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ORIGINAL INVESTIGATION

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Investigation of a cryptic interstitial duplication involving the Prader-Willi/Angelman syndrome critical region

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Abstract A 3-year-old female referred with developmental delay, hypotonia and seizures was found to have a cryptic interstitial duplication of the Prader-Willi/Angelman critical region (PWACR). Her clinical features form part of a common phenotype characteristic of PWACR duplications including developmental delay, behavioural problems and speech difficulties. Microsatellite analysis showed that the duplication had arisen de novo,was maternal in origin and involved the entire 4-Mb PWACR between the common deletion breakpoints. The existence of cryptic rearrangements emphasises the need for molecular tests alongside conventional cytogenetics when investigating abnormalities involving this imprinted region.

Introduction

Structural abnormalities involving 15q11–q13 are relatively common and include interstitial duplications, inverted duplications and deletions. Many, but not all, of these rearrangements are associated with an abnormal phenotype. Paternal deletions of this region result in Prader-Willi syndrome (PWS) and maternal deletions in Angelman syndrome (AS). Interstitial duplications (Bundey et al. 1994; Browne et al. 1997; Cook et al. 1997; Repetto et al. 1998) of maternal origin that include the critical region for PWS and AS (PWACR) produce a more variable phenotype, distinct from PWS and AS, that includes hypotonia, ataxia, seizures, developmental delay and autism or atypical autism with no or only minor dysmorphic features. Paternal duplications of the PWACR are not associated with an abnormal phenotype (Browne et al. 1997; Cook et al. 1997). Many cytogenetically visible apparent interstitial duplications of 15q11–q13 do not include the PWACR but instead comprise multiple repeat units within the 15q pericentromeric region (Ritchie et al. 1998) and segregate in families without phenotypic effect (Browne et al. 1997; Barber et al. 1998).

Most PWS or AS deletions are detectable cytogenetically and include the entire 4-Mb PWACR (Christian et al. 1995; Robinson et al. 1998). Deletions are thus of similar size and share common proximal and distal breakpoints (Christian et al. 1995; Robinson et al. 1998). Interstitial duplications also appear to cover the entire PWACR, although their breakpoints have not been definitively mapped (Browne et al. 1997; Cook et al. 1997; Repetto et al. 1998; Robinson et al. 1997).

We present the cytogenetic and molecular study of a 3 year old girl who carries a cryptic interstitial duplication of the entire PWACR that was not diagnosed by routine cytogenetic analysis. We have defined the breakpoints of the proband's duplication and of five other interstitial duplications that were detected cytogenetically. Using parental and grandparental DNA, we have also investigated the chromosomal origin and structure of the duplication.

Subjects and methods

Patients

Case 1

The proband was referred aged 3 years, with developmental delay, a history of seizures and hypotonia. Her karyotype was normal. Molecular studies were initiated, because of suspected AS, and identified an additional maternally derived allele at D15S11.

Case 2

A male infant was identified at the Wessex Regional Genetics Laboratory (WRGL) while undergoing routine cytogenetic analysis. He was referred aged three with developmental delay and macrocephaly.

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Table 1 Breakpoint mapping (+ duplicated, (+) duplication inferred from marker order, – normal, N/I non-informative)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Centromere						
D15S541	–	–	+	+	+	–
D15S542	N/I	–	+	+	+	–
D15S543	+	+	+	+	N/I	+
D15S11	+	+	(+)	+	+	(+)
D15S128	+	+	(+)	+	(+)	(+)
D15S122	+	+	(+)	+	(+)	(+)
D15S1234	+	+	(+)	+	(+)	(+)
D15S1002	+	+	(+)	+	(+)	(+)
D15S219	N/I	+	+	+	+	+
D15S217	+	+	+	+	+	+
D15S1019	–	N/I	–	–	–	–
D15S1048	N/I	N/I	–	–	–	–
D15S165	–	–	–	–	–	–
D15S118	–	–	–	–	–	–
Telomere						

Cases 3–6

Clinical details and provisional breakpoint mapping of these cases and their families have been reported previously by Browne et al. (1997). All four probands were originally ascertained with developmental delay and speech difficulties.

Fig. 1a, b FISH image of case 1. **a** Detail of the duplicated chromosome 15 showing D15Z3 (*green*) and cos27 (D15S13) signals. There are clearly two resolvable D15S13 signals. **b** FISH of partial metaphase from case 1 with the same probe combination as in **a**. The duplicated chromosome 15 is *arrowed*

Cytogenetics

Karyotypes were determined by analysis of G-banded metaphase chromosomes harvested from peripheral blood lymphocytes. Fluorescence in situ hybridisation (FISH) studies, with probes cos-27 (D15S13) and from cosmid SNRPN (Vysis), were based on the standard method of Pinkel et al. (1988).

Molecular genetics

DNA was extracted from peripheral blood by a salt precipitation technique (Miller et al. 1988). The polymerase chain reaction (PCR) was performed under standard conditions with primers spanning 15q11–q13 (see Table 1). One primer from each pair was fluorescently labelled and the PCR products analysed on an ABI 377 automated DNA sequencer (Perkin Elmer).

