

Triplication of 15q11-q13 with inv dup(15) in a female with developmental delay

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Abstract

A 4 year old female referred with developmental delay was found to have two de novo abnormal derivatives of chromosome 15, a supernumerary inverted duplicated marker chromosome (inv dup(15)) and an interstitial triplication of proximal 15q11-q13 or 14 in one of the two 15 homologues (trip(15)). Fluorescence in situ hybridisation (FISH) using probes within and flanking the Prader-Willi/Angelman syndrome critical region (PWASCR) confirmed the triplication in the abnormal 15 homologue. The inv dup(15) was dicentric, positive for IR39d which maps proximal to the PWASCR, but was negative for all the PWASCR FISH probes used. Results using polymorphic microsatellite repeats confirmed that the additional material in the trip(15) was maternal in origin and included several PWASCR loci. The presence of two de novo abnormalities involving the proximal region of 15q suggests a linked mechanism of origin.

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The proximal long arm of human chromosome 15 is known to contain regions subject to genomic imprinting. Lack of a paternally derived segment of 15q11-q13, either by deletion or by maternal uniparental disomy (UPD) results in Prader-Willi syndrome (PWS). Conversely lack of a maternally derived segment by the same mechanisms results in Angelman syndrome (AS).¹

Distinguishing between normal polymorphic variations in the pericentromeric heterochromatin of proximal 15q and duplications involving euchromatic segments in 15q11-q13 can be very difficult using conventional cytogenetic techniques. Consequently, there have been few reported cases,²⁻⁷ and phenotype/karyotype correlation has proved problematical. However, more recently, three interstitial duplications of the proximal long arm region of chromosome 15 have been reported in which molecular methods were used to confirm that the additional material contained the PWASCR,^{5,6} including a case ascertained with both a dup(15) and a supernumerary inv dup(15).⁷ The phenotypes described in these cases are very similar; all had hypotonia, ataxia, or seizures and developmental delay with no, or very minor, dysmorphic features. The significance of the parental origin of such duplications was recently shown in a family segregat-

ing a proximal 15q duplication in which duplications of maternal origin were associated with an abnormal phenotype, whereas the same duplication when paternally inherited did not have a phenotypic effect.⁸

Supernumerary marker chromosomes (SMC) are found in 0.24 per 1000 live births; however, in the mentally retarded population the frequency rises to 3.27 per 1000.⁹ The most commonly occurring SMCs are derived from inverted duplications of the pericentromeric region of chromosome 15 (inv dup(15)). Overall, the phenotype associated with inv dup(15) varies from normal to severely mentally retarded, with little or no dysmorphism.^{10,11} However, the phenotype of patients ascertained with large inv dup(15)s, shown by FISH or PCR to contain the PWASCR, invariably consist of moderate to severe mental retardation, with more variable reports of autistic behaviour in childhood and seizures. There are usually no dysmorphic features and obesity is not a feature.¹¹⁻¹³ The smaller inv dup(15)s, that is, those which are apparently exclusively heterochromatic, are usually but not invariably associated with a normal phenotype.

As far as we are aware, there have been three reported cases presenting with triplication of 15q11-q13,^{14,15} all of which were characterised using FISH. The phenotype associated with triplication of this region differs from that observed in PWS/AS patients: height and weight are normal and there are minor dysmorphic features. In light of these findings, Schinzel *et al*¹⁴ suggested that the triplication had arisen following an illegitimate meiotic recombination between an inv dup(15) and a normal 15 homologue, following which the marker was lost. We report on the clinical, cytogenetic, and molecular analysis of a patient with triplication of 15q11-q13 and an inv dup(15), and suggest a similar aetiological mechanism to that proposed by Schinzel *et al*,¹⁴ but with retention of the marker.

