ELECTRONIC LETTER

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

F Maggouta, S E Roberts, N R Dennis, M W M Veltman, J A Crolla

J Med Genet 2003;40:e84(<http://www.jmedgenet.com/cgi/content/full/40/7/e84>)

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.¹ Additional copies of the Prader-Willi/Angelman syndrome critical region (PWACR) can occur as interstitial duplications and triplications or as supernumerary marker chromosomes (SMCs).²⁻⁶

SMC(15), often referred to as inv dup(15), is the most common marker chromosome in humans, representing 50% of all markers.^{7,8} 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.^{6,9} 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.^{6,10,11}

Mapping studies have shown that the small SMCs share the same two common proximal breakpoints, BP1 and BP2,¹² 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.^{13,14}

Other rare variants of the inv dup(15) that do not follow the common pattern have also been described. Mignon *et al*¹⁵ and Robinson *et al*¹⁶ each reported a patient with an asymmetrical SMC(15), containing only one copy of the PWACR. The patient described by Mignon *et al*¹⁵ 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

Figure 1 Photograph of the patient aged 8.

interstitial duplication¹⁶ and an interstitial triplication.¹⁷ The presence of two extra marker chromosomes 15 has been reported in all cells of a woman with repeated abortions¹⁸ and in 47% of the cells of a severely retarded patient.⁶ A further patient with mental retardation presented initially with three morphologically distinct markers.¹⁹ 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 pericentromeric 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.

Abbreviations: AS, Angelman syndrome; BP, breakpoint; FISH, fluorescence in situ hybridisation; PWS, Prader-Willi syndrome; PWACR, Prader-Willi/Angelman syndrome critical region; SMC, supernumerary marker chromosome

Key points

- Supernumerary marker chromosome (SMC)15, also referred to as inv dup(15), is the most common SMC in humans, accounting for up to 50% of all SMCs. A main classification of the SMC(15) is based on the presence or absence of the Prader-Willi/Angelman syndrome critical region (PWACR): large SMC(15)s contain two additional copies of the PWACR and are associated with an abnormal phenotype; small SMC(15)s do not contain the PWACR and generally do not have a phenotypic effect.
- We identified and characterised a unique SMC(15) in 70% of the peripheral blood cells of a patient with a severe phenotype. The SMC(15) is dicentric and contains four copies of the PWACR and the pericentromeric IgHV and NF1 pseudogene loci. Dual colour FISH showed that the SMC(15) is composed of two large SMC(15)s resulting in hexasomy of the PWACR and the IgHV and NF1 pseudogenes in the patient's genome.

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 8 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 development²⁰ and the Vinelands adaptive behaviour scales, interview edition survey form,²¹ 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.²² 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^{23, 24} 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*²⁵ with slight modifications. Nick translated biotin or digoxigenin labelled probes used in this study are pTRA25 (centromere 15 specific),²⁶ cos 68 (IgHv),²⁷ P1 (1–4) (NF1 pseudogene),²⁸ cos 27(D15S13),²⁹ 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.³

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). 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–cos27–30G08–30G08–cos27–cos27–30G08–30G08–cos27–cen. This order shows the existence of two contiguous inverted duplications on the SMC(15) (fig 3B). Each inverted duplication contains two copies of the PWACR and IgHV/NF1 pseudogene region, resulting in tetrasomy of that region on the SMC(15). FISH results are summarised in fig 2.

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 cos27 (DIG-TRITC, red signals) and RP11-30G08 (BIO-FITC, 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 located on a single SMC and one copy on each chromosome 15 homologue. The presence of six copies of the entire or parts of the PWACR has been reported previously in rare instances. However, the patients were hexasomic for the region owing to two additional inv dup(15) chromosomes. Three probands had inv dup(15) chromosomes with breakpoints at q12, q13, and q15, respectively, and therefore contained the PWACR.^{10–30} One patient had two extra inv dup(15) in 47% of the cells analysed, each including a single *SNRPN* locus and possibly two *GABRB3* loci.⁶ Four additional people were described to have two extra SMCs with breakpoints at q11 and therefore were without the PWACR.^{18–31–33} By contrast, our patient is carrying the additional four copies of the extended PWACR on a single SMC(15).

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.^{11–13} 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 pericentromeric region of 15q has been shown to carry multiple copies of the pseudogenes NF1, IgH D/V, GABRA5, and BCLA.^{34–35} The four pseudogenes are part of a large amplicon 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 pericentromeric 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 SMC(15)s, but a break in the pericentromeric 15q region with a U type exchange at the breakpoints resulted in the dicentric SMC(15) now seen. The formation of inv dup(15) has been described by several possible mechanisms.^{36–37}

The abnormal phenotype in patients carrying a large SMC is highly variable with no clear relation between the extent of PWACR and clinical severity.^{9–13–15} 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.^{10–38} This indicates that dosage of the PWACR is a major factor contributing to the clinical severity. Our patient, hexasomic for the PWACR and the pericentromeric 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*³⁹ 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.

