American Journal of Medical Genetics 32:285–290 (1989)

ANGELMAN AND PRADER-WILLI SYNDROMES SHARE A COMMON CHROMOSOME 15 DELETION BUT DIFFER IN PARENTAL ORIGIN OF THE DELETION.

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ABSTRACT

Many Prader-Willi syndrome (PWS) and Angelman syndrome (AS) patients have a cytogenetic deletion of 15q11q13. While AS and PWS share a similar cytogenetic anomaly, they have very different clinical phenotypes. DNAs from 4 AS patients were examined using 5 chromosome 15q11q13-specific cloned DNA segments. With the present level of resolution, the molecular deletions between AS and those previously reported for PWS did not appear to differ. However, in contrast to the *paternal* inheritance of the deleted chromosome 15 observed in the majority of PWS patients, *maternal* inheritance of the deleted chromosome 15 was demonstrated in the AS patients by restriction fragment length polymorphisms (RFLPs).

KEY WORDS: Angelman syndrome, Prader-Willi syndrome, chromosome 15, deletion, parental origin

INTRODUCTION

Cytogenetic deletions of chromosome 15q11q13, generally associated with Prader-Willi syndrome (PWS) [Ledbetter et al., 1981; 1982], have recently been reported in Angelman syndrome (AS) [Kaplan et al., 1987; Magenis et al., 1987; Pembrey et al., 1988]. Clinically, the two syndromes are distinct. AS is associated with severe mental retardation, microcephaly, seizures, EEG abnormalities, mild hypotonia, prognathism with tongue protrusion, bouts of inappropriate laughter, 'puppet-like' ataxic gait with jerky arm movements and eye abnormalities including decreased choroid and iris pigmentation [Angelman, 1965; Bower and Jeavons, 1967; Dickinson et al., 1988]. PWS is characterized by hypotonia and failure to thrive in infancy, variable mental retardation, hypogonadism, "almond-shaped" palpebral fissures, narrow bifrontal diameter, decreased retinal pigmentation, short stature, small hands and feet in later childhood and hyperphagia leading to obesity [Cassidy, 1984; Niikawa and Ishikiriyama, 1985]. Parental transmission of the deleted chromosome while paternal in origin for most PWS cases [Butler and Palmer, 1983; Mattei et al., 1983; Niikawa and Ishikiriyama, 1985; Butler et al., 1986; Nicholls et al., 1989], has been shown to be maternal in AS in the few cases that have been examined [Cooke et al., 1988; Williams et al., 1988]. The apparent cytogenetic similarities, differences in parental transmission of the deleted chromosome and clinical differences between these two syndromes led us to examine the deletions in AS patients at the molecular level. Five chromosome 15q11q13-specific DNA segments [Donlon et al., 1986] with known restriction fragment length polymorphisms

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This paper is dedicated to the memory of Professor Samuel A. Latt

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(RFLPs) [Nicholls et al., 1989] were utilized to analyse the DNAs of 4 unrelated AS patients and their parents.

MATERIALS AND METHODS

Patients were diagnosed as having AS at either the Crippled Children's Division of Oregon Health Sciences University, Portland or through the Clinical Genetics and Dysmorphology Program at the Dartmouth Medical School. Patients WJK36 and WJK10 have been reported previously [Magenis et al., 1987; patients 2 and 3 respectively]. Examination of Giemsa trypsin-banded [Seabright, 1971] prometaphase chromosomes [Yunis, 1976] demonstrated that all patients had a cytogenetic deletion of 15q11q13.

High molecular weight DNA was isolated from whole blood or lymphoblasts [Aldridge et al., 1984] and digested with restriction enzymes - Rsa I, Sca I, Taq I and Hind III (New England BioLabs). Digested DNA fragments were separated by agarose gel electrophoresis and transferred to Hybond-N membrane (Amersham). Cloned DNA segments D15S9 (34), D15S10 (3-21), D15S11 (IR4-3R), D15S12 (IR10-1) and D15S13 (189-1) [Donlon et al., 1986; Nicholls et al., 1989] were labelled with α-^{32}P dCTP by the random primer method [Feinberg and Vogelstein, 1983] and hybridized to the membranes (10^6 cpm/ml hybridization fluid). Prehybridization and hybridization conditions were as described by the manufacturer. Hind III-digested DNAs were also hybridized with control probe H2-26 (D13S28) [Lalande et al., 1984]. Following hybridization, the membranes were washed in 0.1XSSC, 0.5%SDS at 55° C 3 times for 20 minutes each, air dried and exposed to X-ray film.