Case report

The patient is the first born child of healthy, non-consanguineous, white parents, the mother aged 26 and the father 28 years. Delivery was induced at 37 weeks for severe back pain. Birth weight was 3540 g (90th centile). The infant had feeding difficulties and hypoglycaemia in the neonatal period and by 1 year had developed episodic vertical head nodding and a rapid vertical nystagmus, later diagnosed as spasmus nutans. Subsequently, there has been concern about all her developmental milestones. At 4 years of age her weight is above the 97th centile, height on the 50th centile, and

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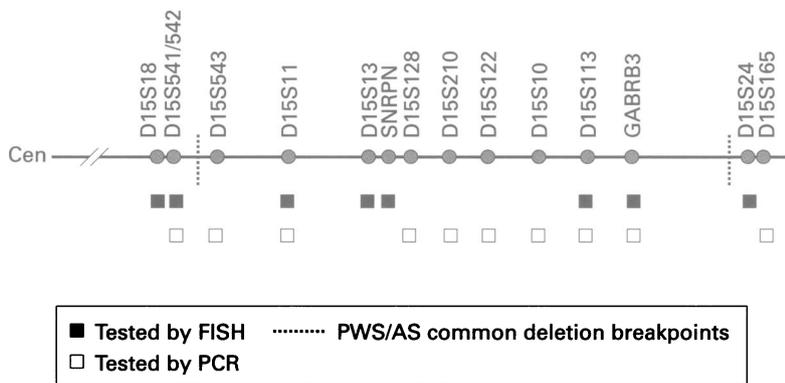


Figure 1 Physical map of 15q11-q13 (not to scale) showing the relative positions of probes and primers used in FISH and PCR analyses.

head circumference on the 75th centile. She is not dysmorphic but has severe learning difficulties. She walks with an unsteady gait, speaks and sings only a handful of words, sometimes with echolalia, is unable to dress herself, requires constant supervision, and attends a special needs school. She has a raised pain threshold, petit mal epilepsy, and is currently being investigated for immunodeficiency as she suffers from cold sores on the right side of her face and is susceptible to wound infections.

Materials and methods

CYTOGENETIC ANALYSIS

Peripheral blood lymphocytes from the patient and both parents were cultured according to standard techniques, followed by staining with GTL, DA/DAPI, CBG, and Ag. Lymphoblastoid cell lines have been established from the patient and her parents and are deposited at the European Cell and Culture Collection, Porton Down, Salisbury, UK (patient DD2586, mother DD2585, father DD2584).

The following probes (p=plasmid, c=cosmid, y=YAC), proximal to distal, were nick translated using the manufacturer's protocols (Amersham and Boehringer): p15 (D15Z1 satellite III, 15 short arm), pTRA-20 (D15Z3, 15 centromere), yIR39d (D15S18), c770C6 (D15S542), cD15S11, c27 (D15S13), a cosmid from SNRPN (Vysis), cE24 (D15S113), yGABRB3, and yCMW1 (D15S24). D15Z1 and pTRA-20 were directly labelled with Fluorogreen (Amersham) and the locus specific probes were labelled with digoxigenin and detected using one layer of antidigoxigenin-TRITC (Boehringer). All probes, including the commercially obtained D15Z1/SNRPN/PML combination, were hybridised for 16 to 18 hours at 37°C, following which slides were stringently washed using a sequential series of 2 × SSC at room temperature (five minutes), 50% formamide/50% 2 × SSC, two five minute washes at 42°C, and a final 2 × SSC wash for five minutes at room temperature. The chromosomal DNA was counterstained with 0.05 mg/ml DAPI suspended in an antifade solution (Vectashield, Vector Labs, UK) and examined using a Carl Zeiss Axioskop epifluorescent microscope fitted with Chroma Technology's Pinkel fluorescent No 83 filter series. Images were captured using a cooled CCD camera and the data analysed using "Smartcapture" soft-

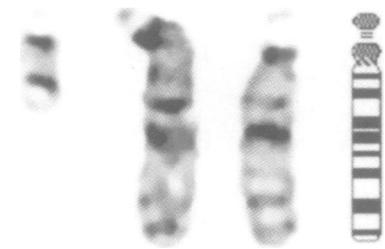


Figure 2 Partial G banded (GTL) karyotype from the patient. From left to right: *inv dup(15)*, *trip(15)*, and normal 15.

ware (Digital Scientific, Cambridge, UK, now VYSIS). The relative positions of the probes used are shown in fig 1.

