

## Electronic letter

### Molecular characterisation of a supernumerary ring chromosome in a patient with VATER association

EDITOR—Supernumerary marker chromosomes are rare with an incidence of 0.3–1.5/1000 newborns. Most carriers have a normal phenotype but in 15% of non-satellited marker cases mental retardation and minor anomalies have been reported.<sup>1</sup> The origin of several supernumerary ring marker chromosomes has been identified by fluorescence in situ hybridisation (FISH).<sup>2</sup> The VATER association is characterised by non-random occurrence of Vertebral anomalies, Anal atresia, Tracheo-oesophageal fistula with Esophageal atresia, Radial limb dysplasia, and Renal defects.<sup>3</sup> The acronym VACTERL is used in cases with additional Cardiac and Limb malformations.<sup>4</sup> VACTERL with hydrocephalus is thought to be an autosomal recessive disorder distinct from the VATER association.<sup>5</sup> Other defects that occur less frequently have been also described.<sup>6</sup> A defect in blastogenesis was suggested as a possible aetiology of this malformation spectrum. Martinez-Frias *et al*<sup>7</sup> proposed that combinations of anomalies of blastogenetic origin, such as VATER/VACTERL, should be considered and called “polytopic field defects” instead of the generic term “association”.

The knowledge that maternal intake of some teratogens, such as oestrogens<sup>8</sup> or methimazole,<sup>9</sup> may be associated with VATER/VACTERL in the newborn, probably affecting blastogenesis, and familial occurrence of VACTER/VACTERL<sup>10,11</sup> suggest heterogeneity in the pathogenesis of the association, although it appears that the underlying causative event takes place at a very early stage of embryonic development.

Only one chromosome abnormality has been described in VATER association, a patient with an interstitial 6q deletion,<sup>12</sup> while an additional case of VATER with 9qh+ has been reported.<sup>13</sup>

We report here an additional patient with malformations characteristic of VATER association and mosaicism for a small supernumerary ring chromosome derived from the pericentromeric region of chromosome 12.

The proband was the term product of an uneventful pregnancy, requiring elective caesarean section because of uterine inertia. Birth weight was 3520 g, length 51 cm, and OCF 33 cm. At birth, an anorectal malformation with urethral fistula was detected and surgical correction was performed on the second day of life. Skeletal *x* ray showed multiple dorsal and lumbar hemivertebrae, sacral agenesis, and multiple left fused ribs. He underwent neurosurgical correction for the presence of a medullary lipoma, thickened terminal filum, and hydromelia. No radial ray defects were observed. Ultrasound examination showed absence of the left kidney. There were no cardiac or tracheal anomalies. Psychomotor development was normal and he now attends primary school. The parents are phenotypically normal.

Analysis of the patient's karyotype showed the presence, in both lymphocytes and fibroblasts, of a supernumerary small ring chromosome in 63% of metaphases. The marker chromosome did not show any specific QFQ or GTG banding pattern and it was DA/DAPI negative. The small

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supernumerary ring chromosome was a de novo anomaly since both parents had a normal karyotype.

The origin of the ring chromosome was ascertained by performing FISH with an alphoid DNA probe specific for several chromosomes and hybridisation was successfully accomplished using the alphoid DNA specific for chromosome 12. The hybridisation signal was present on the centromere of both chromosomes 12 and covered almost the whole ring (fig 1A, B).

In order to establish the presence of euchromatic material, we used some overlapping YAC clones (CEPH Mega-YAC library) specific for the 12q12 (cen-956\_a\_5, 958\_e\_2-tel) and 12p11-p12.1 (cen-832\_f\_14, 952\_a\_6, 753\_f\_12, 922\_d\_9, 927\_g\_11, 806\_c\_2, 792\_b\_12-tel) regions (fig 2). YAC 922\_d\_9 contains the *SOX5* gene.<sup>14</sup> All YAC clones were selected on the basis of the available consensus map<sup>15</sup> and provided by the YAC Screening Center (DIBIT-HSR and IGBE-CNR Milano Italy, <http://www.spr.it/iger/home.html>). FISH analysis with YAC clones specific for the 12q12 region showed that the hybridisation signal of YAC clone 956\_a\_5 was present on the ring with the same intensity as the ones present on both chromosomes 12. This resulted in chimerism since the hybridisation signal was also present on 4q12, 9p23, 6q13, and 11p14. The YAC portion on chromosome 12 is located immediately next to the centromere; the distal YAC 958\_e\_2 gave no signal on the ring chromosome. The 956\_a\_5 and 958\_e\_2 YAC clones overlap for the presence of a STS constructed on the end of the corresponding YAC clone (958e2-R), while the distal 943f6-R STS is present only in the YAC 958\_e\_2.<sup>15</sup> These results allowed us to localise the breakpoint on the long arm of chromosome 12, probably in the region between the YAC clones 956\_a\_5 and 958\_e\_2 containing the two STS constructed on the end of the corresponding YAC clones (958e2-R and 943f6-R).

