LS6–1), D15S10(3–21), and GABA β 3 receptor (GABRB3) distinct from a more proximal PWS critical region.¹⁸ Consistent with this observation, a maternally imprinted candidate gene for PWS, small nuclear ribonucleoprotein polypeptide N, is shown to map within the PWS but outside the AS critical region.^{19–21}

The order of the genetic markers mapping to the PWS and AS critical regions at 15q11–13 has now been defined and determined using a combination of overlapping yeast artificial chromosomes and fluorescent in situ hybridisation.^{18,22} Highly informative dinucleotide repeats, LS6–1CA(D15S113), 3–21 (D15S10), GABRB3, were localised to within the minimal AS critical region, placing IR4–3R (D15S11) and GABA α 5 receptor (GABRA5) on either flank of the minimal AS critical region.^{23–26} Using these (CA)*n* repeats together with conventional RFLP analyses and cytogenetic examinations, we surveyed the relative incidence of the various genetic mechanisms that could give rise to AS in 93 patients. This series consists of previously unreported AS patients as well as families that have previously been reported as examples of the mechanisms described above.^{2,7,12,14,27} As a result of this survey, we propose a staged diagnostic strategy for investigating AS families.

Subjects, materials, and methods

PATIENTS

Ninety-four AS patients from 87 families, referred from a number of centres, were seen by at least one of the authors. One adult affected sib declined to give a blood sample. The diagnoses of AS were confirmed based on clinical history and examination with most diagnoses supported by typical EEG findings. All except three patients had typical clinical features of AS with delayed motor milestones, absent speech, ataxia, and typical facial features. The remaining three patients were atypical in that one (BJ) had behavioural features but not typical facial features, one (RH) had typical facial features but showed marked autistic behaviour, and one (DM) had a hemiplegia as a result of birth asphyxia, making neurological assessment difficult.

Thirty-nine of these patients have been the subjects of previous reports. Of these, 29 have deletions at 15q11–13,^{7,12} five have normal karyotypes,⁷ and two have uniparental disomy.¹⁴ Spontaneous deletion in a maternal pericentric inversion, inv(15)(p11q13), produced an AS child in one family.²⁷

CYTOGENETIC STUDIES

To obtain good chromosome morphology at the 850 band level, samples from each patient were cultured by a variety of methods. Medium RPMI 1640 and FX1 were both used and chromosome elongation achieved either by the addition of 10 μ g/ml of ethidium bromide for the final two hours of a 72 hour culture period or by synchronising cell division with thymidine. For this, an excess of thymidine (300 mg/l) was added 24 hours before harvesting and the block released for four or five hours either by the addition of 5 mg/l of deoxycytidine or by washing the cells with fresh medium. Harvesting and slide making were by standard methods and the chromosomes were GTG banded. Well banded mitoses were then examined for the presence or absence of a deletion in the q11q13 region of chromosome 15. This centromeric region is polymorphic, so comparisons were made between chromosomes 15 from the probands and from their parents in order to determine the extent of familial variation in the size of the 15q11q13 region.

MOLECULAR GENETIC ANALYSES

RFLP analyses

DNA probes IR10–1 (D15S12), p34 (D15S9), 189–1 (D15S13), 3–21 (D15S10), IR4–3R (D15S11),²⁸ CMW–1 (D15S24),²⁹ and cMS620 (D15S86)³⁰ were used for RFLP analyses. Blood samples were collected into 10 ml EDTA tubes and DNA extracted by guanidium hydrochloride and Proteinase K.³¹ DNA (5 μ g) was digested with restriction enzymes according to the manufacturers' instructions. For 3–21(D15S10), 189–1(D15S13), and CMW–1(D15S24), DNA was digested with *Taq*I, while *Sca*I was used for p34(D15S9) and IR10–1(D15S12) and *Sty*I for IR4–3R(D15S11). The DNA was then size fractionated on 0.8% agarose gels, denatured, and blotted onto Hybond N+ filters (Amersham) by alkaline capillary transfer. Filters were hybridised overnight with probes radiolabelled by the random hexanucleotide priming method.³² After washing in 0.1 \times SSC and 0.1% SDS at 65°C for one hour the filters were exposed to Kodak XOMAT films at –70°C.

