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Angelman Syndrome: Three Molecular Classes Identified with Chromosome 15q11q13–specific DNA Markers

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Summary

Angelman syndrome (AS) and Prader-Willi syndrome (PWS) share a cytogenetic deletion of chromosome 15q11q13. To determine the extent of deletion in AS we analyzed the DNA of 19 AS patients, including two sib pairs, with the following chromosome 15q11q13–specific DNA markers: D15S9-D15S13, D15S17, D15S18, and D15S24. Three molecular classes were identified. Class I showed a deletion of D15S9–D15S13 and D15S18; class II showed a deletion of D15S9–D15S13; and in class III, including both sib pairs, no deletion was detected. These molecular classes appear to be identical to those observed in PWS. High-resolution cytogenetic data were available on 16 of the patients, and complete concordance between the presence of a cytogenetic deletion and a molecular deletion was observed. No submicroscopic deletions were detected. DNA samples from the parents of 10 patients with either a class I or a class II deletion were available for study. In seven of the 10 families, RFLPs were informative as to the parental origin of the deletion. In all informative families, the deleted chromosome 15 was observed to be of maternal origin. This finding is in contrast to the paternal origin of the deletions in PWS and is currently the only molecular difference observed between the two syndromes.

Introduction

Angelman syndrome (AS) is characterized by severe mental retardation, inappropriate bouts of laughter, ataxic gait, lack of speech, puppet-like upper-limb positioning and movements, microcephaly, and/or abnormal EEGs (Angelman 1965; McKusick 1989). High-resolution chromosome studies have revealed that at least half of AS patients have a cytogenetic deletion of chromosome 15q11q13, while the others have apparently normal chromosomes (Williams et al. 1989; Pembrey et al. 1989). These cytogenetic findings are shared by patients with Prader-Willi syndrome (PWS), a genetic and dysmorphic form of obesity. The cytogenetic similarities of these two clinically distinct syndromes may reflect different molecular deletions or mutations, different parental origins of the deletions (Butler and Palmer 1983; Knoll et al. 1989*a*), or different parental origin "deletion equivalents" such as uniparental disomy (Nicholls et al. 1989*b*). Previously, molecular studies on chromosome 15q11q13 have focused primarily on PWS. We have now examined DNA of this chromosomal region in 19 AS patients by utilizing eight chromosome 15q11q13–specific DNA markers, in an attempt to differentiate cytogenetically indistinguishable deletions, to determine whether submicroscopic deletions exist and to compare the findings in AS with those in PWS.

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Subjects and Methods

Subjects

Nineteen AS patients (10 males and nine females)

from 17 different families, as well as the parents of 12 of these families, were available for study. Clinical features of 11 of the patients have been described elsewhere. These 11 patients are WJK8 and WJK36 (Magenis et al.'s [1987] patients 1 and 2, respectively); WJK10, WJK24, WJK35, WJK43, WJK70, and WJK67 (Magenis et al.'s [1990] patients 1–4 and 6 and 7, respectively); WJK48 (Kaplan et al. 1987); and sib pair WJK1 and WJK4 (Pashayan et al. 1982). The clinical findings of all patients in the present study were consistent with those described by Angelman (1965).

Laboratory Methods

Cytogenetic studies.—Metaphases from PHA-stimulated peripheral blood lymphocytes or lymphoblasts on most AS patients and on several parents were prepared. The preparations were G-banded by the trypsin-Giemsa method (Seabright 1971). High-resolution chromosome analyses were performed on prometaphase spreads prepared by amethopterin synchronization (Yunis 1976). High-resolution chromosome 15 analyses have been reported on WJK8 and WJK36 (Magenis et al.'s [1987] patients 1 and 2, respectively); WJK48 (Kaplan et al. 1987); WJK10, WJK24, WJK35, WJK43, WJK70, and WJK67 (Magenis et al.'s [1990] patients 1–4 and 6 and 7, respectively); WJK29 (Knoll et al. 1989*a*); and DON34 (Donlon 1988). Prometaphase analyses on one sib pair previously reported by Pashayan et al. (1982) were repeated with focus on the chromosome 15s. In addition, high-resolution cytogenetic analyses were performed on WJK18 and on the sib pair WJK14 and WJK15. Absence of chromosome 15q11.2 was the minimal criterion for diagnosing a cytogenetic deletion.