On the other hand, several YAC clones (832\_f\_14, 952\_a\_6, 753\_f\_12 and 922\_d\_9) specific for the 12p11-p12.1 region gave an intense hybridisation signal on the ring chromosome, indicating that the marker was mainly made up of a portion of chromosome 12 short arm. Additionally, two YAC clones, 927\_g\_11 and 806\_c\_2, have been identified probably containing the breakpoint, since the hybridisation signals on the marker chromosome were less intense than the signals on both chromosomes 12 (fig 1C, D, E, F). The distal YAC 792\_b\_12, which gave no signal on the ring chromosome, overlaps with both YACs 927\_g\_11 and 806\_c\_2 for the presence of the AFM267yc9 marker (Whitehead Institute/MIT site: <http://www.genome.wi.mit.edu/>). Since YAC 927\_g\_11, which contains the breakpoint, and the proximal YAC 922\_d\_9 are positive for the marker AFMb351zh5 (<http://www.genome.wi.mit.edu/>), the breakpoint on the short arm of chromosome 12 could be located between markers AFM267yc9 and AFMb351zh5 (fig 2). On the basis of these molecular data, the patient is trisomic for the 12p12.1-q12 region included between the markers AFM267Yc9 and STS on the end of the 943\_f\_6 YAC clone (943f6-R) and containing the centromere. Thus the ring chromosome is composed of a portion of the q12 region contained in the positive YAC 956\_a\_5 and mainly p arm sequences. Finally, the proband's karyotype was interpreted as follows: 46,XY/47,XY+r(12)(p12.1q12).