(CA)*n* repeats

Six (CA)*n* repeats, IR4–3R (D15S11),²⁴ LS6–1CA (D15S113),²⁶ 3–21 (D15S10),²³ MS14 (D15S97),³³ GABRB3,³⁴ and GABRA5²⁵ were used. MS14 (D15S97) was mapped to a GABRB3 containing yeast artificial chromosome B25D10 (data not presented). The primers used were as published. The dinucleotide fragments were amplified from ~250 ng of genomic DNA in a 20 μ l reaction volume typically containing 10 mmol/l Tris–HCl, pH 8.3, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 100 μ g/ml BSA, 250 μ mol/l dATP, 250 μ mol/l dGTP, 250 μ mol/l dTTP, 2.5 μ mol/l dCTP, 6.67 \times 10^{–14} mol α -³²P dCTP (111TBq/mmol), and 0.5 units of *Taq* polymerase. The cycling conditions were 95°C for two minutes initially,

Genetic mechanisms underlying the development of Angelman syndrome in the 93 patients.

Summary of results

Total number of patients = 94* (87 families)
 Patients with deletions = 60
 18 cytogenetically normal
 42 detected cytogenetically
 Patients with no detectable deletions = 15
 (5 reported to have cytogenetic deletions)
 10 heterozygous for one or more (CA)_n repeats
 5 monoallelic for 4 (CA)_n repeats: MS14(D15S97),
 3-21(D15S10), IR4-3R(D15S11), GABRB3
 Patients from familial cases = 13* (6 families)
 Patients with uniparental disomy = 3
 1 heterodisomy
 2 isodisomy
 Patients with chromosomal abnormalities other than deletion
 2 pericentric inversion of chromosome 15
 1 unbalanced translocation

* Includes one affected sib in whom no blood sample can be obtained.