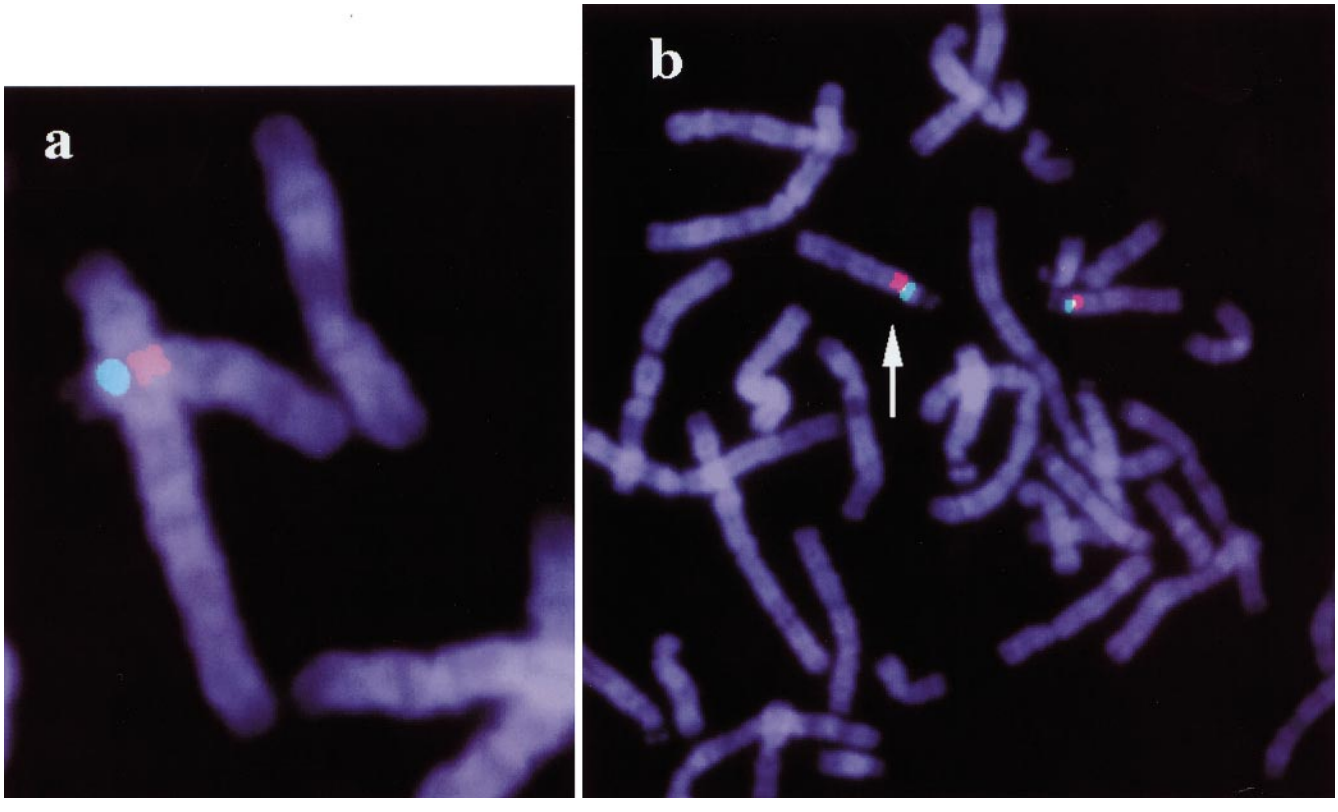
Results

Cytogenetic analysis

Case 1 had a normal karyotype, even on re-inspection after the duplication had been detected by FISH (Fig. 1). The duplication in case 2 involved bands 15q11-q13 and was indistinguishable from the visible interstitial duplications studied previously in our laboratory (cases 3–6; Browne et al. 1997). The duplications in cases 1and 2 had both arisen de novo.

Molecular analysis

Microsatellite analysis within the PWACR confirmed the presence of a duplication in cases 1 and 2. Both duplica-