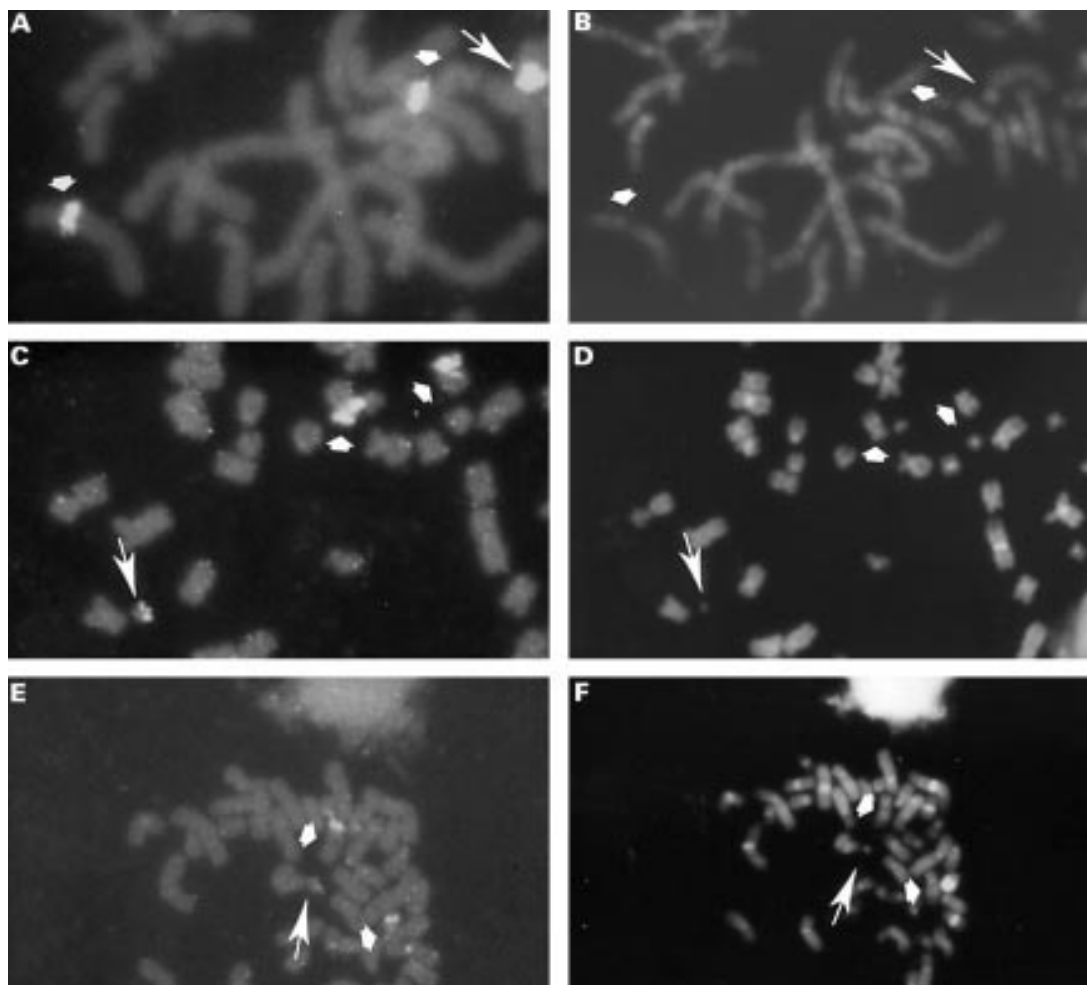


Figure 1 FISH experiments using (A) an alphoid DNA probe specific for chromosome 12; (C) 927\_g\_11, and (E) 806\_c\_2 YAC clones. (B, D, F) The same partial metaphases stained with DAPI. The large arrows show the ring chromosome and the small arrows the normal chromosome 12 pair.

Since the *SOX5* gene maps to 12p12.1,<sup>14 16</sup> immediately next to one of the breakpoints, two BAC clones, bK906D14 and bK943I5 (RPC-11 Human BAC library), containing the 5' genomic sequence of the h-L-*SOX5* gene were also used in FISH experiments. The two BAC clones, like YAC 922\_d\_9, gave a hybridisation signal of the same intensity on both chromosome 12 and on the ring chromosome, indicating that the gene probably does not span one of the breakpoints.

To the best of our knowledge, the present case is the second report of a patient with VATER association and a karyotype anomaly. In order to obtain a karyotype/phenotype correlation, we reviewed all published cases with a supernumerary ring chromosome 12. To date, an extra small ring chromosome 12 mosaicism was described in a patient with delayed development and vesicoureteric reflux and in a fetus.<sup>2</sup> In the latter case, no clinical data were reported because fetal necropsy was not performed after pregnancy termination. In both cases the small ring chromosomes were only defined as deriving from the pericentromeric region of chromosome 12 and were not molecularly characterised in order to verify the presence of euchromatic material from the short and/or the long arm of chromosome 12.

Although the ring chromosome is a different condition from the duplication of the same chromosomal region, nevertheless we reviewed all reported cases with similar duplications. We found only patients with bigger duplications, some of which partially overlapped the region

defined by us. Most reported cases were partially or totally trisomic for the chromosome 12 short arm and their clinical phenotypes were included in the spectrum of 12p trisomy.<sup>17</sup> We selected only the patients who had a "pure" partial or total 12p trisomy, that is, derived from a direct or inverted tandem duplication or from a malsegregation of a parental balanced reciprocal translocation of 12p onto the short arm of an acrocentric chromosome.<sup>18-27</sup> Among these, three were mosaic cases and the full spectrum of 12p trisomy syndrome was present.<sup>19 21 25</sup> Only two patients had a duplication also including the q12 region.<sup>18 26</sup> To the best of our knowledge, no other cases with a duplication of the long arm of chromosome 12 overlapping the critical interval defined by us have been reported.