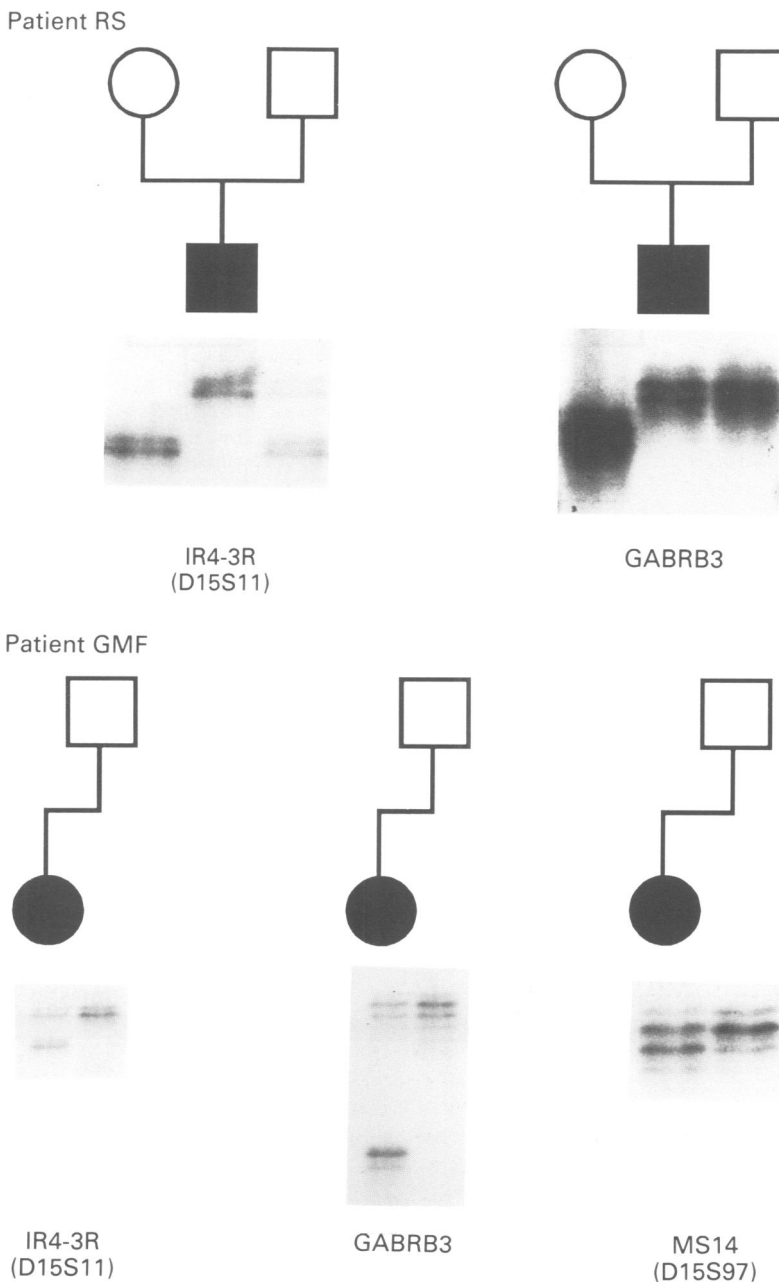


Figure 1 Detection of deletions at 15q11-13 in Angelman syndrome with (CA)_n repeats. (CA)_n repeat genetic markers mapping to the AS/PWS critical region are highly informative for the presence or absence of a deletion at 15q11-13. Patient RS is deleted for the maternal allele at the loci IR4-3R (D15S11) and GABRB3. Since the mother is heterozygous for GABRB3, the deletion in patient RS must have arisen de novo. Patient GMF is reported to have a cytogenetic deletion at 15q11-13. She is heterozygous and therefore cannot be deleted for the loci IR4-3R (D15S11), GABRB3, and MS14 (D15S97).

then 25 cycles of 95°C (denaturation) for 20 seconds, 55°C (annealing) for 20 seconds, 72°C (extension) for 30 seconds before finishing at 72°C for two minutes. Dinucleotide repeats for MS14(D15S97) were amplified using an annealing temperature of 50°C while GABRB3 dinucleotide repeats were amplified in 1.0 mmol/l MgCl₂. The radiolabelled fragments were then size fractionated in the presence of 7 mol/l urea with 6% polyacrylamide. The gels were dried and exposed to Kodak XOMAT films.

Results

The results are summarised in the table.

PATIENTS WITH DELETIONS AT 15q11-13

Sixty patients were found to have molecular deletions at 15q11-13. Of these, 42 had been detected cytogenetically but 18 had been reported as cytogenetically normal (fig 1). Fifty-six (93%) deletions of maternal origin were detected using just three (CA)_n repeats: GABRB3, MS14(D15S97), and IR4-3R(D15S11). Three more deletions were found when three additional (CA)_n repeats, 3-21(D15S10), GABRA5, and LS6-1CA (D15S113), were used. One patient (HuK), who was monoallelic for five (CA)_n repeats, IR4-3R(D15S11), GABRB3, MS14(D15S97), LS6-1CA(D15S113), and 3-21(D15S10), had a deletion at D15S13 shown by 189-1 RFLP analysis. Spontaneous deletions within 15q11-13 were shown in 40 patients since their mothers were heterozygous for at least one of the corresponding loci. Of these 40, spontaneous deletions within the minimal AS critical region, as defined by the loci D15S113(LS6-1), D15S10(3-21), and GABRB3,³ could be confirmed in 33. None of the 60 patients was heterozygous for any of the markers in 15q11-13. Uniparental paternal disomy was excluded in all, except three patients by cMS620(D15S86) RFLP or cytogenetic analyses.

PATIENTS WITH SMALL DELETIONS

Two of the 60 deleted patients had deletions which began distal to or within the locus D15S11 (figs 2 and 3).

