

## ELECTRONIC LETTER

# Distal trisomy 6p and 20q owing to the concurrent transposition of distal 6p and 20q to the 22q telomere: a genomic polymorphism?

M C Bonaglia, R Giorda, A Cavallini, T Pramparo, M Rocchi, R Borgatti, O Zuffardi

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Several papers have recently shown that 6–7% of retarded patients with unclassified malformation syndromes and normal routine cytogenetic analysis have cryptic rearrangements involving subtelomeric regions.<sup>1</sup> About half of these patients have familial unbalanced translocations, the other half have de novo deletions,<sup>2</sup> and very few cases have duplications.<sup>3</sup> The existence of subtelomeric imbalances with only minor or even absent phenotypic effects has been hypothesised and subsequently demonstrated,<sup>3–8</sup> thanks to the increased use of telomeric screening on many groups of patients, tested with different methodologies and selected with different clinical ascertainment criteria.<sup>1</sup> When an unbalanced rearrangement is found both in a proband with an abnormal phenotype and in one of his or her normal parents, the following hypotheses can be made: (1) the rearrangement represents a genomic polymorphism; (2) the imbalance is bigger in the proband than in the phenotypically normal parent; (3) the unbalanced region contains imprinted genes so that the supernumerary/deleted gene is inactive in the normal parent and becomes active in the child; (4) the remaining allele of one of the genes deleted in the submicroscopic rearrangement is mutated in the proband but not in the phenotypically normal parent so that he or she still has a normal copy of that gene. We studied a cryptic rearrangement of chromosome 22q resulting in distal trisomy for both 6p and 20q. The same der(22) was present in the proband, a patient with normal IQ affected by bilateral chorioretinal coloboma and grade IV bilateral vesicoureteric reflux (VUR), and in his clinically normal father. The size of the duplicated 6p and 20q chromosome regions and their gene content were investigated to define the impact of the trisomic segments on the phenotype.

## MATERIALS AND METHODS

### Case history

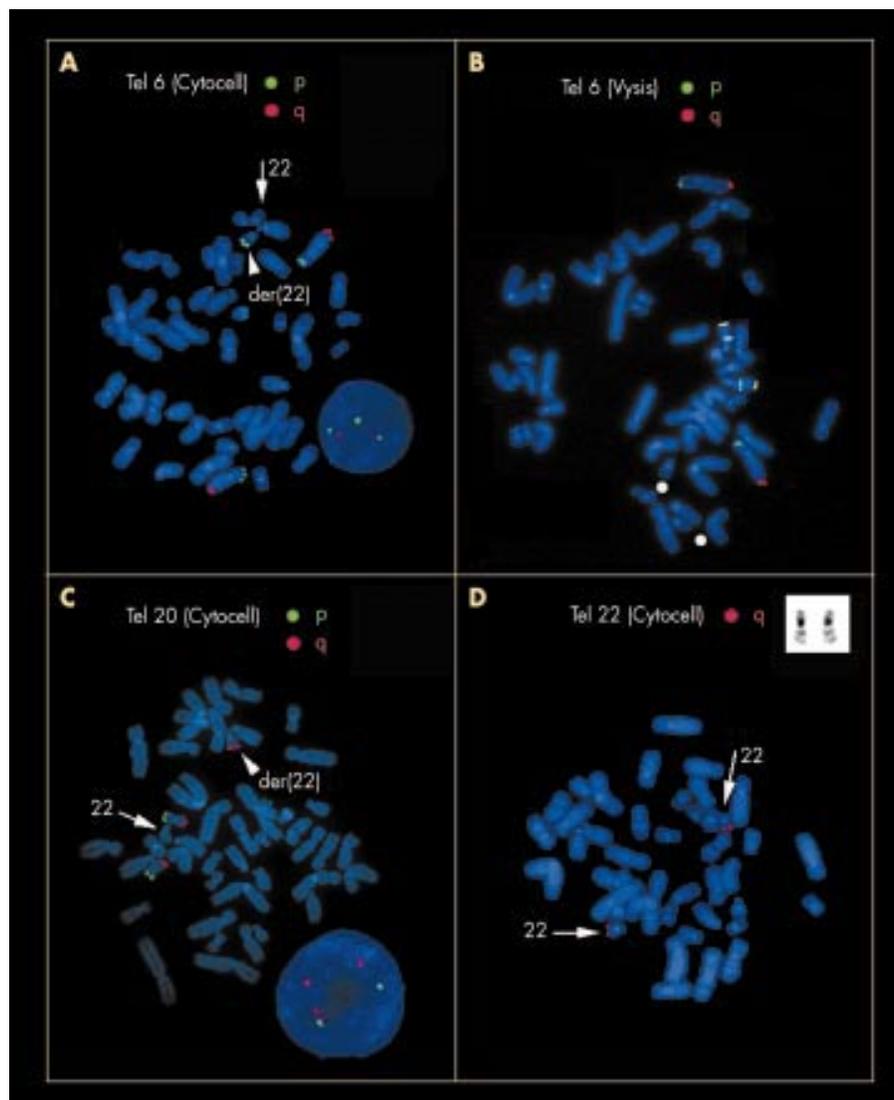
The proband was a 4 year old boy, the only child of non-consanguineous parents. Family history was unremarkable. He was born by normal delivery, after an uncomplicated pregnancy, at the 35th week of gestation because of premature rupture of the membranes. His birth weight was 2700 g (50th centile), length 47 cm (50th centile), and his head circumference was 31 cm (10th centile). At the age of 3 months he underwent an ophthalmological examination because he was not able to fix a target: fundus assessment disclosed the presence of bilateral retinal coloboma. He walked unsupported at the age of 18 months and his language development was normal. At the age of 3 years, neurological examination showed mild generalised hypotonia. His IQ (as measured by the Wechsler Preschool and Primary Scale of Intelligence<sup>9</sup>) was 103, with a score of 97 and 103 for motor and verbal performance, respectively. Ophthalmological re-examination confirmed the presence of bilateral retinal coloboma of the macular region; nyctagmus was also present. Visual evoked

