A supernumerary marker chromosome 15 tetrasomic for the Prader-Willi/Angelman syndrome critical region in a patient with a severe phenotype

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The proximal region of chromosome 15q is predisposed to a wide range of structural rearrangements. Deletions of this region, spanning approximately 4 Mb, can be paternally or maternally derived and result in Prader-Willi syndrome and Angelman syndrome, respectively. Additional copies of the Prader-Willi/Angelman syndrome critical region (PWACR) can occur as interstitial duplications and triplications or as supernumerary marker chromosomes (SMCs). SMC(15), often referred to as inv dup(15), is the most common marker chromosome in humans, representing 50% of all markers. SMC(15) can be classified into two major groups, depending on the presence or absence of the PWACR. Small SMC(15)s do not contain the PWACR, can be either paternal or maternal in origin, and seem to have no phenotypic effect. Large SMC(15)s usually contain two extra copies of the q11–13 region, are exclusively derived from the maternal chromosome 15 homologues, and are associated with various abnormal phenotypes, including mental or growth retardation, seizures, behavioural problems, and dysmorphisms.

Mapping studies have shown that the small SMCs share the same two common proximal breakpoints, BP1 and BP2, as standard PWS/AS deletions, whereas the large SMCs have at least three breakpoints, one similar to the distal breakpoint (BP3) of the PWS/AS deletions. Other rare variants of the inv dup(15) that do not follow the common pattern have also been described. Mignon et al and Robinson et al each reported a patient with an asymmetrical SMC(15), containing only one copy of the PWACR. The patient described by Mignon et al showed several of the classic signs of PWS. The coexistence of SMC(15) with other chromosome 15 derived rearrangements has also been reported including an interstitial duplication and an interstitial triplication. The presence of two extra marker chromosomes 15 has been reported in all cells of a woman with repeated abortions and in 47% of the cells of a severely retarded patient. A further patient with mental retardation presented initially with three morphologically distinct markers. The SMC(15) was subject to dynamic mosaicism with the marker, thought to be unstable during cell division, resulting in morphologically different forms of the SMC.

We describe here a patient with severe developmental delay who was found to have a unique form of an SMC(15) containing four copies of the PWACR and the pseudogene loci IgHV and NF1. The SMC(15) is dicentric and seems to be composed of two large SMC(15)s which have broken and rejoined in the pericentric proximal long arms. The patient is effectively hexasomic for the extended PWACR in 70% of his peripheral blood cells.

**CASE REPORT**

The proband (fig 1), a boy of 8, was the first child of healthy, unrelated parents. He was born by elective caesarian section for face presentation at 38 weeks’ gestation, weighing 4400 g.
He was sleepy and hypotonic from birth and needed tube feeding for 10 days. Feeding difficulties and slow weight gain persisted during infancy. Electrophysiological studies carried out at the age of 7 months because of reduced visual fixation showed normal retinal activity but a reduction in evoked responses over the posterior temporal regions. He sat alone for the first time at 2 years and has never walked unaided. At the age of 2 years, surgery was carried out to correct bilateral undescended testes.

At the age of 7 years he developed generalised seizures which became less frequent on treatment with sodium valproate and lamotrigine. Brain MRI, performed at 15 months of age, showed thinning of the corpus callosum and delayed myelination of the cerebral hemispheres. When seen by us at the age of 18 years 9 months, the proband was able to pull himself to stand and move around the furniture, but he was unable to walk alone. He had no words and few social responses. His visual responses were limited: he would reach out for objects but was unable to judge distances accurately. His favourite toys were those he was able to recognise by touch.

On examination, the proband's height was on the 25th centile and his head circumference was on the 50th centile. He had bilateral epicanthic folds, lateral displacement of the inner canthi, and downward slanting palpebral fissures. His palate was narrow, his upper incisors were large, and his upper lip was prominent, with a short philtrum. His nose was short and upturned with a broad tip, and his cheeks were full. His chin was long and somewhat prominent. There was a moderate pectus excavatum. Hands and feet were normally formed but there was laxity of the finger joints and pes planus with a valgus deformity of the ankle on weight bearing. Muscle tone was reduced in the upper limbs but normal in the lower limbs, with normal deep tendon reflexes.