MOLECULAR ANALYSIS

Genomic DNA was extracted from peripheral blood by standard methods.¹⁶ PCR analysis was performed by published techniques using primers which amplify polymorphic microsatellite repeat sequences located on chromosome 15.¹⁷ The loci of the primers used are shown in fig 1. PCR products were separated on a 6% polyacrylamide/urea gel and visualised by autoradiography.

Results

CYTOGENETIC RESULTS

High resolution conventional chromosome analysis following GTL banding showed a female karyotype with 47 chromosomes in all 30 cells examined. One of the 15 homologues had an approximate 15% increase in the length of the long arm with an apparent duplication of bands q11-q13 or 14 and, in addition, all cells contained a bisatellited, dicentric marker chromosome which from initial GTL and DAPI staining was interpreted to be a dicentric *inv dup(15)* (fig 2). Both parents were karyotypically normal.

The FISH results with probes D15Z1 and pTRA-20 confirmed that the supernumerary marker was a dicentric *inv dup(15)* and contained two copies of the satellite III proximal 15p and centromeric alphoid sequences. The locus specific probes showed that the *inv dup(15)* was positive for the most proximal probe (IR39, D15S18, fig 3A) but negative for all the PWASCR probes (fig 3B). The interpretation of the signal distribution on the abnormal 15 homologue showed three distinct signals at D15S18 (fig 3A) and SNRPN (fig 3B) and what could be interpreted as two or three signals at D15S11, D15S13, GABRB3, and D15S24 (data not shown). Furthermore, the distribution of the signals at D15S18 and SNRPN suggest that the additional material in the abnormal 15 consists of an inverted repeat. Using ISCN (1995)¹⁸ nomenclature, the patient's karyotype can be described as: 47,XX,dup(15)(q11-q13or14),+*inv dup(15)* de novo.ish trip(15)(pTRA-20+, D15S18+++ , D15S542+, D15S11++(+), D15S13++(+), SNRPN+++ , D15S113++(+), GABRB3++(+), D15S24++(+)),+*inv dup(15)*(pTRA-20+, D15S18+, D15S542-, pTRA-20+).