### Key points

- It has become evident that subtelomeric cryptic rearrangements may provide an explanation for previously unexplained cases of mental retardation and unclassified malformation syndromes.
- Among the more than 1500 cases analysed, few were found with the same cryptic imbalance present in the proband and in one normal parent making the association between abnormal phenotype and chromosome rearrangement puzzling.
- We describe a patient with features of the papillorenal syndrome (MIM 120330). After the exclusion of *PAX2* mutations and of gross cytogenetic anomalies, telomere screening was performed showing a chromosome 22 with a bar of FISH subtelomeric signals related to 22q, 6p, and 20q.
- The proband's phenotypically normal father had the same rearrangement.
- Molecular characterisation with BAC/PAC clones from each rearranged chromosome region has shown that 6p was trisomic for 250 kb and 20q for 300 kb, showing that there were no genomic differences between the proband and his father. The malformations of the proband, essentially consisting of bilateral chorioretinal coloboma and grade IV bilateral VUR, did not overlap with the reported 6p and 20q duplication phenotypes. Molecular data showed that the duplicated 6p region contains only two olfactory receptor genes whereas the duplicated 20q region, although still sketchy, does not seem to contain any gene.
- We hypothesise that the chromosomal rearrangement is not related to the patient's phenotype and is just an unusual genomic polymorphism.

potentials (VEPs) showed decreased amplitude. The proband has had gradually worsening VUR from birth. At 4 years he has grade 4 VUR with no haematuria or proteinuria and is currently receiving antibiotic treatment. His kidneys did not show any morphological or structural anomalies by echography. Echocardiography and tonal audiometric and impedentiometric examinations were normal. Neurological examination showed mild generalised hypotonia. The proband shows very mild dysmorphic features such as round face with

**Abbreviations:** BAC, bacterial artificial chromosome; FISH, fluorescence in situ hybridisation; PAC, P1 artificial chromosome; PCR, polymerase reaction; VEP, visual evoked potential; VUR, vesicoureteric reflux



**Figure 1** Proband's FISH analysis with specific subtelomeric probes for chromosome 6 (A and B), chromosome 20 (C), and chromosome 22 (D). p arm probes are labelled in green and q arm in red. Both 6p (A) and 20q (C) subtelomeric probes (Cytocell) show hybridisation to the long arm of one chromosome 22 (arrowhead). (B) FISH analysis with the subtelomeric 6p probe (TelVysion, Vysis) shows signals only on both chromosome 6 homologues. White dots pinpoint chromosome 22 homologues. The probe mix also contains a second set of differentially labelled/coloured subtelomeric probes, which were localised to different chromosomes: tel13q (spectrum yellow) and 13q (spectrum aqua). These probes served as internal controls for the hybridisation efficiency. (D) FISH with tel22q (Cytocell) probe shows fluorescent signals on both chromosome 22 homologues. In the upper right corner is shown a G banding cut out of chromosomes 22 showing the impossibility of distinguishing the der (22) from the normal chromosome 22.

bulbous nose tip, sparse eyebrows and eyelashes, large, posteriorly rotated ears, thin helix, grooved philtrum, thin lips, and hypoplastic toenails.

