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Molecular Analysis of an Extra inv dup(15)(q13) Chromosome in Two Patients with Angelman Syndrome

T. Buchholz[1], S. Schuffenhauer[1], K. Evans[2], L. Robson[3], B. Appleton[4], A. Smith[3]

[1]Abteilung für pädiatrische Genetik, Ludwig-Maximilians Universität München, Germany; [2]Queensland Medical Laboratory, Australia; [3]Cytogenetics Unit, Children's Hospital, Camperdown, NSW, Australia; [4]Department of Paediatrics, Children's Hospital, Brisbane, Australia

INTRODUCTION

Angelman syndrome (AS) is caused by the loss of function of yet unidentified gene(s) which map within 15q11-13 and show monoallelic expression from the maternal allele. Lack of the maternal allele(s), due to either a deletion on the maternal chromosome 15 (about 70% of AS patients) or a paternal uniparental disomy (UPD)15 (<5%), are the most common molecular defects in AS. Prader-Willi syndrome (PWS) also maps to proximal 15q, but is caused by the loss of function of paternally expressed gen(s) [1]. Here we describe clinical, cytogenetic and molecular data for two non-related patients with AS who carry a nonmosaic extra cromosome inv dup(15).

Case reports

AS was diagnosed in a male (DH, 6 years of age) and a female (GT, 13 years of age) patient who showed typical AS features including severe mental retardation with absent speech, happy disposition with paroxysms of laughter and seizures. The craniofacial features of AS-Elmicrocephaly, flat occiput, midface hypoplasia and wide mouth with protruding tongue H were present in GT, while DH showed only a subset of features.

Neither patient was able to walk.

Cytogenetics and fluorescence in situ hybridization (FISH)

A supernumerary bisatellited marker chromosome was found in both patients in all 50 cells investigated by GTG banding. FISH with probes for D15Z2 and GABRB3 (Oncor) confirmed the bisatellited marker chromosome as inv dup (15). Thus, both patients carry 4 copies of the PWS/AS chromosomal region (Fig. 1).

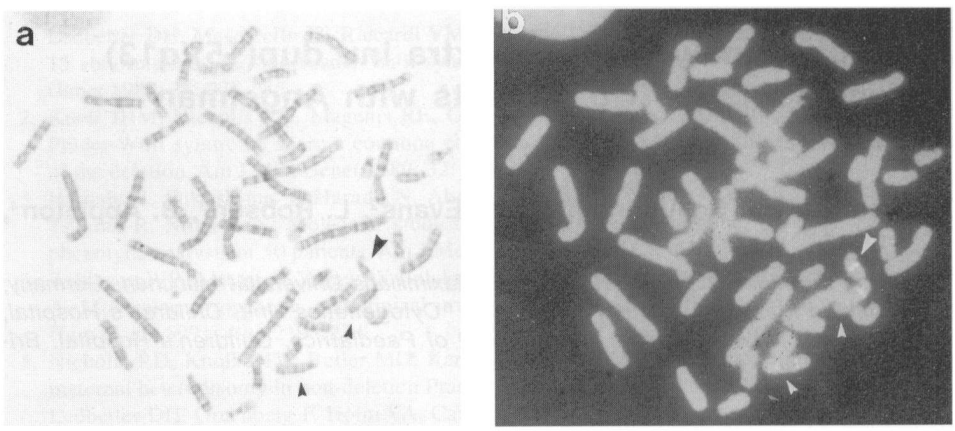

Fig. 1 - FISH. ***a*) GTG-banded metaphase of patient DH showing an extra bisatellited marker chromosome (large arrow head) and two normal chromosomes 15 (small arrow heads).** ***b*) FISH on the same metaphase with a cosmid of the GABRB3 gene and a control cosmid from distal 15q. The GABRB3 probe shows a stronger signal on the inv dup(15) compared to the normal chromosomes 15, indicating the presence of two GABRB3 copies on the marker chromosome.**