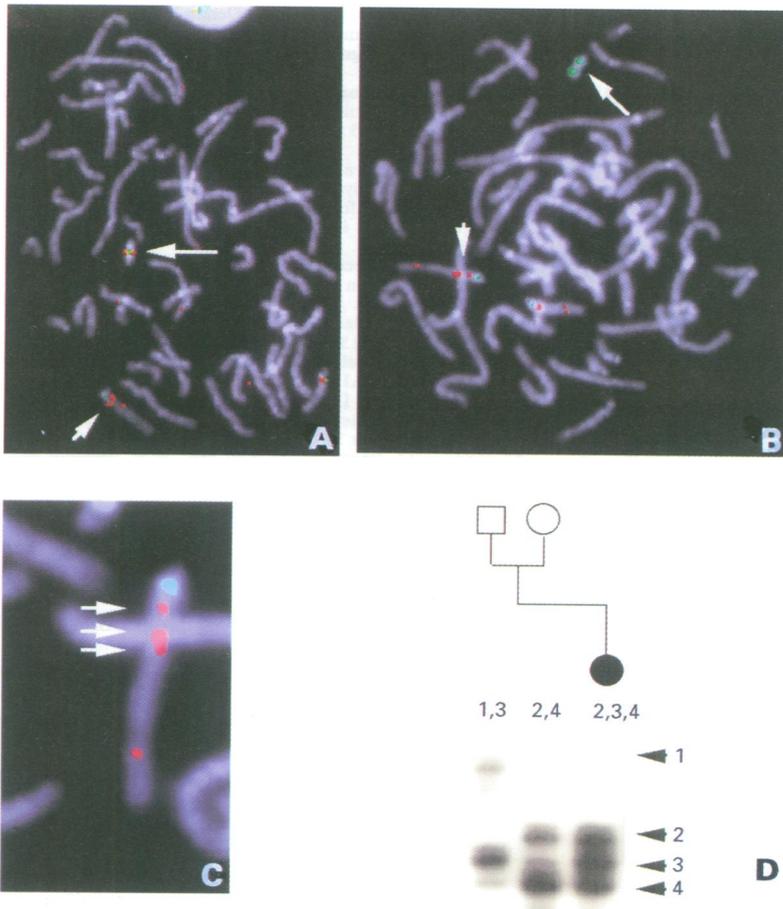


Figure 3 Results of *in situ* hybridisation. (A) FISH with a YAC from IR39d (D15S18) proximal to the PWASCR and pTRA-20, specific for the 15 centromere. The inv dup(15) is dicentric (green signal, arrow) and positive for yIR39d (red signal). The abnormal 15 (arrowhead) shows three copies of yIR39d (red signal), two proximal and adjacent with a third, single and more distal. (B) Following hybridisation with the chromosome 15 short arm probe, D15Z1 (green) and SNRPN (red), from within the PWASCR, together with PML (red) which maps to distal 15q. The inv dup(15) shows two copies of D15Z1 but is negative for SNRPN (arrow). The abnormal 15 has three copies of SNRPN compared with a single signal on the normal homologue. In contrast to the result with yIR39d, the signal distribution shows one proximal and two distal but adjacent signals. These results suggest that the triplication includes an inverted repeat. (C) An enlarged view of the abnormal 15 shown in (B) with arrowheads indicating the points of hybridisation with the SNRPN cosmid. (D) Autoradiograph showing the PCR result obtained at D15S122. The distribution of alleles is fully informative for parental origin and confirms that the additional PWASCR euchromatin is maternally derived.

MOLECULAR RESULTS

The PCR analyses were carried out using CA and tetranucleotide repeat polymorphisms from several loci from within the PWASCR (fig 1). The results at D15S122 (fig 3D), D15S128, and GABRB3 were fully informative for parental origin and in each case the additional material was maternally derived. Variability both within and between PCR autoradiographs, however, made quantification of the allele (band) intensities too variable for precise estimates of dosage.

Discussion

Our results show that the additional material from the PWASCR present in the abnormal 15 homologue is maternal in origin and that it results from an interstitial triplication. The absence of PWASCR euchromatin within the inv dup(15) suggests that the trip(15) is responsible for the patient's abnormal phenotype.

In previously reported molecular studies of proximal 15q duplications,⁵⁻⁷ the additional

material has also been shown to be maternal in origin and the clinical phenotypes are very similar to that seen in our patient, that is, moderate to severe developmental delay, seizures, ataxia, but no dysmorphism. These abnormalities are also strikingly similar to those seen in patients presenting with inv dup(15)s or other SMC(15)s containing PWASCR euchromatin. In all cases where molecular studies have been undertaken, the origin of the additional material is maternal.¹¹⁻¹³ Thus, the inheritance of additional maternal copies of the PWASCR either by interstitial duplication or triplication or by the presence of an inv dup(15) results in very similar phenotypic effects. More recently, Cook *et al*⁸ have reported a family in which the maternal transmission of a proximal 15q duplication containing PWASCR euchromatin resulted in two children with features of autism, whereas the carrier mother, who had inherited the same duplication from her father, was phenotypically normal.

In a previous report of triplication of proximal 15q the patient was found to have a selective defect in the antibody response to polysaccharide antigens without associated T cell immunodeficiency.¹⁴ In this context, it is interesting to note that the patient described here is currently being investigated for immunodeficiency.

The presence of two *de novo* abnormalities involving proximal 15q suggests a linked mechanism of origin. We propose that a large inverted duplicated marker 15 originally formed by U type exchange either during a premeiotic oogonial mitotic division or during the early prophase of oocyte meiosis, following which an illegitimate recombination occurred between the inv dup(15) containing the PWASCR and a normal 15 homologue. The PWASCR euchromatin from the inv dup(15) was thereby inserted into the 15 homologue giving rise to a chromosome 15 with an interstitial triplication, including an inverted repeat (fig 3) and the small inv dup(15) lacking PWASCR euchromatin.