A psychological assessment using the Mullen scales of early development and the Vinelands adaptive behaviour scales, interview edition survey form, was carried out on the proband when he was 7 years and 11 months old. The results indicated that the proband had developmental delays consistent with a severe to profound level of learning difficulties. The results also showed slightly different levels of developmental functioning in different areas, with his motor skills being more advanced than his communication and social skills.

A family history interview was conducted with the proband's mother. This indicated that the proband was able to sit at 2 years old, was able to crawl at around 5 years old, and was beginning to pull himself up to stand from about 7 years. When younger he had a marked lack of spontaneous affection or emotional reciprocity and was, at the time of assessment, still socially awkward and isolated. The proband did show some further behaviour usually associated with an autism spectrum disorder, for example, a lack of eye contact, a relative difficulty with reciprocal conversation and social play, an interest in the sensory property of water, repetitive patterns of movement, and self-injurious behaviours such as scratching and biting himself. However, owing to the proband's level of learning difficulty it would be difficult to determine whether these behaviours can be attributed to an autism spectrum disorder or to his delayed development.

METHODS

Chromosomal preparations were made from peripheral blood cultures using standard methods. Fluorescence in situ hybridisation (FISH) was performed according to the method described by Pinkel et al with slight modifications. Nick translated biotin or digoxigenin labelled probes used in this study are pTRA25 (centromere 15 specific), cos 68 (IgHV), P1 (1–4) (NF1 pseudogene), cos 27 (D15S13), and bacterial artificial chromosomes (BAC) 30G08 (D15S156) and 758N13 (D15S1010) (http://dev.ensembl.org) (fig 2).

The results indicate that in our patient the quadruplicated region extends from a site proximal to cos 68 (IgHV) to a distal breakpoint that lies between RP11–30G08 (D15S156) and RP11–758N13 (D15S1010). The physical distance between the probes cos 68 and RP11–758N13 was estimated by contig information (www.ensembl.org) as 10 Mb.

Dual colour FISH using the probes cos27 (D15S13) and RP11–30G08 (D15S156) determined the following order for the probes: cen–cos27–30G08 (D15S156) and bacterial artificial chromosomes (BAC) 30G08 (D15S156) and 758N13 (D15S1010) (http://dev.ensembl.org) (fig 2).

Figure 2 FISH results showing the locus copy number of corresponding probes on the SMC(15) of the proband. Probes are listed in order centromere (top) to telomere (bottom). The relative position of the probes is shown on the map (not drawn to scale).

FISH slides were analysed with a Zeiss Axioskop microscope with a cooled CCD camera and applied imaging MacProbe software. Molecular analysis was performed using microsatellite repeat markers from chromosome 15q11–14 as described previously.

RESULTS

Cytogenetic analysis identified the presence of a uniquely large SMC(15) in 70% of the metaphase cells examined. The parental chromosomes were normal, indicating that the marker had arisen de novo. The chromosome 15 centromere probe pTRA-25 showed the SMC to be dicentric.

Additional chromosome 15 FISH probes were used to determine the nature of the chromosome 15 material contained in the SMC (fig 2). Hybridisations with cos 68 (IgHV) and P1–4 (NF1 pseudogene), both located between the centromere and the common proximal breakpoint, BP1, showed four fluorescent signals on the SMC(15). Four fluorescent signals were also seen using the probes cos 27 (D15S13) and RP11–30G08 (D15S156), which map to the proximal and distal ends of the PWACR, respectively. A representative example of these results is shown in fig 3 A and B.

No signals were found using the more distal probe RP11–758N13 (D15S1010), located outside the PWACR. These results indicate that in our patient the quadruplicated region extends from a site proximal to cos 68 (IgHV) to a distal breakpoint that lies between RP11–30G08 (D15S156) and RP11–758N13 (D15S1010).
By using the probes cos 27 and 30G08 simultaneously we showed that the two middle copies of the marker are inverted in orientation with respect to the two flanking copies. The distal breakpoint is located between 30G08(D15S156) and 758N13(D15S1010), a site which has been reported to harbour several hot spots for chromosome breakage. At least three different breakpoints, placed in this interval, are involved in the distal boundary of the large inv dup(15), one being equivalent to the common deletion breakpoint (BP3) of the PWS/AS deletion patients.

