American Journal of Medical Genetics 57:61–65 (1995)

# Supernumerary inv dup(15) in a Patient With Angelman Syndrome and a Deletion of 15q11–q13

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**We have studied a patient with Angelman syndrome (AS) and a 47,XY,+inv dup(15) (pter→q11::q11→pter) karyotype. Molecular cytogenetic studies demonstrated that one of the apparently normal 15s was deleted at loci D15S9, GABRB3, and D15S12. There were no additional copies of these loci on the inv dup(15). The inv dup(15) contained only the pericentromeric sequence D15Z1. Quantitative DNA analysis confirmed these findings and documented a standard large deletion of sequences from 15q11–q13, as usually seen in patients with AS. DNA methylation testing at D15S63 showed a deletion of the maternally derived chromosome. AS in this patient can be explained by the absence of DNA sequences from chromosome 15q11–q13 on one of the apparently cytogenetically normal 15s, and not by the presence of an inv dup(15). This is the fourth patient with an inv dup(15) and AS or Prader Willi syndrome, who has been studied at the molecular level. In all cases an additional alteration of chromosome 15 was identified, which was hypothesized to be the cause of the disease. Patients with inv dup(15)s may be at increased risk for other chromosome abnormalities involving 15q11–q13.** 

**KEY WORDS: inv dup(15), Angelman syndrome, DNA deletion**

## INTRODUCTION

Angelman (AS) and Prader-Willi (PWS) syndromes are both associated with loss of genetic sequences from 15q11–q13. In AS there is deletion of maternally derived genetic information from 15q11–q13 in most patients [Knoll et al., 1989; Hamabe et al., 1991a], and absence of maternal sequences as a result of paternal uniparental disomy in some of the nondeletion patients [Malcolm et al., 1991]. In PWS, there is deletion of paternally derived genetic information from 15q11–q13 in 60–70% of patients [Nicholls et al., 1989a; Hamabe et al., 1991b; Robinson et al., 1991], and maternal uniparental disomy of this region in most nondeletion patients [Nicholls et al., 1989a,b; Mascari et al., 1992].

Inverted duplicated chromosome 15s (inv dup(15)s) are one of the most common supernumerary chromosomes, and they have been demonstrated in patients with PWS [Fujita et al., 1980; Ledbetter et al., 1982; Mattei et al., 1984], in normal individuals [Stetten et al., 1981; Knight et al., 1984], and in individuals with multiple clinical problems, including mental retardation and seizures [Schreck et al., 1977; Kirkilionis and Sergovich, 1987]. Normal individuals have small inv dup(15)s containing only the most proximal sequences on chromosome 15q, while most individuals with mental retardation and other signs have larger inv dup(15)s with duplication of many 15q11q13 loci [Cheng et al., 1994; Leana-Cox et al., 1994]. The relationship of the inv dup(15) to PWS has not been elucidated in most patients studied. Recently, 2 patients with PWS and small inv dup(15)s were studied at the molecular level and found to have uniparental (maternal) inheritance of the 2 cytogenetically normal chromosome 15s [Robinson et al., 1993; Cheng et al., 1994]. Similarly, a patient with AS and a small inv dup(15) was found to have uniparental inheritance (paternal) of the 2 cytogenetically normal 15s [Robinson et al., 1993]. None of these patients demonstrated additional chromosome 15q sequences (aside from the pericentromeric D15Z1) on the inv dup(15). In all 3 patients, it was hypothesized that the inv dup(15) was noncontributory to the patient's phenotype. We present our findings on a second patient with Angelman syndrome and an inv dup(15), who does not demonstrate uniparental disomy.

## CLINICAL REPORT

The propositus was first seen for consultation at age 2½ years, for evaluation of encephalopathy with mental

Received for publication July 14, 1994; revision received December 5, 1994.

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retardation, severe global developmental delay, hypotonia, and frequent mixed seizures. Developmental delay was noted from age 6 months, and infantile spasms developed at 1 9/12 years. At age 2 5/12 years he did not speak, had fits of giggling, and abnormal movements. Physical examination demonstrated short stature, microcephaly, prognathism, wide-spaced teeth, and Brushfield spots. He had fair hair and skin and an abnormal EEG. A clinical diagnosis of AS was made. Family history was unremarkable and there was one sib (age 6 months) in good health.

## MATERIALS AND METHODS

### Cytogenetic and Molecular Cytogenetic Studies

Cytogenetic studies were carried out on G-banded chromosomes using standard methods.

Probe D15Z1 was obtained from Oncor, Inc., (Gaithersburg, MD) and fluorescence in situ hybridization (FISH) was carried out according to the supplier's protocol.