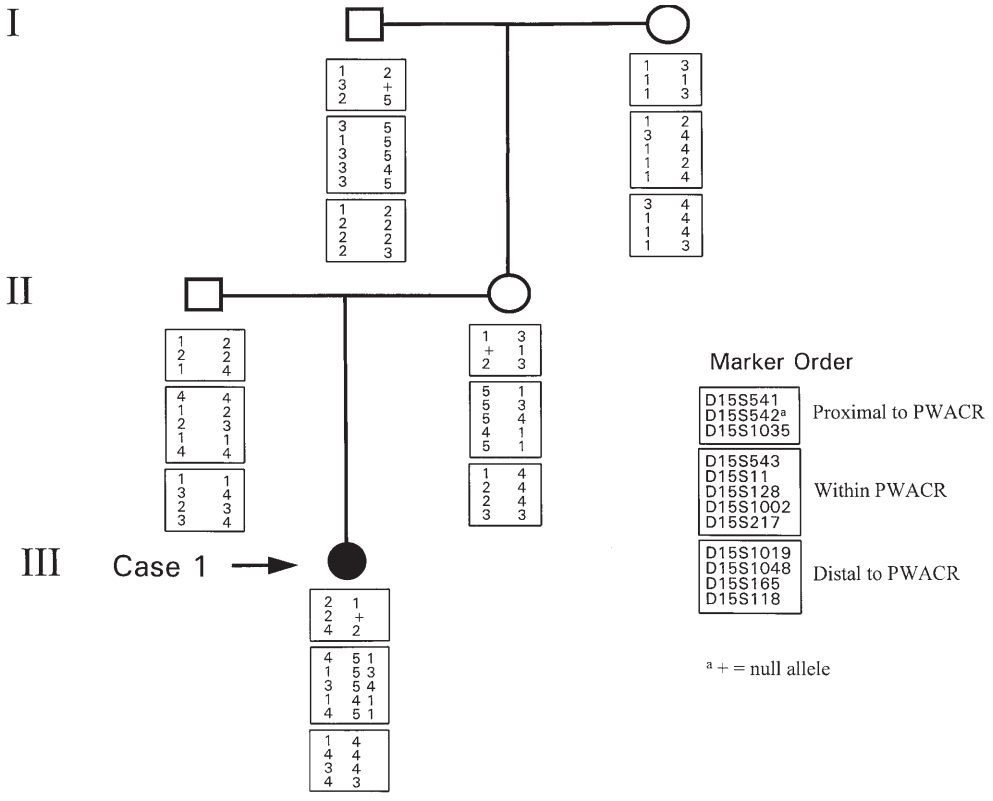


Fig. 2 Pedigree of case 1 showing recombination of markers proximal to the

tions were maternal in origin and involved both maternal chromosome 15 homologues. For all microsatellite markers proximal to the PWACR, the maternal allele inherited by case 1 was from the grandfather, whereas for all informative markers distal to the PWACR, the maternal allele was from the grandmother (Fig. 2).

Further studies defined two proximal duplication breakpoints (Table 1). Cases 1, 2 and 6 include D15S543 but not D15S541/D15S542; the other three extend beyond D15S541/D15S542 towards the centromere. All six duplications share a common distal breakpoint between D15S217 and D15S1019; however, the breakpoint of case 2 may extend distally to D15S165.

Discussion

We present, as far as we are aware, the first molecular characterisation of a cryptic interstitial duplication of the entire 4-Mb PWACR. Distinguishing between normal and duplicated chromosome 15 homologues at 15q11–q13 is difficult (Browne et al. 1997; Cook et al. 1997), because of differences in the conformation of the euchromatic region and the presence of large repeat units between the centromere and the PWACR (Barber et al. 1998; Ritchie et al. 1998). Approximately 25% of deletions causing PWS and AS are also cryptic, being identifiable only by FISH or PCR (Chan et al. 1993; Saitoh et al. 1994).

The cryptic duplication was maternal in origin, consistent with all other duplications of this region producing an abnormal phenotype (Browne et al. 1997; Cook et al. 1997; Repetto et al. 1998). Most patients with additional maternally inherited copies of the PWACR present with developmental delay, hypotonia, seizures, ataxia and learning difficulties, particularly with regard to speech and language. Case 1 was not dysmorphic but was referred with developmental delay, hypotonia and seizures. Autism or atypical autism have frequently been associated with rearrangements involving chromosome 15q (Gillberg 1998), although these features were not obvious in any of our duplication patients.

Our results show that the cryptic duplication was identical in size to the five visible duplications and shared the same proximal breakpoints reported for deletions (Christian et al. 1995). All six cases appeared to share a single distal breakpoint between D15S217 and D15S1019 consistent with the common distal breakpoint reported in PWS and AS deletions between D15S12 and D15S24 (marker order: Cen – D15S217 – D15S12 – D15S24 – D15S1019 – Tel). The distal breakpoints of four reported duplication cases included D15S12 but not D15S24 (Repetto et al. 1998; Robinson et al. 1997), whereas in three cases, the duplications extended beyond D15S24 (Bundey et al. 1994; Robinson et al. 1997).

The de novo duplications of cases 1 and 2 were each derived from two maternal chromosome 15 homologues and, for case 1, we have demonstrated maternal recombination of markers immediately proximal to the duplication. This provides the first direct evidence that duplications (and reciprocal deletion products) can arise by un-

equal meiotic exchange, following mispairing of repeat sequences identified flanking the PWACR (Christian et al. 1999). This mechanism may account for the majority of de novo interstitial duplications reported (Bundey et al. 1994; Cook et al. 1997; Repetto et al. 1998), although interstitial duplications and deletions can also arise through intrachromosomal rearrangements (Carrozzo et al. 1997; Repetto et al. 1998; Robinson et al. 1998).

Additional maternal copies of the PWACR produce an abnormal phenotype that includes developmental delay and often speech problems and seizures. Screening individuals with these clinical features could identify new cases with chromosome 15 rearrangements and further define the phenotype and origin of PWACR duplications.

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