Molecular studies.—Genomic DNA was extracted from lymphocytes or lymphoblasts (Aldridge et al. 1984). Restriction-enzyme digests of 1–3 μg of DNA were performed (5 U/μg), according to the method suggested by the manufacturer (New England Biolabs) by using *Hin*dIII or enzymes appropriate for identifying RFLPs (Nicholls et al. 1989*a*). Digested DNA fragments were separated by agarose-gel electrophoresis, transferred to nylon membranes (Hybond N; Amersham), and hybridized with DNA probes radiolabeled with α-^{32}P dCTP by the random-primer method (Feinberg and Vogelstein 1983). Following overnight hybridization at 42°C, the filters were washed in 0.1 × SSC, 0.5% SDS at 55°C for 1 h and exposed to X-ray film.

The following chromosome 15q11q13–specific DNA probes were used for this study: 34 [D15S9], 3-21 [D15S10], IR4-3R [D15S11], IR10-1 [D15S12], 189-1 [D15S13], IR29-1 [D15S17] (Donlon et al. 1986), IR39d—a *Sac*I/*Hin*dIII subfragment of IR39 [D15S18] (Nicholls et al. 1989*b*), and CMW-1 [D15S24] (Rich et al. 1988). RFLPs for these probes have been described elsewhere (Nicholls et al. 1989*a;* Rich et al. 1988). In addition, a control probe, H2-26 [D13S28] (Lalande et al. 1984), was hybridized to *Hin*dIII-digested DNA.

Deletions in AS DNA samples, detected by comparing polymorphic alleles or by quantitative-dosage blot hybridization, were evaluated either as the absence of an allele in families with informative RFLPs or as a 50% reduction in the intensity of the hybridization band detected by the DNA probe.

Results

DNA samples from 19 AS patients and from 23 of their parents were analyzed by using cloned chromosome 15q11q13–specific markers. The patients could then be grouped into three classes as shown in figure 1. Class I and class II have deletions which differ only by the presence or absence of D15S18. The patients within these two classes had a cytogenetic deletion of chromosome 15q11q13, as shown in table 1. No deletions were detected when the cloned DNA markers were used in class III individuals, and no cytogenetic deletions were observed in these patients (table 1). There was complete concordance between the molecular and cytogenetic data. No deletions of D15S17 were observed in the three classes, and no deletions of D15S24 were detected in the 13 AS patient samples examined.

The distinction between class I and class II is seen

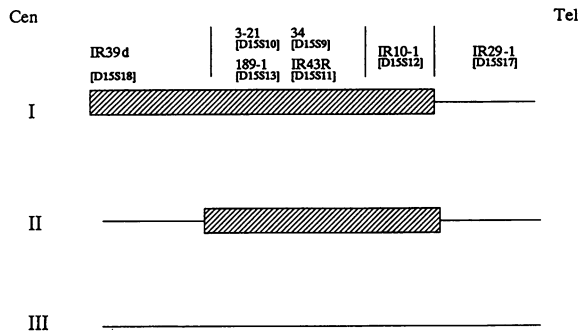

Figure 1 Schematic representation of three molecular DNA classes in AS. A cross-hatched box (▨) denotes a deleted section; a line (—) denotes an intact section. Class I is deleted for D15S9–D15S13 and D15S18. Class II is deleted for D15S9–D15S13, and class III has no detectable deletion. D15S17 is intact in all classes. Deletion analysis in patients with cytogenetic deletions and in a translocation PWS patient (Tantravahi et al. 1989) has allowed orientation of the probes with respect to the centromere (Cen) and the telomere (Tel).

Table 1

Relationship between Molecular Classes and Cytogenetic Data

Class and AS Patient(s)	Cytogenetics[a]
I:	
WJK8, WJK29, WJK43, DON34, WJK67, WJK70, and WJK36	−
II:	
WJK10, WJK18, WJK24, WJK48, and WJK35	−
WJK53	NT
III:	
WJK1 and WJK4 (sib pair)	+
WJK14 and WJK15 (sib pair)	+
WJK52 and WJK81	NT

[a] A minus sign (−) = del 15(q11q13); a plus sign (+) = no deletion; NT = not tested.

in figure 2. Class I is deleted for IR39d, and class II is intact for IR39d. This defines the extent of the critical region at the centromeric end. At the telomeric end there is less variability, for probes IR29-1 (fig. 3*A*) and CMW-1 were always intact. Probes 34 (fig. 3*B*), 3-21, 189-1, IR10-1, and IR43R are within the cytogenetic deletion and are deleted in all class I and class II patients.