### Cytogenetic and molecular analysis

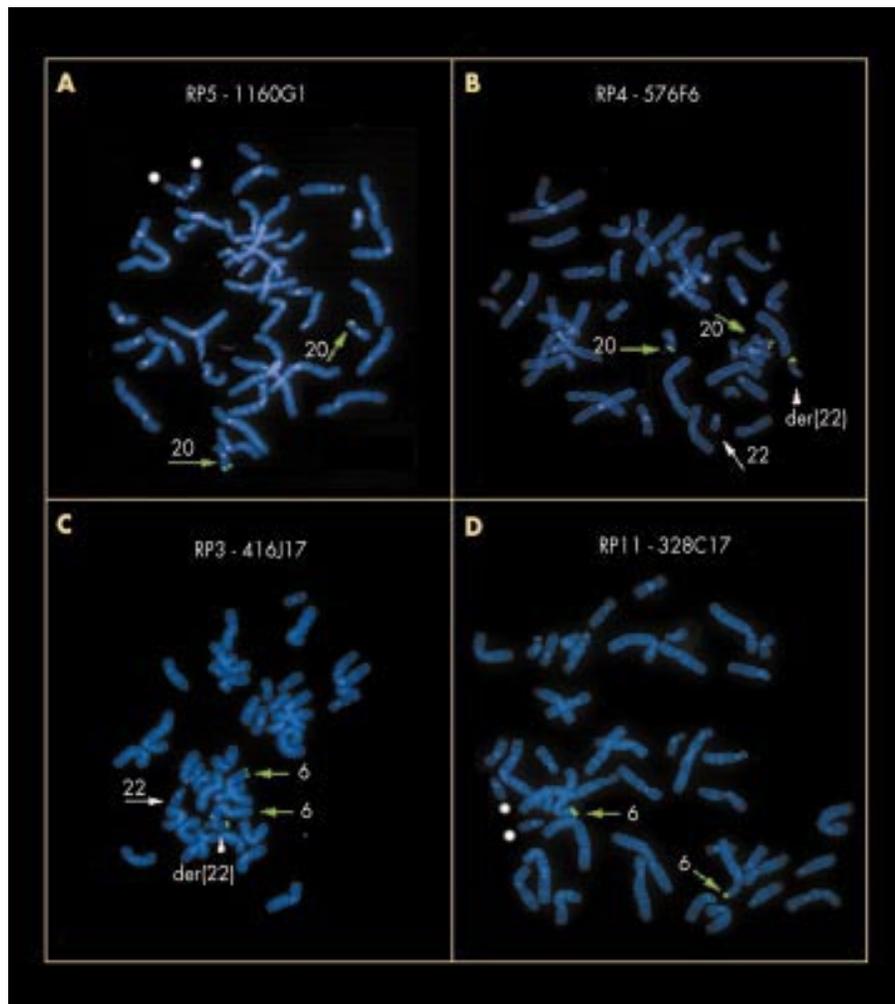
Routine cytogenetic analysis (no less than 550 bands) was performed on blood from the proband and the parents using standard high resolution techniques.<sup>10</sup>

The Chromoprobe-T kit with telomere specific probes was used according to the supplier's instructions (Cytocell, Adderbury, England). The 6p, 20q, and 22q AquariusT probes (Cytocell) and the TelVysion subtelomeric probes (Vysis, Downers Grove, IL, USA) were also used to retest the chromosomes of the proband.