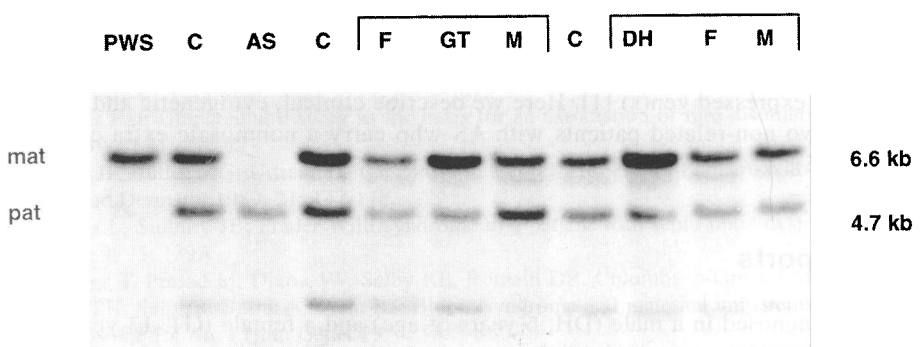

Fig. 2 - DNA methylation analysis of locus D15S63. DNA was digested with *Hind*III and *Hpa*II and probed with pW71B. The inv dup(15) carriers GT and DH show an increased dosage of the maternal 6.6-kb allele compared to the paternal 4.7-kb allele. The parents (F = father; M = mother) and unrelated controls show normal biparental fragments. The PWS patient with deletion D15S9-S12 lacks the paternal band (pat) the AS deletion patient lacks the maternal band (mat).

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Molecular data

DNA methylation analysis of loci D15S9 and D15S63 in both patients showed an increased dosage of the maternal in comparison to the paternal fragment. This indicates that the normal chromosomes are biparental and that the inv dup(15) is maternally imprinted at these loci (Fig. 2). With the polymorphisms detected within the PWS/AS chromosomal region (D15S9, D15S11, D15S13, D15S210, D15S10, D15S113, GABRB3, D15S12, D15S24), it was not possible to confirm the suggested maternal origin of the inv dup(15). Gene dosage analysis demonstrated an increased dosage of the loci D15S9, D15S13, D15S63, D15S10 and D15S12, confirming the tetrasomy of this region in both patients. DH shows a normal dosage at the locus D15S24 excluding this locus from the inv dup(15). In GT, a 2:1 ratio of the two polymorphic alleles at D15S24 was observed, indicating that only one copy of D15S24 is present in the asymmetric inv dup(15).

Discussion

Supernumerary inv dup(15) chromosomes account for approximately half of all bisatellited marker chromosomes [2]. On the basis of molecular breakpoint analysis, they have been classified into subgroups [3, 4]. The markers of the present report – *with breakpoints located distal to GABRB3 – contain two copies of the whole* PWS/AS chromosomal region and are therefore type 3 inv dup(15) chromosomes [3]. These inv dup(15) chromosomes are associated with different phenotypes. Despite some overlapping AS features of the inv dup(15) type 3 carriers, they usually do not fulfill the diagnostic criteria of AS. In contrast, the patients described here meet the diagnostic criteria of AS, thus broadening the spectrum of inv dup(15) phenotypes. The wide spectrum of inv dup(15) phenotypes can be due to different breakpoints, mosaicism, parental origin and imprinting effects. All type 3 inv dup(15), including those of the present probands, are of maternal origin. With respect to imprinted genes, a gain of function of the maternally expressed AS gene(s) is expected, and this should not significantly affect the phenotype, as suggested from PWS and AS patients with UPD. There is also no evidence for a rearrangement within the critical AS region between D15S10 and D15S113, but mutations in all three copies of the maternally inherited AS gene(s) cannot be excluded. Finally, the features of these inv dup(15) carriers might represent a phenocopy of AS, due to a dosage effect of non-imprinted genes located in the duplicated region.

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Correspondence: Dr. Arabella Smith, RAHC-The Children's Hospital, Pyrmont Bridge Road, Camperdown NSW 2050 Australia.