Single copy probes used for FISH (with their corresponding locus names) include: 34-10 (D15S9), 16B-3 (GABRB3), and 1R10-1-45 (D15S12) [Knoll et al., 1993]. These phage clones were labeled by nick translation with either biotin-16-dUTP or digoxigenin-11-dUTP (Boehringer-Mannheim), hybridized and detected as previously reported [Knoll et al., 1993]. Chromosomes were counterstained with 4,6-diamino-2-phenylindole (DAPI) for chromosome identification.

### Molecular Studies

For quantitative DNA analysis, genomic DNA was extracted from lymphocytes, digested with *Hind*III, separated on an agarose gel, transferred to nylon membrane, and hybridized using 15q11–q13-specific radiolabelled probes. Probes used in these studies (and their corresponding loci) include IR39b (D15S18), IR4-3R (D15S11), 3-21 (D15S10), 28B3-H3 (GABRB3), and IR10-1 (D15S12) [Donlon et al., 1986; Nicholls et al., 1989a; Wagstaff et al., 1991]. A chromosome 13-specific probe, H2-26 (D13S28) [Lalande et al., 1984], served as a 2 copy per diploid genome control and was cohybridized with each 15q11q13-specific probe. Quantitation was carried out as previously described [Cheng et al., 1994].

The methylation status of sequences at D15S63 was examined after digestion of genomic DNA from lymphocytes of the patient and his parents with *Hind*III and *Hpa*II as previously described [Dittrich et al., 1992]. The *Hind*III/*Hpa*II restriction site at D15S63 is methylated (6.0 kb allele) and not cut on the maternally inherited chromosome, but is unmethylated and cut on the paternally inherited chromosome (4.4 kb allele).

## RESULTS

### Cytogenetics and Molecular Cytogenetics

Fifty metaphases from peripheral blood lymphocytes were studied by G-banding, and they all demonstrated a supernumerary bisatellited marker chromosome. The marker was about the size of a G-group chromosome (Fig. 1) and there were no obvious abnormalities of any other chromosomes. Parental lymphocyte chromosomes were studied and both were found to be normal (46,XX in the mother and 46,XY in the father) in 30/30 cells studied by G-banding.

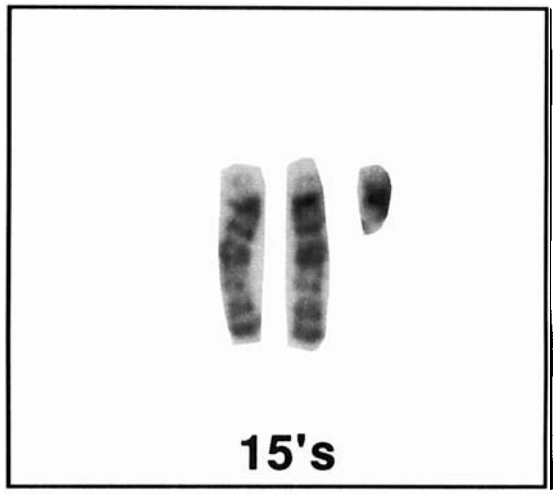


Fig. 1. Partial G-banded karyotype showing 15s and inv dup(15) from the patient with AS.

FISH with D15Z1 on chromosomes from the propositus demonstrated 2 regions of hybridization on the supernumerary chromosome (Fig. 2a,b). This suggested the chromosome 15 origin of the marker. Parental lymphocyte chromosomes were also studied using this probe to rule out low-level mosaicism, and there was no evidence for cells with more than 2 signals in 100 interphase and metaphase cells examined.

In order to study the DNA sequences present on the inv dup(15) and on the apparently normal 15s, we carried out FISH using a number of single-copy sequences that map to 15q11–q13. Sequences for D15S9, GABRB3, and D15S12 were absent from the inv dup(15) and from 1 of the apparently cytogenetically normal 15s (Fig. 2c,d). This finding is consistent with one of the apparently cytogenetically normal 15s having a submicroscopic deletion.

The patient's karyotype is therefore 47,XY,del(15)(q11q13),+inv dup(15)(pter→q11::q11→pter).

### Molecular Studies

By quantitative hybridization, the patient had 2 copies of the proximal locus D15S18, and only 1 copy of the more distal loci D15S11, D15S10, GABRB3, and D15S12 (data not shown). Therefore he is deleted for D15S11, D15S10, GABRB3, and D15S12 on one of the apparently cytogenetically normal chromosome 15s, as has been seen in other patients with AS [Knoll et al., 1990].