ACKNOWLEDGEMENTS

We thank Professor Patricia Jacobs and Dr N Simon Thomas for helpful discussions. We are also grateful to Dr Peter Christie for providing clinical details. This work was part of a MRC funded project.

Authors' affiliations

F Maggouta, S E Roberts, J A Crolla, Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury SP2 8BJ, UK
N R Dennis, Department of Human Genetics, University of Southampton, Southampton SO9 4HA, UK
M W M Veltman, Section of Developmental Psychiatry, University of Cambridge, Cambridge CB2 2AH, UK

Correspondence to: Dr F Maggouta, Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury SP2 8BJ, UK; f.maggouta@salisbury.nhs.uk

REFERENCES

- 1 **Knoll JH**, 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.
- 2 **Clayton-Smith J**, Webb T, Cheng XJ, Pembrey ME, Malcolm S. Duplication of chromosome 15 in the region 15q11–13 in a patient with developmental delay and ataxia with similarities to Angelman syndrome. *J Med Genet* 1993;**30**:529–31.
- 3 **Browne CE**, Dennis NR, Maher E, Long FL, Nicholson JC, Sillibourne J, Barber JCK. Inherited interstitial duplications of proximal 15q: genotype-phenotype correlations. *Am J Hum Genet* 1997;**61**:1342–52.
- 4 **Schinzel AA**, Brevecic L, Bernasconi F, Binkert F, Berthet F, Wuilloud A, Robinson WP. Intrachromosomal triplication of 15q11–q13. *J Med Genet* 1994;**31**:798–803.
- 5 **Roberts SE**, Dennis NR, Browne CE, Willatt L, Woods CG, Cross I, Jacobs PA, Thomas NS. Characterization of interstitial duplications and triplications of chromosome 15q11–q13. *Hum Genet* 2002;**110**:227–34.
- 6 **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.
- 7 **Buckton KE**, Spowart G, Newton MS, Evans HJ. Forty four probands with an additional marker chromosome. *Hum Genet* 1985;**69**:353–70.
- 8 **Blennow E**, Bui T-H, Kristofferson U, Vujic M, Anneren G, Holmberg E, Nordenskjöld M. Swedish survey on extra structurally abnormal chromosomes in 39105 consecutive prenatal diagnoses: prevalence and characterization by fluorescence in situ hybridization. *Prenat Diagn* 1994;**14**:1019–28.
- 9 **Leana-Cox J**, Jenkins L, Palmer CG, Plattner R, Sheppard L, Flejter WL, Zachowski J, Tsien F, Schwartz S. 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.
- 10 **Robinson WP**, Binkert F, Gine R, Vazquez C, Muller W, Rosenkranz W, Schinzel A. Clinical and molecular analysis of five inv dup(15) patients. *Eur J Hum Genet* 1993;**1**:37–50.
- 11 **Robinson WP**, Dutly F, Nicholls RD, Bernasconi F, Penaherrera M, Michaelis RC, Abeliovich D, Schinzel AA. The mechanisms involved in formation of deletions and duplications of 15q11–13. *J Med Genet* 1998;**35**:130–6.
- 12 **Huang B**, Crolla JA, Christian SL, Wolf-Ledbetter ME, Macha ME, Papenhausen PN, Ledbetter DH. Refined molecular characterization of the breakpoints in small inv dup(15) chromosomes. *Hum Genet* 1997;**99**:11–17.
- 13 **Wandstrat AE**, Leana-Cox J, Jenkins L, Schwarz S. Molecular cytogenetic evidence for a common breakpoint in the largest inverted duplications of chromosome 15. *Am J Hum Genet* 1998;**62**:925–36.
- 14 **Christian SL**, Fantes JA, Mewborn SK, Huang B, Ledbetter DH. Large genomic duplicons map to sites of instability in the Prader-Willi/Angelman syndrome chromosome region (15q11–q13). *Hum Mol Genet* 1999;**8**:1025–37.
- 15 **Mignon C**, Malzac P, Moncla A, Depetris D, Roeckel N, Croquette M-F, Mattei M-G. Clinical Heterogeneity in 16 patients with inv dup 15 chromosome: cytogenetic and molecular studies, search for an imprinting effect. *Eur J Hum Genet* 1996;**4**:88–100.
- 16 **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 chromosomes. *Eur J Hum Genet* 1995;**3**:49–55.
- 17 **Long FL**, Duckett DP, Billam LJ, Williams DK, Crolla JA. Triplication of 15q11–q13 with inv dup(15) in a female with developmental delay. *J Med Genet* 1998;**35**:425–8.
- 18 **Shim SH**, Lee C-H, Park YJ, Lee HJ, Park W, Cho YH. Two inv dup(15) chromosomes in a woman with repeated abortions. *Am J Med Genet* 2001;**104**:303–6.