Fluorescence in situ hybridisation (FISH) to chromosomal preparations from the patient and his father was carried out with bacterial artificial chromosome (BAC) and P1 artificial chromosome (PAC) clones containing chromosome 6p and 20q specific sequences from several locations according to the

publicly available genome resources (NCBI Map Viewer: <http://www.ncbi.nlm.nih.gov>; Santa Cruz Human Genome Browser: <http://genome.ucsc.edu>; (The Wellcome Trust) Sanger Institute: <http://www.sanger.ac.uk>). DNA clones were labelled with biotin-dUTP (Vector Laboratories, Burlingame, CA, USA) or diethylaminocoumarin-5-dUTP (DEAC/Aqua, Perkin Elmer Life Sciences, Boston, MA, USA) using standard nick translation reactions. The biotin-dUTP labelled probes were visualised with FITC-avidin (Vector) and the chromosomes were counterstained with DAPI (Sigma Aldrich, Milano, Italy).

Three colour FISH was performed using cosmid n85a3 (AC000036: 22q13.3) labelled with diethylaminocoumarin-5-dUTP (DEAC/Aqua), probe AquariusT 6p (Spectrum Green, Cytocell) and AquariusT 20q (Spectrum Red, Cytocell). Hybridisations were analysed with an Olympus BX61 epifluorescence microscope and images were captured with the Power Gene FISH System (PSI, Newcastle upon Tyne, UK).



**Figure 2** FISH analysis from patient's lymphocytes with BAC and PAC clones from the chromosome 20q (A and B) and 6p (C and D) telomeric regions. Chromosome 20q probes: PAC clone RP5-1160G1 (A) shows signals only on both chromosome 20q homologues (green arrows) whereas no signals are visible on chromosome 22 (white dots); PAC clone RP4-576F6 (B) confirmed the partial trisomy 20q showing signals on both chromosomes 20q (green arrows) and on one chromosome 22q (white arrowhead). Chromosome 6p probes: PAC clone RP3-416J17 (C) confirmed the partial trisomy 6p showing signals on both chromosomes 6p (green arrow) and on one chromosome 22q (white arrowhead); BAC clone RP11-328C17 (D) shows signals only on both chromosome 6p homologues (green arrow) whereas no signals are visible on chromosome 22 (white dots).

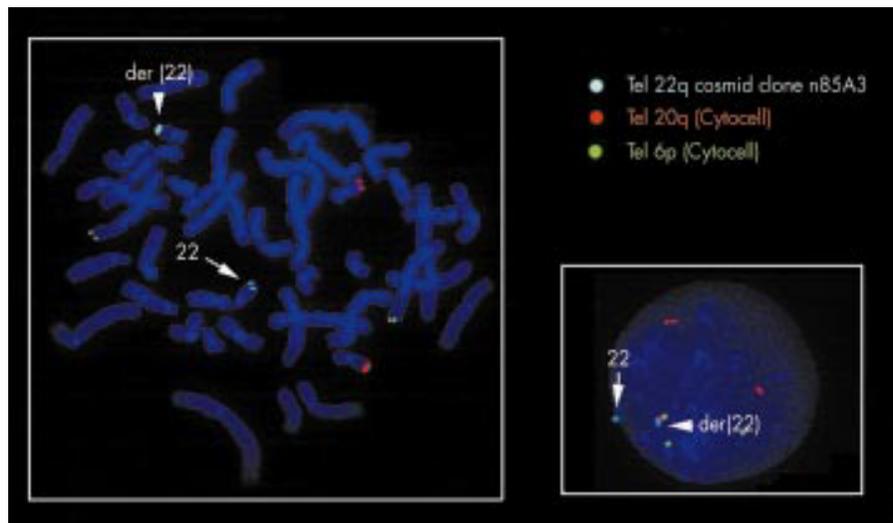
Analysis of the *PAX2* gene was performed according to Parsa *et al*<sup>11</sup> and Schimmenti *et al*.<sup>12</sup> Polymerase chain reaction (PCR) products were directly sequenced using the BigDye Cycle Terminator Sequencing kit (Applied Biosystems) on an ABI Prism 310 Genetic Analyser.

## RESULTS

Classical cytogenetic investigation on the patient and his parents gave normal results. The proband's FISH analysis for all subtelomeric regions (Kit Multi-T Cytocell) showed concomitant hybridisation of the 6p (6ptel48, fig 1A) and 20q (20qtel14, fig 1C) subtelomeric probes to the long arm of one chromosome 22 that maintained its fluorescent 22q signals (fig 1D). All other chromosomes showed a normal hybridisation pattern.