The 12p trisomy syndrome has a well recognised spectrum of anomalies; congenital malformations of the internal organs are rare in "pure" 12p duplications. The frequency of malformations, however, seems to increase with the extent of the imbalance. The patient described here does not show the 12p syndrome phenotype. Our clinical and molecular findings could support the hypothesis previously reported that the critical duplicated segment causing 12p trisomy syndrome is located distally to our 12p breakpoint, in p13.1-p13.3.<sup>21</sup> Our proband has a complex phenotype with multiple dorsal and lumbar hemivertebrae, sacral agenesis, anorectal malformation, and unilateral kidney agenesis. According to the criteria of Quan and Smith,<sup>3</sup> we conclude that our patient has VATER association.

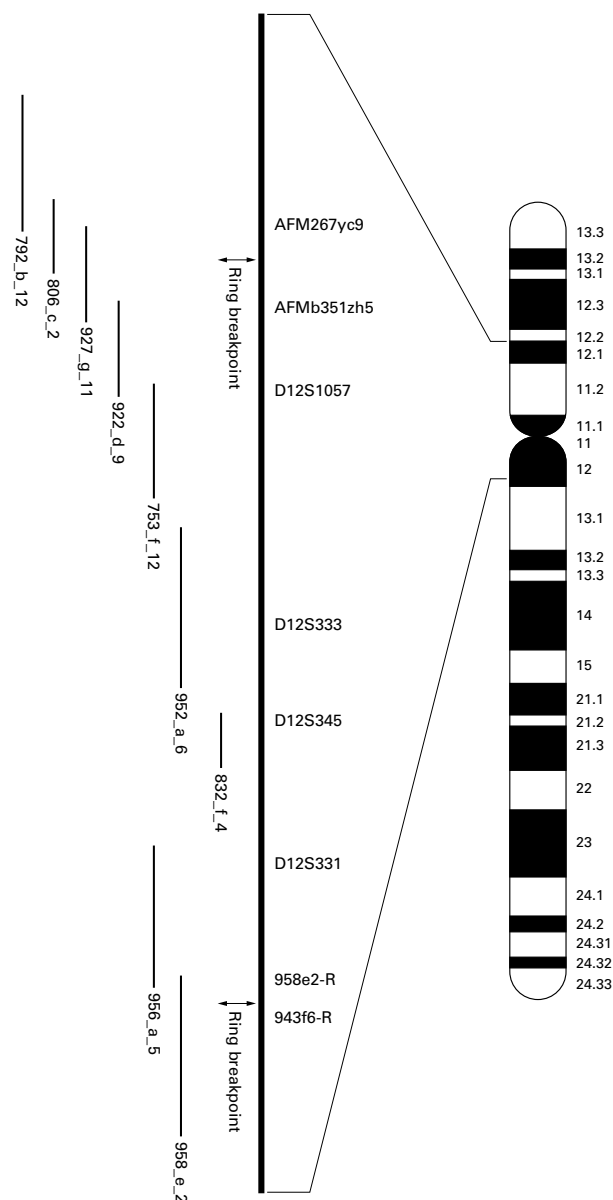


Figure 2 Schematic representation of all the YAC clones specific for the 12p12.1-q12 region used in FISH experiments. The physical data are summarised from Mostert *et al.*,<sup>13</sup> Krauter *et al.*,<sup>14</sup> and from the Whitehead Institute/MIT site (<http://www.genome.wi.mit.edu/>).

One of the reported patients with the larger duplication including the 12q12 region had bilateral hydronephrosis with a rudimentary left kidney,<sup>26</sup> similar to the present case.

Table 1 Clinical features of reviewed cases with supernumerary ring 12 chromosomes and larger partially overlapping duplications compared with present case. The proband's phenotype and similar features observed in the other cases are in bold