DNA samples from the parents of 10 of the deletion patients were available for study. The maternal origin of the deleted chromosome 15 was reported elsewhere (Knoll et al. 1989*a*) for WJK10, WJK29, WJK35, and WJK36 and has been extended to include three more AS families. WJK18 and WJK67 showed informative segregation of RFLPs for probes 3-21 and IR43R, respectively, while WJK70 showed informative segregation of alleles for probe 3-21, as shown in figure 4, and for probes 34 and IR10-1 (not shown). Parental origin of the deleted chromosome 15 could not be determined in WJK48 (Knoll et al. 1989*b*), WJK8, and WJK24 by using RFLPs. In all patients with informative RFLPs, the deletion was of maternal origin.

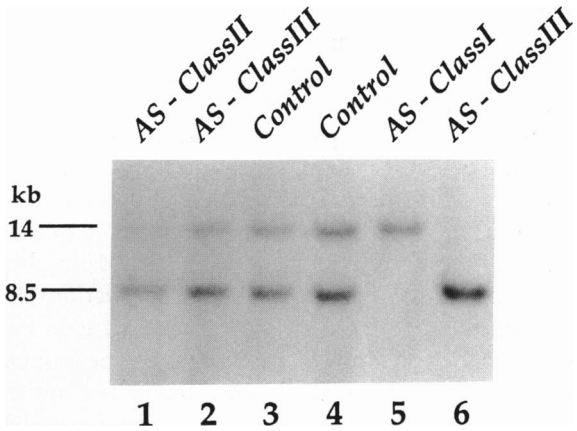

Figure 2 Delineation of classes I and II in AS. Probe IR39d (D15S18) detects a two-allele polymorphism at 14 and 8.5 kb on *Sac*I-digested DNA. The presence or absence of IR39d distinguishes cytogenetic deletions into classes I and II. DNAs in lanes 1–4 are heterozygous for IR39d and therefore are not deleted. DNA in lane 1 is from a class II patient (WJK53), that in lane 2 is from a class III patient (WJK52), and that in lanes 3 and 4 is from control heterozygotes (WJK37 and WJK38). AS patient DNA in lane 5 is deleted for IR39d and is therefore class I (DON34), and the AS patient DNA in lane 6 is homozygous and intact for the 8-kb allele and is from class III (WJK81). The distinction between class II and class III is dependent on the results of D15S9–D15S13. DNA loading was equivalent in lanes 2 and 3 and in lanes 4–6.

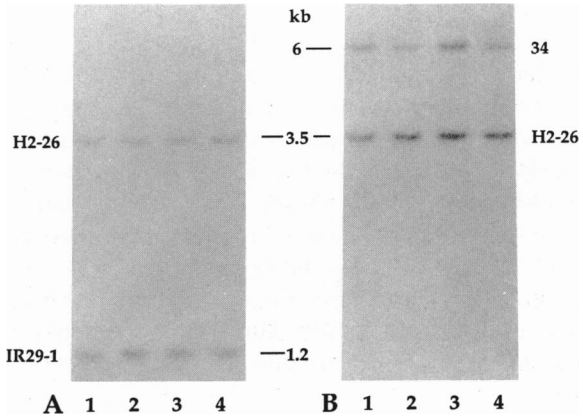

Figure 3 Quantitative hybridization of IR29-1, a chromosome 15q11q13–specific marker distal to the deletion, and 34, a chromosome 15q11q13 marker within the critical region. *A*, Hybridization of probe IR29-1 and of a control chromosome 13 probe, H2-26, to *Hin*dIII-digested DNAs. *B*, Rehybridization of the same filter with probe 34. DNA samples are from AS patients (lane 2, WJK70; lane 4, WJK67) and from AS parents (lane 1, WJK65; lane 3, WJK71). H2-26, IR29-1, and 34 detect 3.5-, 1.2-, and 6-kb fragments, respectively. IR29-1 does not detect a deletion in the DNA of AS patients, whereas 34 does.