To confirm the rearrangement, the patient and his father were tested with another subtelomeric probes kit (TelVysion, Vysis). The 20q signals were still present on both 20q chromosomes and on 22qter whereas those related to 6p were present on both 6p chromosomes only (fig 1B). The discrepancy between the two 6p probes was again shown using the single Aquarius T 6p (Cytocell) and TelVysion 6p (Vysis) telomere probes. The first probe gave two normal signals on 6p and a

third one on 22q whereas the second gave signals on 6p only. We were not able to clarify the different results obtained using Cytocell and Vysis Tel6p probe because in the suppliers' instructions both companies gave the same bibliographic reference<sup>13</sup> for the telomeric clone 6p. In that paper, two telomere 6p clones, GS-62-111 and GS-196-15, anchored to the same 6ptel48 STS are cited. BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>) with its forward and reverse sequence primers localised 6ptel48 in clone RP3-416J17 (AL035696, contig NT\_034880), and overlapping clone RP1-62111 (dj62111, <http://www.sanger.ac.uk/HGP/Chr6/>) located within 300 kb from the end of the chromosome (fig 4). FISH experiments with different BAC and PAC clones from each rearranged chromosome region were performed to define the size of the 6p and 20q trisomy. FISH with PAC clones RP3-416J17 (fig 2 C) and RP1-62111 confirmed the partial 6ptel trisomy showing signals on both chromosomes 6p and on one chromosome 22q. We were not able to localise clone GS-196-15,<sup>13</sup> because none of the genome databases contains it. Thus, we can only deduce that Cytocell uses a clone located within the telomeric 300 kb, whereas Vysis uses a different clone (that can only be GS-196-15 as stated in the supplier's instructions) located beyond the telomeric 300 kb. The overlapping BAC/PAC clones RP11-45C18 (AL359496) and



**Figure 3** Proband's metaphase and interphase three colour FISH. The clones used are tel22q cosmid clone n85A3 (spectrum aqua), AquariusT 6p probe (Cytocell, spectrum green) and AquariusT 20q probe (Cytocell, spectrum red). (Left) metaphase FISH analysis shows one chromosome 22 with aqua signals (arrow) and the derivative chromosome 22 (arrowhead) with a mixture of aqua, green, and red signals. (Right) in the nucleus is visible the orientation of the translocated 6p and 20q segments versus the subtelomeric region 22q on der (22) (arrowhead). The orientation is 22q-20q-6p.

RP4-609N19 (AC004842), also belonging to contig NT\_034880, gave signals on both chromosomes 6p and one chromosome 22q but also on several other regions containing olfactory receptor gene clusters (Weizmann Institute of Science: <http://bioinformatics.weizmann.ac.il/HORDE/humanGenes/>) (fig 4A). The clone RP11-328C17 (AL365272 contig NT\_034880) (fig 2D) showed signals exclusively on both chromosome 6 homologues narrowing the size of the trisomic 6p segment to the terminal 250 kb (fig 4A).

FISH with BAC RP11-245N9 and PAC RP4-576F6 (fig 2B) clones, mapping on the very distal 20q, confirmed the trisomy of the distal part of chromosome 20q, showing signals on both chromosome 20q homologues and on the chromosome der(22). PAC clone RP13-152O15 (AL355803, ctg: NT\_011333) located at about 62.5 Mb and RP5-1160G1 (fig 2A) (dj1160g1, <http://www.sanger.ac.uk/HGP/Chr20/>) partially overlapping the duplicated clone RP4-576F6 gave a normal set of signals on 20q only, restricting the duplicated region to the terminal 300 kb (fig 4B). Analysis by FISH of the proband's parents established the father to be a carrier of the same rearrangement. Metaphase three colour FISH showed one chromosome 22 with the aqua signals and the der(22) with a mixture of aqua, green, and red signals (fig 3) thus showing that both 6p and 20q were translocated to the same chromosome 22q. Interphase three colour FISH (fig 3) showed that the orientation of the translocated subtelomeric 6p and 20q segments versus the subtelomeric region 22q on der (22) was 22q-20q-6p. Although less likely, the orientation could also be 22cen-6p-20q-22q (insertion rather than translocation). However, this possibility was excluded by three colour FISH using the cosmid clone n66c4 (AC000050), that overlaps the proximal part of the PAC clone 99k24,<sup>14</sup> labelled with aqua, the Cytocell tel6p labelled in green, and the Cytocell tel 20q labelled in red. In some prometaphases it was possible to discern that the aqua signal was located proximally compared with the mix of green and red signals (data not shown). FISH analysis with cosmid clone n94h12 (AC002056),<sup>14</sup> which covers the most telomeric region of 22q, excluded any possible 22q terminal deletion.