Reported cases	Partial trisomy	Phenotype
Present case	12p12.1-q12 (ring chromosome)	<b>Dorsal and lumbar hemivertebrae, sacral agenesis, anorectal malformation, unilateral kidney agenesis, rib fusion</b>
Callen <i>et al.</i> , case 5 <sup>2</sup>	Chr 12 pericentromeric region (ring chromosome)	Delayed development, <b>vesicoureteric reflux</b>
Callen <i>et al.</i> , case 4 <sup>2</sup>	Chr 12 pericentromeric region (ring chromosome)	Undetermined
Rethoré <i>et al.</i> <sup>26</sup>	12pter-q12	<b>Bilateral hydronephrosis, rudimentary left kidney</b>
Kondo <i>et al.</i> <sup>25</sup>		12p syndrome
Stengel-Rutkowski <i>et al.</i> , case 1 <sup>18</sup>	12pter-q12	12p syndrome Congenital heart disease, incomplete intestinal rotation, Meckel diverticulum, 12p syndrome
Stengel-Rutkowski <i>et al.</i> , case 2 <sup>18</sup>	12pter-p11	<b>Anal atresia</b> , hypospadias, 12p syndrome
Guerrini <i>et al.</i> <sup>19</sup>	12pter-p11	Cerebral malformation, 12p syndrome
Biederman <i>et al.</i> <sup>27</sup>	12pter-p11	Congenital heart disease, 12p syndrome
Rauch <i>et al.</i> , <sup>21</sup> Rivera <i>et al.</i> <sup>24</sup>	12pter-p11	12p syndrome
Rauch <i>et al.</i> , case 1 <sup>21</sup>	12pter-p11.21	12p syndrome
Pfeiffer <i>et al.</i> <sup>20</sup>	12p13.3-p11.2	12p syndrome
Rauch <i>et al.</i> , <sup>21</sup> Allen <i>et al.</i> , <sup>22</sup> Back <i>et al.</i> <sup>23</sup>	12pter-12.1	12p syndrome

Among the reported cases with complete duplication of 12p, one patient had anal atresia,<sup>18</sup> another one had cerebral ventricular dilatation,<sup>19</sup> and two other patients had cardiac anomalies,<sup>18, 27</sup> while the remaining cases did not show any internal organ malformations (table 1).

Although ring chromosomes have been well characterised, the pathogenetic mechanism remains to be understood. We could postulate three hypotheses. The first one is that the patient's phenotype could be the result of the presence of the supernumerary ring chromosome. On reviewing all the above mentioned published cases, we could not define a precise karyotype/phenotype correlation. However, on the basis of the two previously described patients, one with the bigger duplication including the 12q12 region and a rudimentary left kidney<sup>26</sup> and the other with a supernumerary ring 12 chromosome and vesicoureteric reflux,<sup>2</sup> we cannot rule out the possibility that the presence of the trisomic q12 segment might mainly determine the patient's phenotype.

In the 12p12.1-q12 region, several genes and as yet uncharacterised ESTs have been identified (Human GeneMap '99) whose triple dosage could determine a developmental defect causing a pathological phenotype. Among the genes, possible candidates are *SOX5*<sup>16</sup> and the human homologue (*BICD1*) of the *Drosophila Bicaudal* gene (*Bic-d*)<sup>28</sup> because of their role during embryonic development. Particularly, gain of function mutations of *Bic-d* in *Drosophila* disrupt the establishment of anterior and posterior polarity in the early embryo.<sup>29</sup> However, we do not know if the genes contained in this supernumerary chromosome are normally expressed.

The second hypothesis is that the breakpoints of the rearrangement could interrupt the coding sequence of a gene with a negative dominant effect by the eventually translated truncated protein. Since the *SOX5* gene maps in 12p12.1,<sup>14, 16</sup> immediately next to one of the breakpoints, we performed FISH experiments to verify this hypothesis. Our results indicated that the gene was unlikely to span the breakpoint. However, these data do not exclude the involvement of other genes. Additionally, a dominant negative or a deleterious effect could be determined by the presence of a chimeric gene caused by the fusion of two coding sequences spanning the breakpoints.

The third hypothesis is that the cytogenetic anomaly and the pathological phenotype could be randomly associated, although the supernumerary ring chromosome was observed in a large percentage, 63%, of mitoses. In this respect, mosaicism for a small de novo supernumerary 12 marker chromosome was reported in a normal child.<sup>30</sup> Thus, the features of VATER association presented by our patient could be the result of a germinal or somatic mutation of a gene not localised in the critical interval defined by

us. To the best of our knowledge, one subject with VACTERL has been found to have a somatic point mutation in mitochondrial DNA from kidney tissue.<sup>31</sup>

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