Patient BP was deleted for MS14(D15S97) and IR4-3R (CA)_n repeats but was heterozygous for the IR4-3R *StyI* RFLP. He was monoallelic for 189-1(D15S13), GABRB3, 3-21(D15S10), IR39(D15S18), and IR10-1(D15S12). These results indicated that the breakpoint on his chromosome 15 lies within the locus D15S11 flanked by IR4-3R *StyI* RFLP and IR4-3R (CA)_n repeats (figs 2 and 3). This patient's DNA was digested with five different restriction enzymes and analysed by Southern hybridisation. No altered fragments were seen. These results showed that IR4-3R (CA)_n repeats must map distal to the *StyI* RFLP.

Patient CE was deleted for the GABRB3 and MS14 (CA)_n repeats (D15S97) but was heterozygous for the IR4-3R (CA)_n repeat (D15S11). Spontaneous deletion spanning the minimal AS

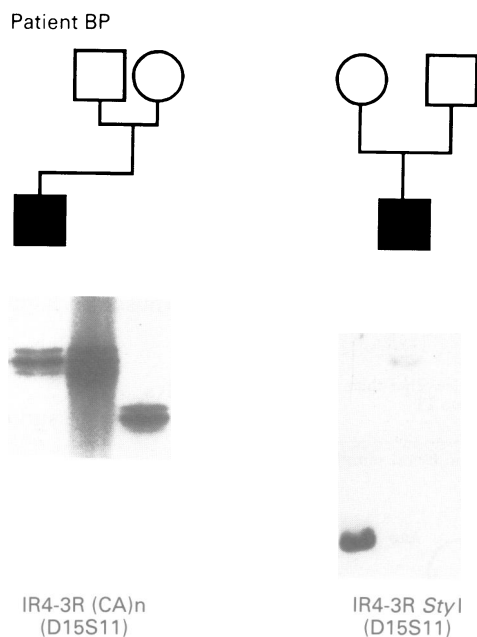


Figure 2 The breakpoint in AS can occur within or distal to D15S11 (IR4-3R). Patient BP is deleted for the maternal loci D15S97 (MS14) (results not shown) and IR4-3R (D15S11) (CA)n repeat. However, he is clearly heterozygous (not deleted) for the IR-4-3R (D15S11) StyI RFLP. The IR4-3R (D15S11) (CA)n repeats must therefore lie distal to the IR4-3R (D15S11) StyI RFLPs. Patient CE is deleted for the loci D15S97 (MS14) and GABRB3 but is heterozygous (not deleted) for IR4-3R (D15S11) (CA)n. His breakpoint is therefore more distal than that of patient BP. When the DNA of these two patients was digested by five different restriction enzymes and analysed by the Southern technique, no altered fragments were seen. This suggests that the breakpoints in both patients are probably > 20 kb from D15S11.