The postulated mechanism leading to the formation of the two *de novo* chromosome 15 abnormalities seen in this case was previously described by Schinzel *et al*.¹⁴ In that paper, the authors suggested that the interstitial triplication in their patient had arisen following an illegitimate meiotic recombination between an unstable inv dup(15) and a normal 15 homologue following which the supernumerary marker was lost. The results presented in this paper add further support to this proposed aetiology for the origin of proximal 15q triplications involving the PWASCR.

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- Knoll JHM, Nicholls RD, Magenis RE, Graham JM Jr, Lalande M, Latt SA. Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. *Am J Med Genet* 1989;32:285-90.
- De France HF, Beemer FA, Ippel PF. Duplication in chromosome 15q in a boy with the Prader-Willi syndrome; further cytogenetic confusion. *Clin Genet* 1984;26:379-82.

- 3 Shohat M, Shohat T, Rimoin DL, et al. Rearrangement of chromosome 15 in the region q11.2-q12 in an individual with obesity syndrome and her normal mother. *Am J Med Genet* 1990;37:173-7.
- 4 Rauch LA, Nevin NC. Duplication of 15q11.2-15q13 in five cases with different phenotypes. *J Med Genet* 1991;28:573-4.
- 5 Bunday S, Hardy C, Vickers S, Kilpatrick MW, Corbett JA. Duplication of the 15q11-13 region in a patient with autism, epilepsy, and ataxia. *Dev Med Child Neurol* 1994;36:736-42.
- 6 Clayton-Smith J, Webb T, Cheng XJ, Pembrey ME, Malcolm S. Duplication of chromosome 15 in the region 15q11-q13 in a patient with developmental delay and ataxia with similarities to Angelman syndrome. *Am J Med Genet* 1993;30:529-31.
- 7 Abeliovich D, Dagan J, Werner M, Lerer I, Shapira Y, Meiner V. Simultaneous formation of inv dup(15) and dup(15) in a girl with developmental delay: origin of the abnormal chromosome. *Eur J Hum Genet* 1995;49:49-55.
- 8 Cook EH Jr, Lindreen Y, Levarthal BL, et al. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am J Hum Genet* 1997;60:928-34.
- 9 Buckton KE, Spowart G, Newton MS, Evans HJ. Forty four patients with an additional "marker" chromosome. *Hum Genet* 1985;69:353-70.
- 10 Webb T. Inv dup(15) supernumerary marker chromosomes. *J Med Genet* 1994;31:585-94.
- 11 Crolla JA, Harvey JF, Sitch FL, Dennis NR. Supernumerary marker 15 chromosomes: a clinical, molecular and FISH approach to diagnosis and prognosis. *Hum Genet* 1995;95:161-70.
- 12 Cheng SD, Spinner NB, Zackai EH, Knoll JHM. Cytogenetic and molecular characterisation of inverted duplicated chromosomes 15 from 11 patients. *Am J Hum Genet* 1994;55:753-9.
- 13 Leana-Cox J, Jenkins L, Palmer CG, et al. Molecular cytogenetic analysis of inv dup(15) chromosomes, using probes specific for the Prader-Willi/Angelman syndrome region - clinical implications. *Am J Hum Genet* 1994;54:748-56.
- 14 Schinzel AA, Brecevic L, Bernasconi F, et al. Intrachromosomal triplication of 15q11-q13. *J Med Genet* 1994;31:798-803.
- 15 Holowinsky S, Black SH, Howard-Peebles PN, et al. Triplication 15q11-q13 in two unrelated patients with hypotonia, cognitive delays and visual impairment. *Am J Hum Genet* 1993;53:A125.
- 16 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- 17 Hudson TJ, Engelstein M, Lee MK, et al. Isolation and chromosomal assignment of 100 highly informative human simple sequence polymorphisms. *Genomics* 1992;13:622-9.
- 18 ISCN (1995). In: Mitelman F, ed. *An international system for human cytogenetic nomenclature*. Basel: Karger, 1995.