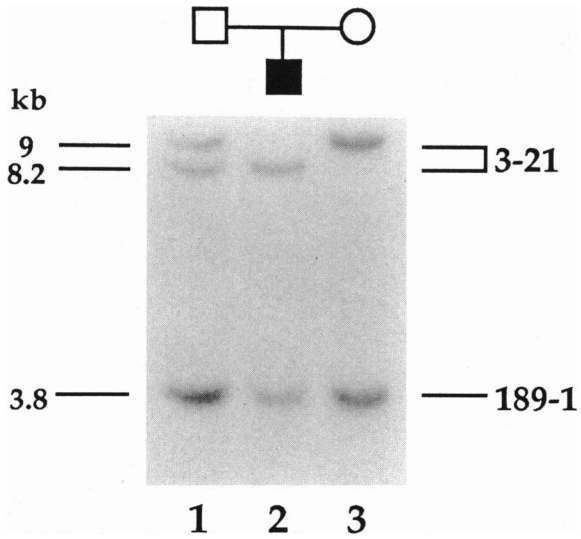

Figure 4 Segregation of RFLPs in an AS family. Probe 3-21 (D15S10) detects a three-allele polymorphism at 9, 8.9, and 8.2 kb on *Taq*I-digested DNA. Probe 189-1 (D15S13) detects 3.8- and 2-kb alleles on *Taq*I-digested DNA. The patient (WJK70; lane 2) is informative for probe 3-21 and shows the deletion to be of maternal origin, as the patient did not inherit a 9-kb maternal allele (WJK71; lane 3) but inherited only an 8.2-kb paternal allele (WJK65; lane 1). DNA marker 189-1 is not informative for parental origin of the deletion in this family but shows quantitatively that 189-1 is deleted in the patient. All lanes were loaded with 3 μg of DNA.

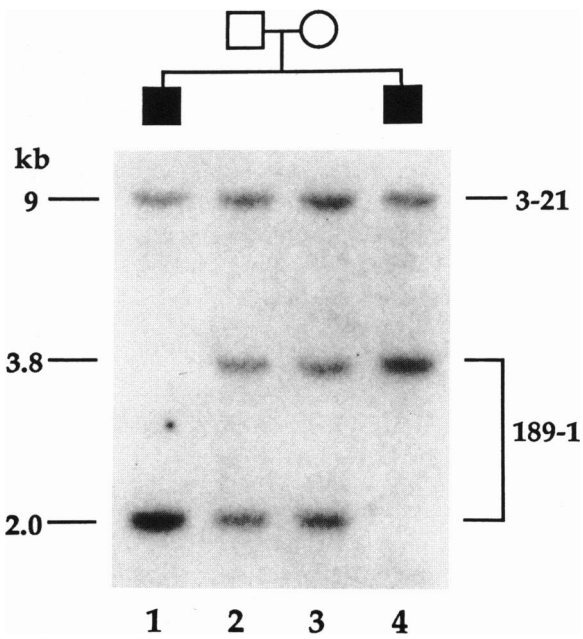

Figure 5 Inheritance of chromosome 15s in affected sibs. Each sib received different paternal and different maternal chromosome 15s. When probe 189-1 is used on *Taq*I-digested DNA, the parents (WJK2 and WJK3; lanes 2 and 3, respectively) are heterozygous for probe 189-1, and each son (WJK1 and WJK4; lanes 1 and 4, respectively) is homozygous for different alleles of probe 189-1. The parental origin of the chromosome 15s cannot be determined by using probe 3-21, as all individuals are homozygous for a 9-kb allele. All lanes were loaded with 3 μg of DNA.

Of the AS patients with apparently normal chromosomes, DNA samples from the parents of the two sib pairs were available. In one sib pair (WJK1 and WJK4) it was determined cytogenetically that each sib inherited a different paternal chromosome 15, as the father had two heteromorphic chromosome 15s. Molecular analyses with probe 189-1 confirmed the cytogenetics and revealed that each sib also had received a different maternal chromosome 15, as shown in figure 5. The parental origin of the chromosome 15s in the other sib pair (WJK14 and WJK15) was determined by DNA analyses. Both patients received the same maternal chromosome at IR39d and CMW-1. All other loci were homozygous and could have been either maternally or paternally derived.

Discussion

The molecular data reported here substantiate the cytogenetic findings that deletions of chromosome 15q11q13 occur in AS (Kaplan et al. 1987; Magenis et al. 1987). The higher frequency (12/14) of families with visible deletions in our sample, compared with those reported by others (Pembrey et al. 1989; Williams et al. 1989), may reflect our initial sample selection, in which we analyzed individuals with known cytogenetic deletions. All patients with microscopic deletions had molecular deletions. Conversely, no molecular deletions were detected in nondeleted cytogenetic cases.