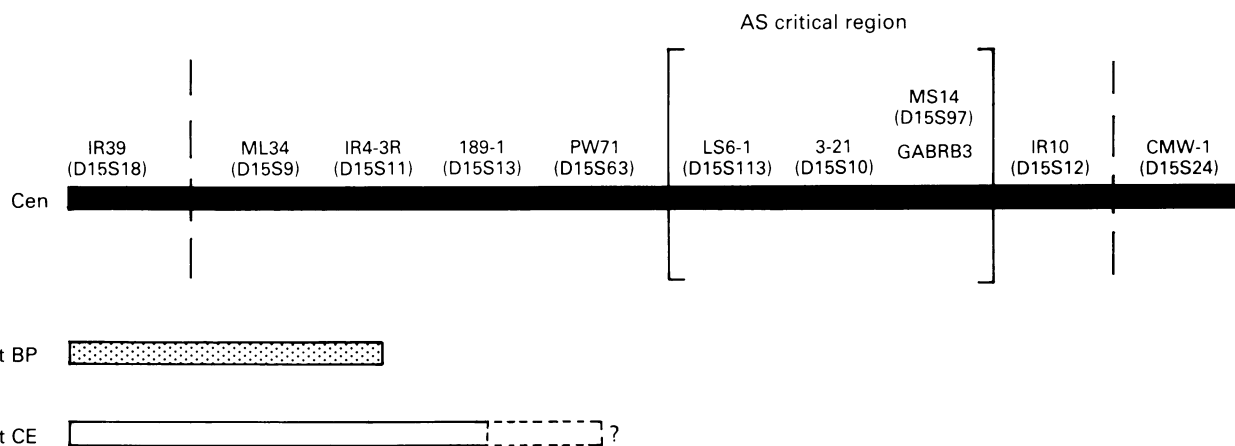
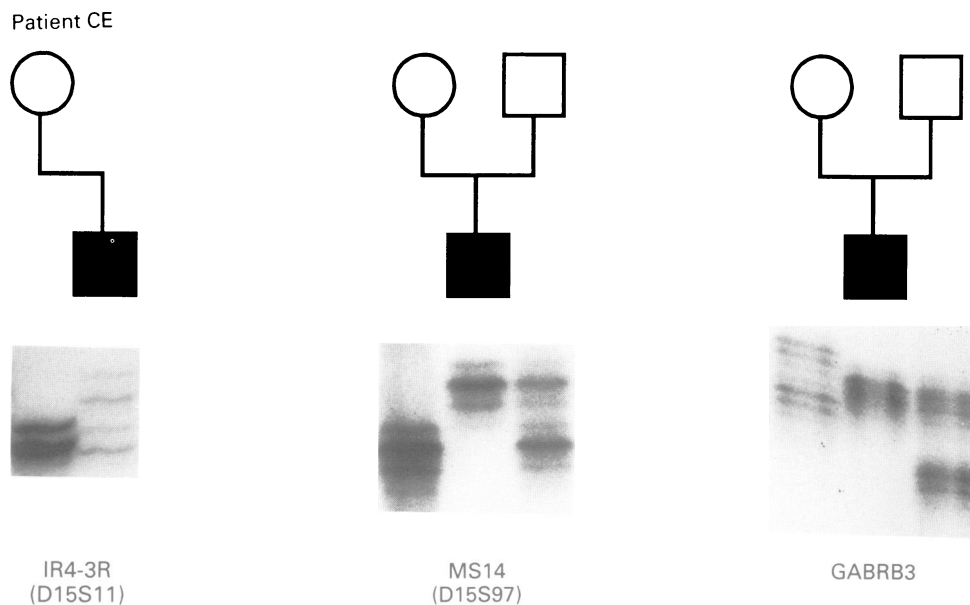


Figure 3 Schematic map of the PWS and AS critical regions, showing the order of the loci as reported by Kuwano et al.¹⁸ and Knoll et al.²² The brackets indicate the minimal AS critical region while the vertical dotted lines show the common breakpoints for AS and PWS patients. The breakpoint for patient BP (dotted bar) maps to within D15S11 (IR4-3R) while that of patient CE maps distal to D15S11 (IR4-3R). Cen = centromere.

critical region was confirmed since his mother is heterozygous for GABRB3 (fig 2). He was monoallelic for 189-1(D15S13), 3-21(D15S10), IR10-1(D15S12), IR39(D15S18), and CMW-1(D15S24) (fig 2). His breakpoint must therefore lie more distal than that of patient BP (fig 3).

FAMILIAL CASES OF AS

Thirteen patients were seen from six families that had more than one child affected with AS. Only one of these families had a normal child but one of her two affected sibs declined to give a blood sample. Of the 12 who were analysed, all were cytogenetically normal and heterozygous for one or more loci within the minimal AS critical region. No molecular deletion was found. Analyses with IR4-3R(D15S11) *S_{ty}I* RFLP, IR4-3R(D15S11) (CA)_n repeats, MS14(D15S97), and GABRB3 showed that the affected children always received the same maternal chromosome 15q11-13. This strongly supports the previous hypothesis that the pattern of inheritance is consistent with an autosomal dominant mode of inheritance modified by imprinting.