- 19 **Cockwell AE**, Davalos IP, Rivera HR, Crolla JA. FISH characterization of dynamic mosaicism involving an inv dup(15) in a patient with mental retardation. *Am J Med Genet* 2001;**103**:289–94.
- 20 **Mullen E**. *The Mullen scales of early learning*. AGS ed. Circle Pines, Minnesota: American Guidance Service, 1995.
- 21 **Sparrow S**, Balla D, Cicchetti D. *Vineland scales of adaptive behavior*. Survey from manual. Circle Pines, Minnesota: American Guidance Service, 1984.
- 22 **World Health Organization**. *The international statistical classification of diseases and related health problems, 10th revision (ICD-10)*. Geneva, Switzerland: WHO, 1992.
- 23 **Bolton PF**, Macdonald H, Pickles A, Rios P, Goode S, Crowson M, Bailey A, Rutter M. A case-control family history study of autism. *J Child Psychol Psychiatry* 1994;**35**:877–900.
- 24 **Fombonne E**, Bolten P, Prior J, Jordan H, Rutter M. A family study of autism: cognitive patterns and levels in parents and siblings. *J Child Psychol Psychiatry* 1997;**38**:667–83.
- 25 **Pinkel D**, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J. Fluorescence in situ hybridisation with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc Natl Acad Sci USA* 1988;**85**:9138–42.
- 26 **Choo KH**, Earle E, Vissel B, Filby RG. Identification of two distinct subfamilies of alpha satellite DNA that are highly specific for human chromosome 15. *Genomics* 1990;**7**:143–51.
- 27 **Tomlinson IM**, Cook GP, Carter NP, Elavarapu R, Smith S, Walter G, Buluwela L, Rabbits TH, Winter G. Human immunoglobulin VH and D segments on chromosomes 15q11.2 and 16p11.2. *Hum Mol Genet* 1994;**3**:853–60.
- 28 **Purandare SM**, Huntsman-Breidenbach H, Li Y, Zhu XL, Sawada S, Neil SM, Brothman A, White R, Cawthon R, Viskochil D. Identification of neurofibromatosis 1 (NF1) homologous loci by direct sequencing, fluorescence in situ hybridisation, and PCR amplification of somatic cell hybrids. *Genomics* 1995;**30**:476–85.
- 29 **Donlon TA**, Lalande M, Wyman A, Bruns G, Latt SA. Isolation of molecular probes associated with the chromosome 15 instability in the Prader-Willi syndrome. *Proc Natl Acad Sci USA* 1986;**83**:4408–12 (correction in **83**:6964).
- 30 **Van Dyke DL**, Weiss L, Logan M, Pai GS. The origin and behavior of two isodicentric bisatellited chromosomes. *Am J Hum Genet* 1977;**26**:294–300.
- 31 **Martin-Lucas MA**, Perez-Castillo A, Abrisqueta JA. Infertility associated with two accessory bisatellited chromosomes. *Hum Genet* 1986;**73**:133–6.
- 32 **Manenti E**. Two extra inv dup(15) chromosomes and male infertility: second case. *Am J Med Genet* 1992;**42**:402–3.
- 33 **Gentile M**, Susca F, Resta N, Stella A, Cascone A, Guanti G. Infertility in carriers of two bisatellited marker chromosomes. *Clin Genet* 1993;**44**:71–5.
- 34 **Ritchie RJ**, Mattei M-G, Lalande M. A large polymorphic repeat in the pericentromeric region of the human chromosome 15q contains three partial gene duplications. *Hum Mol Genet* 1998;**7**:1253–60.
- 35 **Fantes JA**, Mewborn SK, Lese CM, Hedrick J, Brown RL, Dyomin V, Chaganti RSK, Christian SL, Ledbetter DH. Organisation of the pericentromeric region of chromosome 15: at least four partial gene copies are amplified in patients with proximal duplication of 15q. *J Med Genet* 2002;**39**:170–7.
- 36 **Schreck RR**, Breg WR, Erlanger BF, Miller OJ. Preferential derivation of abnormal human G-group-like chromosomes from chromosome 15. *Hum Genet* 1977;**36**:1–12.
- 37 **Martinsson T**, Johansson T, Vujic M, Sjostedt A, Steffenburg S, Gillberg C, Wahlstrom J. Maternal origin of inv(15) chromosomes in infantile autism. *Eur Child Adolesc Psychiatry* 1996;**5**:185–92.
- 38 **Webb T**, Hardy CA, King M, Watkiss E, Mitchell C, Cole T. A clinical, cytogenetic and molecular study of 10 probands with supernumerary inv dup (15) marker chromosomes. *Clin Genet* 1998;**53**:34–43.
- 39 **Herzig LBK**, Cook EH Jr, Ledbetter DH. Allele-specific expression analysis by RNA-FISH demonstrates preferential maternal expression of UBE3A and imprint maintenance within 15q11–q13 duplications. *Hum Mol Genet* 2002;**11**:1707–18.