While submicroscopic deletions not covered by these sequences or point mutations cannot be ruled out, these data suggest that there are no cytological deletions that could not be detected by these DNA sequences. Further, AS patients with visible deletions can be grouped into two classes (I and II) that overlap by five probes: IR10-1, 34, IR4-3R, 189-1, and 3-21. Class I and class II differ only by the absence (class I) or presence (class II) of DNA segment IR39d. Probes IR29-1 and CMW-1 are intact in classes I–III. While there is complete concordance between the cytogenetic and molecular genetic analyses of classes I–III in both AS and PWS, at the cytogenetic level there is evidence of differences in the sizes of deletions in the two syndromes (Magenis et al. 1990). One possible explanation for this apparent discrepancy is that differential modification of the two parental chromosome 15q11q13 subregions may affect chromatin condensation such that the extent of the deletion in AS appears larger than that in PWS. Alterna-

tively, there may be differences in the distal extent of the deletions in AS and PWS which are not detectable using the cloned DNA markers IR10-1, CMW-1, and IR29-1. In fact, we now have additional data, from use of other chromosome 15q11q13 probes, that demonstrate variation in the distal extent of the deletions (J. H. M. Knoll, K. Glatt, and M. Lalande, unpublished data).

The deletion overlap in class I and class II defines the outer limits of the critical region for syndrome expression. These limits in AS are the same as those in PWS (Donlon et al. 1986; Donlon 1988; Nicholls et al. 1989*b*; Tantravahi et al. 1989), with the exception of a PWS translocation patient who is intact for IR10-1 (Tantravahi et al. 1989).

Although the absence of a DNA segment (gene or genes) can be important in disease expression, the different extent of deletions between AS patients, the similarity of deletions in AS and PWS, and, in particular, the difference in parental origins of the deletions in AS and PWS suggest that chromosomal imprinting may be critical in the expression of these two syndromes. Currently, the only difference between AS and PWS class I and class II deletions is the parental origin of the deleted chromosome: in AS the deleted chromosome is of maternal origin (Cooke et al. 1989; Knoll et al. 1989*a*; Williams et al. 1989; Magenis et al. 1990), and in PWS the deletion is of paternal origin (Butler and Palmer 1983; Mattei et al. 1983; Niikawa and Ishikiriyama 1985; Butler et al. 1986; Nicholls et al. 1989*b*). Our recent observation that several PWS patients with normal chromosomes do not have a molecular deletion for chromosome 15q11q13 but do have a paternal deletion equivalent—i.e., maternal disomy (Nicholls et al. 1989*b*)—is in support of this hypothesis. Genes within chromosome 15q11q13 may be regulated differently, depending on the parent from whom they originated.

Class III AS patients have no detectable molecular and cytogenetic deletions. Within this class of patients, there are families with one affected individual ("sporadic") and families with more than one affected individual ("familial"). We examined the parental origin of chromosome 15q11q13 in only familial cases. In one family the affected sibs each received a different maternal chromosome 15 and a different paternal chromosome 15. In the second family each affected son received the same maternal chromosome 15q11q13. The origin of the other chromosome 15q11q13 in this family could have come, at least in part, from either the father or the mother. However, preliminary data on a newly characterized probe, mapping distal to the critical region (J. H. M. Knoll, K. Glatt, and M. Lalande, unpublished data), revealed an unambiguous paternal contribution in one of the affected sibs. The findings in the first family are suggestive of a non–chromosome 15 syndrome but do not rule out recombination distal to the informative DNA marker. The findings in the second family exclude uniparental disomic inheritance of the whole chromosome 15 but do not exclude uniparental disomy for part of chromosome 15. If uniparental paternal disomy does exist in class III, it may occur in sporadic class III cases but not in familial class III cases. Alternatively, uniparental disomy could include a region smaller than that defined by the deletion overlap in class I and class II. If this is the case, it would define the region critical for AS.

It remains to be determined whether familial and sporadic class III patients have a common etiology and how that etiology compares with that of deletion cases. At the clinical level all classes have the same phenotype. At the cytogenetic level class III patients have apparently normal chromosome 15s, while class I and class II patients have cytogenetic deletions of chromosome 15. AS, like aniridia (Francke et al. 1979; Ferrell et al. 1987), Wilms tumor (Grundy et al. 1988; Huff et al. 1988), and tuberous sclerosis (Haines et al. 1989; Sampson et al. 1989; Smith et al. 1989), may be genetically heterogeneous. The defect in class III AS patients (familial and/or sporadic) could involve (*a*) a different gene(s) on either the same or a different chromosome or (*b*) different regulation as compared with class I and class II AS patients. The existence of heterogeneity will not be resolved until there is a marker for AS.

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