RESULTS

Five chromosome 15 specific cloned DNA segments mapping to proximal 15q have been isolated [Donlon et al., 1986], characterized and used in identifying deletions or duplications in PWS [Nicholls et al., 1989]. In this study, these DNA segments were used to identify deletions in AS and to determine the parental origin of the deletions.

The hybridization of probe IR4-3R to the DNA of 3 AS patients, one PWS patient and 5 normal individuals is shown in Fig. 1. Probe IR4-3R detects a 2 allele RFLP

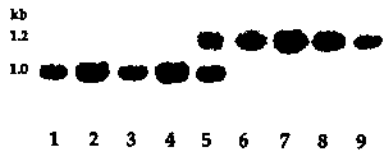


Fig. 1. Hybridization of probe IR4-3R to Rsa I digested DNAs. Each lane contains 3 μg of DNA. DNA samples are from AS patients (lane 1, WJK24; lane 3, WJK29; lane 9, WJK35), AS parents (lane 2, WJK25; lane 4, WJK30; lane 5, WJK31; lane 8, WJK34), a PWS patient (lane 6, WJK32) and her mother (lane 7, WJK33). Note the reduced hybridization intensity in the DNAs of patients relative to those of parents which serve as controls for two copy (per genome) hybridization intensity.

with Rsa I. Single copies of each of 2 alleles (1.0 and 1.2 kb) are detected in a normal individual (lane 5). For the other normal controls, 2 copies (per genome) of allele 1 (1.0 kb) are detected in lanes 2 and 4 while 2 copies (per genome) of allele 2 (1.2 kb) are observed in lanes 7 and 8. A single copy (per genome) of the 1.0 kb allele is detected in AS patient WJK29 (lane 3) and another AS patient (WJK24, lane 1); and a single copy (per genome) of the 1.2 kb allele is detected in AS patient WJK35 (lane 9) and a PWS patient (lane 6). This indicates that one IR4-3R allele is deleted in each of 3 AS patients and in the one PWS patient due to a deletion of one 15q11q13 chromosome subregion. DNA screening of 4 AS patients using the 5 15q11q13 specific cloned DNA segments is summarized in Table I. All patients had a deletion of the 5 cloned DNA segments.

Parental origin of the deleted chromosome 15 could be determined in all patients and is shown in Fig. 2. All showed the deleted chromosome 15 to be of maternal origin since in no case was inheritance of a maternal allele observed. WJK10 inherited a paternal 16.0 kb allele (Fig. 2A). WJK35 inherited a non-maternal

Table I. DNA Analysis of Four Angelman Syndrome Patients

	Chromosome 15q11q13 Probes				
Patient DNA	3-21	IR4-3R	189-1	34	IR10-1
WJK10	-	-	-	-	-
WJK29	-	-	-	-	-
WJK35	-	-	-	-	-
WJK36	-	-	-	-	-

'-' = deletion

Deletions in AS DNAs were detected either as the absence of an allele in families with informative RFLPs or as a 50% reduction in DNA probe hybridization intensity.