PATIENTS WITH NO DETECTABLE DELETIONS AT 15q11-13

Molecular deletions could not be shown in a further 15 patients. Five patients were monoallelic for four (CA)_n repeats: MS14(D15S97), GABRB3, IR4-3R(D15S11), and 3-21(D15S10). Ten patients were heterozygous for one or more loci within 15q11-13. Three of these 10, BJ, DM, and RH, had small variations from the classical features of AS (see Subjects, materials, and methods).

UNIPARENTAL DISOMY

RFLP analyses with cMS620(D15S86), a marker that maps to the distal end of chromosome 15, showed that three patients developed AS as a result of uniparental disomy. Two of the UPD patients were the subjects of a previous report.¹⁴ One of these patients, who was previously shown to have uniparental heterodisomy, was also heterozygous for IR4-3R (CA)_n repeat (D15S11), CMW-1(D15S24), and cMS620(D15S86). This indicates that his uniparental paternal heterodisomy extends proximal to and probably covers the minimal AS critical region. The remaining two patients were monoallelic at all loci within 15q11-13 and CMW-1(D15S24) plus cMS620(D15S86), consistent with uniparental paternal isodisomy.

CHROMOSOMAL ABNORMALITIES

Cytogenetic analyses showed that two families had pericentric inversions of chromosome 15. Both resulted in cytogenetically visible deletions in two AS offspring (LD and EM) plus a PWS patient (BA) in one branch of the pedigree.

Deletion in the affected child (EM) of a

family carrying pericentric chromosome 15 inversion^{7,27} was confirmed by using three (CA)_n repeats, GABRB3, MS14(D15S97), and IR4-3R(D15S11).

In a second previously unreported family, inheritance of an inverted chromosome 15 led to a cytogenetically visible deletion at 15q11-13, resulting in PWS and AS in two different branches of the pedigree. On one branch, the normal father inherited the inverted chromosome 15 from the maternal grandmother. This resulted in a deletion at 15q11-13 and the development of PWS in his son (BA). His normal daughter also inherited the inverted chromosome 15. In the other branch, the normal mother gave birth to an AS child (LD) who was also found to have a deletion at 15q11-13, confirmed by GABRB3 (CA)_n repeat analysis. The other unaffected child received a normal chromosome 15.

An AS child was born to a mother with the karyotype 46,XX,t(9;15)(p24;q15). Cytogenetic analysis showed that the karyotype of the child was 45,XX,-9,-15,+der(9),t(9;15)(p24;q15)mat. She had AS since she was monosomic for 15q11-13.

Discussion

Although Angelman syndrome commonly results from the lack of maternal contribution because of deletions or uniparental disomy, familial transmissions do occur in a minority of cases. To date, only one recurrent AS family has been shown to be transmitting a small deletion, involving the loci D15S113(LS6-1), 3-21(D15S10), and GABRB3.^{3,18,35} No other cases of familial AS have demonstrable deletions.^{1,2,17} For these reasons, sporadic cases with no observable deletions can have a recurrence risk as high as 50%. Families in which the absence of maternal alleles in the minimal AS critical region cannot be shown to be the result of spontaneous deletions can be transmitting a constitutional deletion.³ We therefore surveyed our panel of 93 AS patients in order to assess relative contributions of various genetic mechanisms in causing AS. We also present a staged diagnostic strategy based on the use of highly informative (CA)_n repeat markers.

DELETION DETECTION

Consistent with previous reports,^{6,7} we found that most (64.5%, 60 of 93 patients) of our patients with Angelman syndrome are the result of de novo maternal deletions at 15q11-13. However, when uniparental disomy, chromosomal, and familial cases were excluded, 60 out of 75 (80%) of our patients have demonstrable deletions. Detecting deletions at 15q11-13 cytogenetically can be difficult even in skilled hands and molecular confirmation is essential. A number of highly polymorphic dinucleotide (CA)_n repeats have been described recently^{23-26,33,34} that map to the PWS/AS critical region¹⁸ with heterozygosities that range from 73% to 82.5%.^{23-26,34} These, together with conven-

tional RFLPs, were exploited to characterise our panel of 93 AS patients.