Interestingly, the proximal breakpoint located between cos 68 (IgHV) and the centromere is unique and has not been previously reported in rearrangements resulting in interstitial duplications, triplications, or SMCs. However, the pericentric region of 15q has been shown to carry multiple copies of the pseudogenes NF1, IgH D/V, GABRA5, and BCLA. The four pseudogenes are part of a large amplification the copy number of which varies within the normal population and is increased in people with an apparent duplication of proximal 15q.

Our FISH and molecular data suggest that the SMC is composed of two maternally derived inv dup(15)s, each containing the PWACR and the IgHV/NF1 region. The structure of this SMC could be explained by a pericentric break of a pre-existing inv dup(15), followed by a U type reunion between the sister chromatids. Alternatively, our patient may originally have had two SMCs, with a break in the pericentric 15q region with a U type exchange at the breakpoints resulting in the dicentric SMC(15) now seen. The formation of inv dup(15) has been described by several possible mechanisms.

The abnormal phenotype in patients carrying a large SMC is highly variable with no clear relation between the extent of PWACR and clinical severity. However, the phenotype associated with tetrasomy of the PWACR in people with an interstitial triplication or an SMC is in general significantly more severe than that associated with trisomy in patients with an interstitial duplication. This indicates that dosage of the PWACR is a major factor contributing to the clinical severity. Our patient, hexasomic for the PWACR and the pericentric IgHV/NF1 pseudogene loci in 70% of cells, has severe developmental abnormalities, supporting the relation between increased dosage and severity of phenotype.

Recently, Herzig et al have shown increased expression levels of the imprinted gene UBE3A in cell lines carrying a maternal interstitial duplication, maternal triplication, or a large SMC(15) marker. UBE3A expression was increased in proportion to the UBE3A copy number with the highest expression level in the cell line containing an inv dup(15). These results suggest that overexpression of UBE3A may contribute to the abnormal phenotypes in patients with additional copies of the 15q11–13 region.

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Figure 3 Results of in situ hybridisation on the proband’s peripheral blood metaphases. (A) Metaphase spread hybridised with cos 68 (DIG) and pTRA-25 (BIO). The SMC(15) (arrow) is dicentric (green signals) and contains four copies of cos 68 (red signals). (B) Partial metaphase spread hybridised with cos 27 (DIG-TNF1, red signals) and RP11–30G08 (BIO-TNF1, green signals). One hybridisation signal of each probe is seen on each chromosome 15 homologue, but four signals of each probe appear on the SMC(15) (arrow). An enlarged view of the SMC is shown in the inset. The order of the signals shows the existence of two inverted duplications on the SMC.

Figure 4 ABI 377 Genescan peak data for molecular marker D15S822 in the proband and parents.

Molecular analysis of the proband and parents using polymorphic markers from within the PWACR confirmed that the patient had additional copies of this region. The extra alleles were maternal in origin (fig 4 shows the allele peaks for marker D15S822) but no extra alleles were found in the mother, showing that this SMC(15) had arisen de novo. Exact assessment of the allele copy numbers when using polymorphic molecular markers is difficult and the combination of the high copy number with mosaicism in the proband made exact assessment impossible in this case.

DISCUSSION

In this study we have identified and characterised a unique, very large dicentric SMC in a patient with a severely affected phenotype consisting of inability to walk alone, seizures, cortical blindness, and lack of speech. The SMC is derived from chromosome 15 and is of maternal origin consistent with virtually all reported large SMC(15)s.

As far as we are aware, this is the first patient described to be hexasomic for the proximal 15q11–13 region, with four copies of the extended PWACR on a single SMC(15). These results suggest that overexpression of UBE3A may contribute to the abnormal phenotypes in patients with additional copies of the 15q11–13 region.
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