On retrospective analysis, high resolution G banding failed to detect the abnormal chromosome 22 (fig 1D).

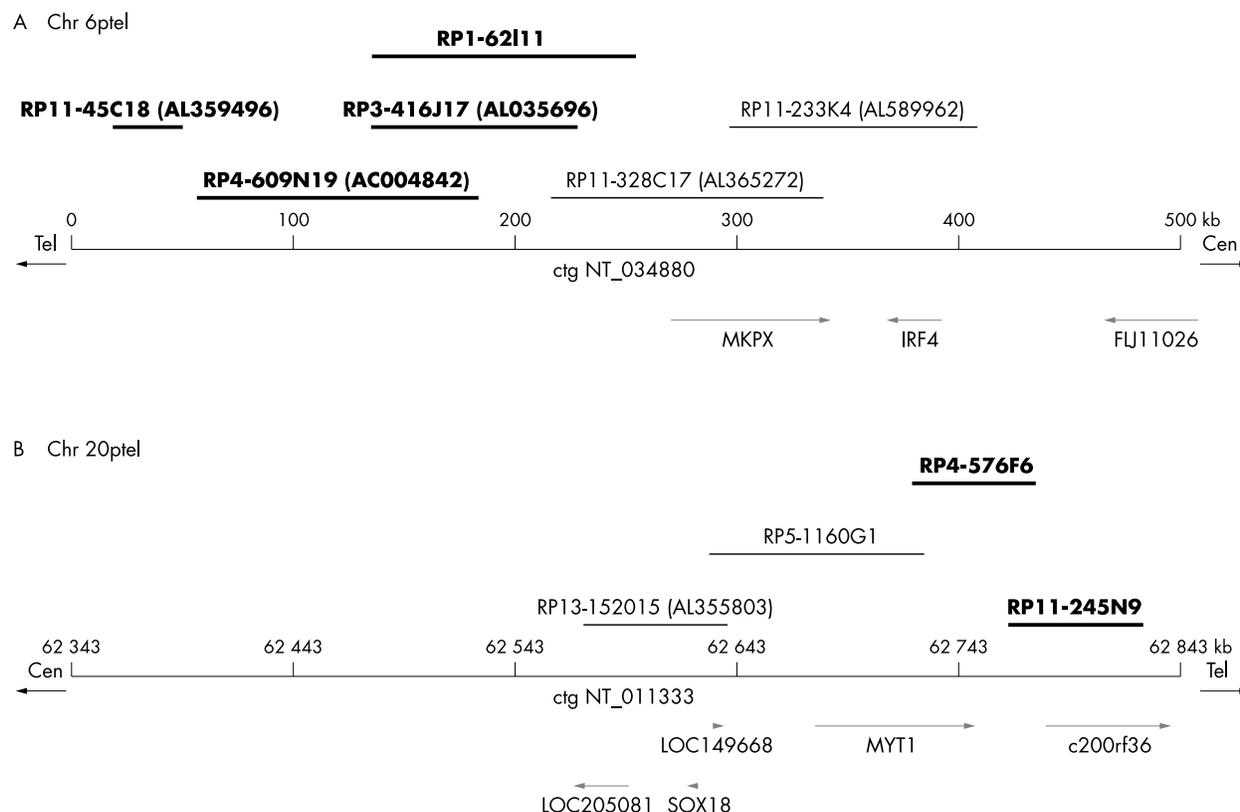
According to the entire data, the final cytogenetic interpretation was 46,XY,ish der(22)t(22;20;6)(q13.33;q13.33;p25.3) (D22S1726+, RH38993+, RH12503+).

Sequence analysis of exons 1-12 of the *PAX2* gene<sup>11-12</sup> did not show any mutation. FISH with BAC clones RP11-179B2 (AL138762 ctg NT\_033279) and GS-165F21 (AL589862 ctg NT\_033279) excluded the deletion of one *PAX2* allele.