Our experience showed that a combination of six highly polymorphic (CA)_n repeats detected over 90% of all deletions. Of the 60 patients with demonstrable deletions, only one (HK) was not detected by these (CA)_n repeats but by a traditional RFLP. The deletions in the two patients from the families with pericentric inversion were confirmed. Five other patients are monoallelic for MS14(D15S97), IR4-3R(D15S11), LS6-1CA (D15S113), and GABRB3. These patients probably have unconfirmed deletions since the heterozygosities at IR4-3R(D15S11), LS6-1CA(D15S113), and GABRB3 are 74%, 73%, and 83%, respectively.^{24,26,34} If this is the case, we have detected 91% (61 out of 67 deletions) of patients with true deletions. It is interesting that with just three (CA)_n repeats, IR4-3R(D15S11), MS14(D15S97), and GABRB3, we can already identify 85% of all the deletion patients. Since the mothers of 47 deleted patients are heterozygous for the loci deleted in their children, these deletions must have arisen *de novo* during oogenesis. That is, these mothers are not harbouring constitutional deletions as in the family reported by Hamabe *et al.*³

During the course of our study, parental origin specific methylation differences have been described with probes from D15S9(ML34)³⁶ and D15S63(PW71)³⁷ which will be of considerable use in the screening of AS and PWS patients for deletions. This potentially provides an alternative strategy for at least detecting large deletions. Our finding of AS patients whose deletions do not extend to ML34 is interesting and poses a potential problem for the interpretation of methylation studies.

Our results also showed that, because of its subjective nature, cytogenetic assessment at 15q11-13 can be misleading. Nineteen patients with deletions were thought to be cytogenetically normal while five patients who were thought to have cytogenetic deletions were heterozygous for at least one (CA)_n repeat. Two patients with reported cytogenetically observed deletions, GMF and CK, were heterozygous for IR4-3R(D15S11), MS14(D15S97), and GABRB3, probes extending right across the minimal AS critical region and beyond.

VARYING BREAKPOINTS IN AS PATIENTS

Knoll *et al.*³⁸ described three classes of AS patients. Class I patients are deleted for the loci D15S9(ML34), D15S10(3-21), D15S11(IR4-3R), D15S13(189-1), D15S18(1R39), and D15S12(IR10) that map within 15q11-13, while class II patients are deleted for all loci except D15S18(IR39). Class III patients have no detectable deletions. Two of our patients, however, have deletions which started from within (BP) or distal (CE) to the locus D15S11(IR4-3R) (fig 3). In contrast, the deletion in the AS family reported by Hamabe *et al.*³ involved only D15S10(3-21),

D15S113(LS6-1), and GABRB3.¹⁸ Thus the breakpoints in AS patients can occur at multiple points within 15q11-13.

FAMILIAL CASES

AS is known to recur in families. In contrast to sporadic cases, the pattern of AS transmission suggests that their constitutional genetic defects were transmitted in an autosomal dominant fashion.³⁹ However, because of genetic imprinting the manifestations are restricted only to the offspring of females who have inherited the abnormal gene(s). Six out of 87 (6.9%) of our families have more than one AS child. No deletions were found in these patients, consistent with the fact that the family described by Hamabe *et al.*³ is still the only one in which a deletion has been detected. Within each of our families, all the affected children received the same maternal alleles at 15q11-13, indicating that it is possible in these families to predict the development of AS in a subsequent offspring.

NON-DELETION CASES

We could not detect deletions in 15 of our patients. All our patients with proven deletions, except patients CE and BP, are monoallelic for all loci spanning IR39(D15S18) to IR10(D15S12) which cover the minimal AS critical region. Five of these 15 are also monoallelic across the AS minimal critical region, from D15S11(IR4-3R) to GABRB3. These patients probably have undetected deletions. However, the remaining 10 are heterozygous for at least one locus within the minimal AS critical region, suggesting that like the non-deletion familial cases, they have either point mutations or very small deletions. It is interesting that three of these 10 have small variations from the classical features of AS.