Examination of the DNA methylation pattern at D15S63 showed that the propositus had only the paternal 4.4 kb allele, and not the maternal 6.0 kb contribution (Fig. 3), as has been reported in other AS patients [Dittrich et al., 1992]. The paternally inherited chromosome is unmethylated at the *Hind*III/*Hpa*II restriction site of D15S63, and the maternally inherited chromosome is methylated.

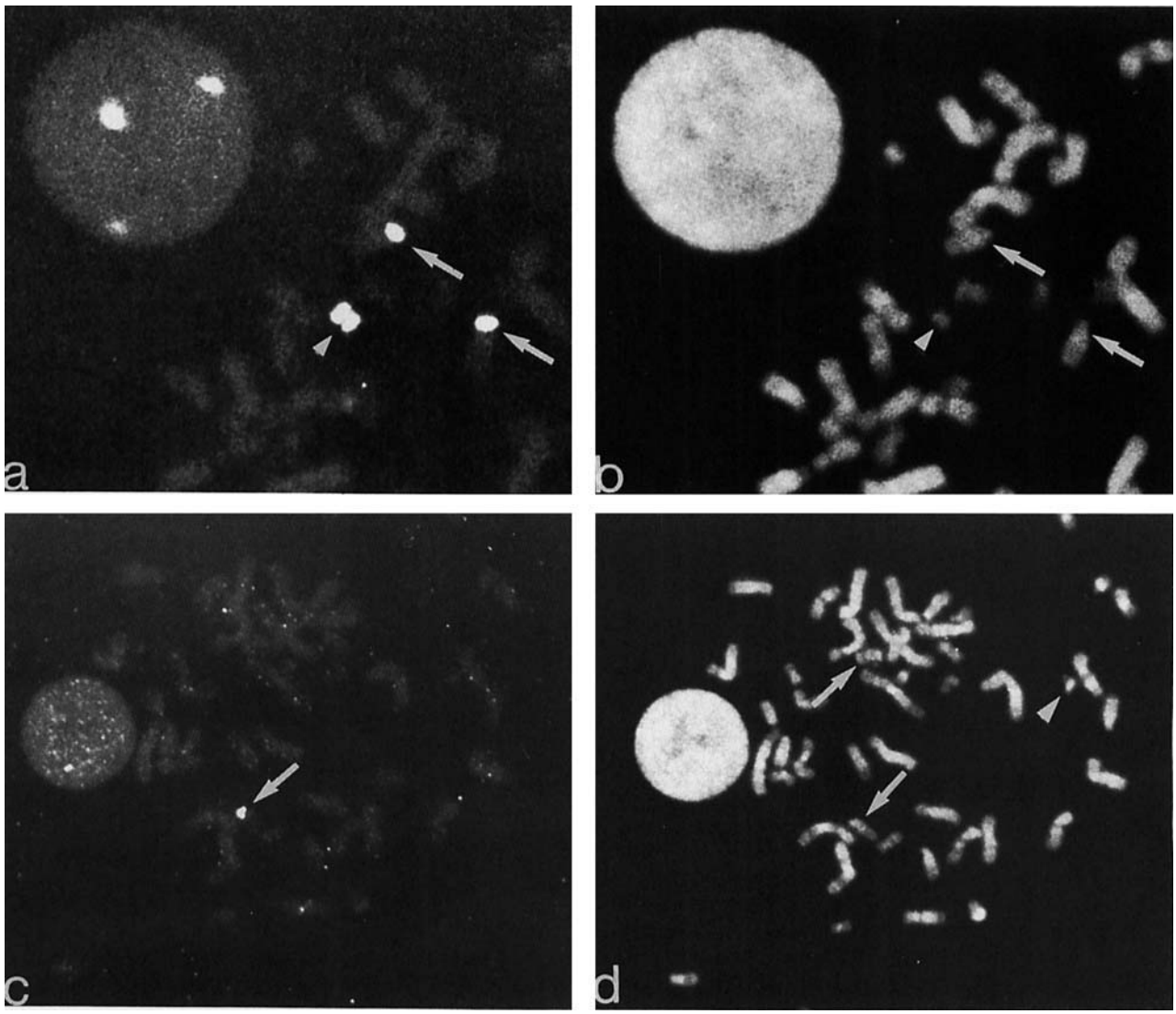


Fig. 2. Hybridization of a satellite DNA probe (D15Z1) **(a)** and a genomic clone IR10-1-45 (D15S12) **(c)** to interphase and metaphase cells from the propositus with AS. D15Z1 recognizes the centromeric regions of both chromosomes 15 (arrows) and the inv dup (arrowhead), while IR10-1-45 hybridizes to only one chromosome 15 and not to the inv dup (c). Chromosomes were counterstained with DAPI for identification **(b,d)**.