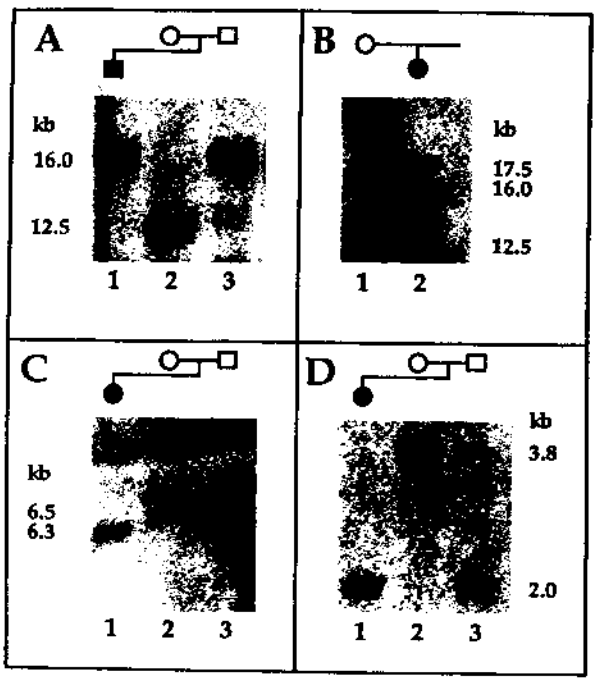


Fig. 2. Informative segregation of RFLPs in AS families. A) Segregation of Sca I alleles by probe IR10-1 is shown. The AS patient (lane 1, WJK10) inherits a paternal 16.0kb allele (lane 3, WJK12) but does not inherit a maternal 12.5 kb allele (lane 2, WJK11). B) Segregation of Sca I alleles detected by probe IR10-1 in a second family is shown. The AS patient (lane 2, WJK35) has a 17.5 kb allele whereas the mother (lane 1, WJK34) is a heterozygote for the 16.0 kb and 12.5 kb alleles. C) Segregation of Sca I alleles detected by probe 34 is shown for AS patient WJK29 (lane 1). She inherits the paternal 6.3 kb allele (lane 3, WJK31) but does not inherit a maternal 6.5 kb allele (lane 2, WJK30). D) Segregation of Taq I alleles detected by probe 189-1 is shown for AS patient WJK36 (lane 1). She inherits the paternal 2.0 kb allele (lane 3, WJK38) but none from her mother (lane 2, WJK37).

17.5 kb allele (Fig. 2B) and a non-maternal 6.3 kb allele for probe 34 (data not shown). The father of WJK35 was not available for study. WJK29 inherited a paternal 6.3 kb allele (Fig. 2C) and WJK36 inherited a 2.0 kb paternal allele (Fig. 2D).

DISCUSSION

AS and PWS share the same apparent cytogenetic lesion yet the clinical phenotypes are very different. This observation is not surprising when one considers that 15q11q13 spans several megabases of DNA and that kilobase size differences are not detectable with present light microscopy techniques. The molecular data reported here (see Fig. 1; Table I) substantiate the cytogenetic findings that deletions of 15q11q13 occur in AS [Kaplan et al., 1987; Magenis et al., 1987; Pembrey et al., 1988]. While the DNA probes used do not precisely delineate the deletion breakpoints in AS and PWS, the present data indicate that the molecular deletions in AS overlap with those previously demonstrated in PWS patients with cytogenetic deletions of 15q11q13 [Donlon et al., 1986; Tantravahi et al., 1989].

In the informative families in this study, all 4 showed that the chromosome 15 donated by the mother was the one in which the deletion had occurred. Maternal transmission of the deleted chromosome 15 in 5 additional cases is known. Of these additional cases, 4 were detected cytogenetically [Cooke et al., 1988] including three by one of us [REM; unpublished data] and one molecularly [Williams et al., 1988]. When these additional cases are included with our 4 informative cases the probability by chance that the mother would transmit the deleted chromosome 15 becomes less than 0.2% ($1/2^9$). In contrast, deletions in 21 of 24 PWS patients have been shown either cytologically [Butler and Palmer, 1983; Mattei et al., 1983; Niikawa and Ishikiriyama, 1985; Butler et al., 1986] or molecularly [Nicholls et al., 1988] to be paternal in origin. A preponderance of paternal deletions has also been observed in other microdeletion syndromes such as retinoblastoma [Ejima et al., 1988] and Miller Dieker Syndrome [Schwartz et al., 1988; VanTuinen et al., 1988].

Our data indicate that while AS and PWS are associated with as yet indistinguishable molecular deletions, the parental origin of the deletion seems to be critical. The reasons for the apparent differences in parental transmission of the deleted chromosome in the 2 syndromes are speculative. Swain et al. [1987] have demonstrated parental *imprinting* in transgenic mice. They have shown autosomal gene expression is dependent on the parent from which the gene was inherited. A similar situation may be occurring in AS and PWS with clinical expression of either AS or PWS depending on parental inheritance.

While our findings suggest that AS and PWS may depend on the parental origin of the mutation, in order to understand the cause of AS and PWS, the extent of the cytogenetic deletion overlap between the 2 syndromes must be determined at the DNA level. Deletion and physical maps of 15q11q13 are presently being constructed to define more precisely the extent of the deletions in the 2 syndromes. Such maps should prove useful in identifying the gene(s) associated with AS and PWS and eventually, in understanding the mechanisms of chromosome deletion and gene expression in AS and PWS.

ACKNOWLEDGEMENTS

This investigation was supported by the National Institute of Health (HD18658) and the Howard Hughes Medical Institute, of which SAL was an investigator. Technical assistance of K.Glatt and D.Shook is gratefully acknowledged.

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Edited by John M. Opitz and James F. Reynolds