UNIPARENTAL DISOMY

As a result of the paternal imprinting (inactive paternal allele) in the AS critical region, AS can also result from uniparental paternal disomy 15. Most non-deletion cases of PWS arise from uniparental maternal disomy⁴⁰; in contrast, uniparental paternal disomy in AS is rare.^{14,41} Only three of our 93 AS patients are the result of uniparental paternal disomy. One of these three is heterodisomic for the proximal as well as distal regions of chromosome 15 as he has inherited both paternal alleles proximal to the minimal AS critical region as well as on the distal end of chromosome 15.

DIAGNOSTIC STRATEGY

Defining the underlying genetic mechanism that gives rise to the disorder in a sporadic AS patient is fundamental in the accurate assessment of recurrence risks as well as in the confirmation of diagnosis. The risk of having a second AS child in families with spontaneous deletions or uniparental disomy is low and no such example has been reported. Certain chro-

mosomal rearrangements, such as inversions or balanced translocations, carry a higher recurrence risk. The majority of cases of familial AS have no detectable deletions,^{1,39} but it is now shown that deletion within 15q11–13, albeit small, can also occur in familial AS.³ This poses a counselling difficulty, as demonstration of absent maternal alleles cannot automatically lead to the conclusion that families with a sporadic AS child have low recurrence risks.

We suggest a staged diagnostic approach for newly diagnosed AS families. Patients with clinical features suggestive of AS should be screened cytogenetically for chromosomal rearrangements, such as inversions and balanced translocations. Subjective cytogenetic screening for deletions at 15q11–13 should be supported by screening with the six (CA)_n repeats that are currently available. In addition, detection of parental origin specific methylation at D15S9(ML34)³⁶ and D15S63(PW71)³⁷ may be used to detect large deletions. At the same time, uniparental disomy should be excluded by the highly polymorphic probe cMS620 that maps to the distal end of chromosome 15 or through cytogenetically visible polymorphisms.

Demonstrations of heterozygosity at any one of the loci within the AS critical region, such as D15S113(LS6), D15S10(3-21), and GABRB3, implies that such patients do not have deletions. Since no familial cases of AS have detectable deletions except one,³ such families may have recurrence risks as high as 50%. Demonstration of absent maternal alleles in the presence of maternal heterozygosity in the corresponding loci at 15q11–13 suggests that de novo deletion must have occurred and these families have a low recurrence risk. Similarly, the demonstration of uniparental disomy also implies low recurrence risk as no such examples have been reported.

However, difficulties arise if the absence of a maternal allele in the patient is accompanied by a mother monoallelic for all the loci mapping within the minimal AS critical region. Spontaneous deletion in the affected child cannot be automatically assumed. There is a small, though distinct, possibility the mother has a constitutional deletion in her non-functional paternal chromosome 15q11–13, and has transmitted this to the affected child.³

Although our experience showed that over 90% of patients with deletions can be quickly identified with just three (CA)_n repeats, IR4-3R(D15S11), MS14(D15S97), and GABRB3, we found that a number of patients will remain undefined, despite using all six (CA)_n repeats. This is often because of the pattern of inheritance of the alleles so that no definitive conclusions could be drawn but a deletion is strongly suspected because loci across 15q11–13 are all monoallelic.

In these circumstances, methylation specific fragments at D15S63(PW71) and D15S9(ML34) will identify those with large undetected deletions. However, in the absence of such confirmation, a high recurrence risk must be advised. Kuwano *et al.*¹⁸ suggested that FISH

may be applied to assess AS and PWS patients and resolve these problems; unfortunately, this specialised technique is not yet universally available. Our strategy, with slight modifications, can equally be applied to newly diagnosed PWS patients.

Cell lines from the mother of the patient with an unbalanced translocation (No 8689), the inversion patient EM (No 5705), and the father of the inversion PWS patient BA (No 5769) are available from Paul Rutland, Unit of Molecular Genetics, Institute of Child Health, 30 Guilford Street, London WC1N3JH, UK.

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