## DISCUSSION

We have studied a patient with AS, an inv dup(15), and a deletion of 15q11–q13. The inv dup(15) chromosome was relatively small, and on molecular and molecular cytogenetic studies only D15Z1 was duplicated. However, loci D15S9, D15S11, D15S10, GABRB3, and D15S12, which are consistently deleted in patients with AS, were also found to be deleted from one of the apparently cytogenetically normal 15s in this patient. Additionally, we were able to demonstrate that the maternally inherited allele for D15S63 was absent. This finding is consistent with the patient's AS resulting from deletion of maternally inherited alleles from 15q11–q13 [Knoll et al., 1989; Magenis et al., 1990; Hamabe et al., 1991b].

This patient is only the second AS patient reported with an inv dup(15). However, he is the first patient reported with a deletion of 15q11–q13 and a small inv dup(15) chromosome. The other AS patient with an inv dup(15) showed paternal isodisomy, of chromosome 15. The most likely explanation for the origin of the isodisomy in that case was thought to be monosomy for chromosome 15 (in addition to the inv dup(15)) in the original zygote, with duplication of the single paternal chromosome [Robinson et al., 1993]. An alternative hypothesis that accounts for the presence of uniparental disomy for chromosome 15 in conjunction with an inv dup(15) is that formation of inv dup chromosomes may rescue trisomic fertilizations [Robinson et al., 1993]. The original zygote is trisomic, and then one of the 15s forms an inverted dup(15) increasing the viability of the zygote. This relationship could explain the fact that the frequency of inv dup(15)s appears to be higher (25-fold) in the PWS population [1/40, Ledbetter et al.,

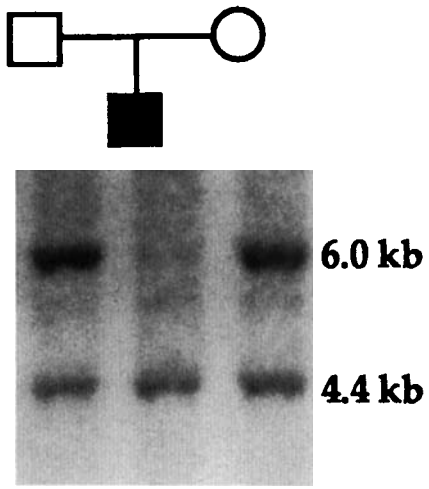


Fig. 3. Maternal origin of deletion in AS patient using methylation-sensitive probe PW71. Hybridization of probe PW71 (D15S63) on *Hpa*II/*Hind*III-digested genomic DNA from the propositus and his parents. PW71 recognizes a maternally inherited 6.0-kb DNA fragment and a paternally inherited 4.4-kb DNA fragment. Since each parent has a maternal and a paternal chromosome, both fragments are evident. The patient does not have a maternal 6.0 kb contribution at D15S63 and has only a paternal 4.4 kb contribution. Equivalent amounts of DNA are loaded in all lanes.

1982] than in the general population [~1/1,000, Sachs et al., 1987]. Since the frequency of inv dup(15) in AS is unknown, it is difficult to determine if there is a causal relationship between the occurrence of either a 15q11–q13 deletion or uniparental inheritance along with an inv dup(15) in AS patients. In the case of a deletion and inv dup(15), one could speculate that one cytogenetic abnormality occurred and caused the abnormal pairing of 15q11–q13 during meiosis. The aberrant pairing could predispose this region to the formation of the second abnormality. There are 2 other examples of inv dup chromosomes in conjunction with structural abnormalities of the homologous chromosome region. Leana-Cox et al. [1994] studied 26 inv dup(15)s, and 1 patient demonstrated a duplication of D15S11 and GABRB3 on one of the apparently cytogenetically normal 15s. A similar finding has also been made in a patient with a supernumerary inv dup(22) ("cat eye" chromosome) and a duplication of proximal 22q sequences on one of the apparently cytogenetically normal 22s [Mears et al., 1994]. These 3 cases of inv dup chromosomes, and a deletion or duplication of the homologous region on an apparently cytogenetically normal chromosome, are provocative. It is necessary to examine additional patients with inv dup(15)s at the molecular level to determine whether there is an increased frequency of uniparental disomy, deletion, and/or duplication involving 15q11–q13 in patients with these markers. Our findings suggest that patients with inv dup(15)s detected prenatally should be tested for other chromosomal rearrangements involving 15q11q13.

## ACKNOWLEDGMENTS

We gratefully acknowledge Rochelle Kline for cytogenetic analysis of the propositus and his family, and Donna McDonald-McGinn for assistance in counseling and acquisition of samples.

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