## DISCUSSION

### Genotype/phenotype correlations in the proband

The phenotype of our patient is essentially characterised by retinal coloboma and VUR. Coloboma can occur as an isolated finding in an otherwise healthy person, can be part of complex malformation syndromes with all possible types of mendelian inheritance,<sup>15-16</sup> or can occur as part of complex syndromes.<sup>17</sup> Different modes of inheritance have been suggested, including dominant single gene<sup>18</sup> and polygenic inheritance.<sup>19</sup> In our patient, the association of ocular coloboma and VUR was suggestive of papillorenal syndrome (MIM 120330), which is caused by loss of function mutations of *PAX2* on chromosome 10q.<sup>20</sup> The predominant abnormalities associated with this syndrome are bilateral colobomas of the optic nerve and retina and renal hypoplasia with or without renal failure.<sup>21</sup> VUR is often found and tends to resolve with age. Most patients present with a normal IQ. All these findings were in agreement with our patient's phenotype. After the finding of two normal *PAX2* alleles, subtelomere screening was requested, an analysis that some clinicians consider helpful in difficult diagnoses. The unusual finding of a der (22) with distal 20q and 6p regions attached to its long arm telomere opened the possibility of associating the malformation syndrome to a chromosomal cause. However, the presence of the same anomaly in the normal father of the proband made the association between abnormal phenotype and chromosomal rearrangement puzzling. In any case, to determine if the proband's malformations could be related to his chromosomal imbalance, we performed an exhaustive review of 6p and 20q duplications and investigated whether the paternal or maternal origin of the rearrangement was associated with different malformation phenotypes. To our knowledge, 36 patients with partial trisomy 6p have been reported: 26 resulted from a parental balanced translocation,<sup>22-23</sup> five from a familial pericentric inversion (case 5A and B),<sup>24-25</sup> and five were de novo.<sup>24-26-28</sup> The 6p duplicated segment involved different regions ranging from 6p22.1 to 6pter and the severity of the clinical findings correlated with the increased size of the



**Figure 4** Maps of the terminal 500 kb of chromosomes 6p (A) and 20q (B) drawn on the basis of the public databases. Bold bars indicate duplicated BAC and PAC clones, thin bars indicate single copy clones. The grey arrows represent genes located beyond the terminal 300 kb of chromosome 6p and putative genes located within the terminal 300 kb of chromosome 20q. For the 22qter map see Anderlid *et al.*<sup>14</sup>

duplication but not with its parental origin. The smallest telomeric 6p duplication resulting from a paternal pericentric inversion was described by Anderlid *et al.*<sup>25</sup> (patients 5 A and B), where the clinical findings highlighted severe mental retardation and autism. Both patients had wide supratentorial ventricles and heterotopias determined by MRI; in addition one had a thin corpus callosum determined by MRI. In none of the patients was eye coloboma reported whereas in about half of them renal abnormalities (small kidney, proteinuria, or cystic abnormalities of Potter type III) were described. Our patient has grade 4 bilateral VUR without proteinuria and no other morphological renal malformation. Moreover, the presence of a urinary tract malformation locus on chromosome 6p<sup>29-31</sup> was not confirmed by a more recent genome wide search.<sup>32</sup> Thus, the proband's phenotype does not seem to be related to the 6p duplication. This conclusion is also in agreement with the finding that the 6p duplicated region contains only two olfactory receptor genes (*OR4F16* and *OR4F1P*, Weizmann Institute of Science: <http://bioinformatics.weizmann.ac.il/HORDE/humanGenes/>) in which variability in copy number and chromosomal location near the ends of human chromosomes has been documented.<sup>33</sup> As to the 20q duplication, only nine clinical reports have been published. Some duplications were of maternal origin,<sup>34-40</sup> others paternal,<sup>37-41</sup> and two were de novo<sup>25</sup> (patient 9<sup>42</sup>). The smallest 20q duplication was the subtelomeric rearrangement described by Anderlid *et al.*<sup>25</sup> (patient 9), where the patient showed severe mental retardation, lack of emotional contact, dysmorphic features (low set ears, upward slanting palpebral fissures, flat base of the nose, thin philtrum) and brain abnormalities (thin corpus callosum and pathological changes in the white matter). None of these patients had the malformation picture of our patient with retinal coloboma and VUR; thus the 300 kb 20q duplication does not seem to be responsible for them. However, the

20q terminal region is still not well defined (UCSC map and Human Genome Database) so we cannot exclude the possibility that undefined genes located in the last 300 kb could contribute to the patient's phenotype.

#### The rearrangement

The rearrangement we studied is very unusual because it involves three subtelomeric chromosomal regions. It must be stressed that the involvement of the third chromosome (6p) has been disclosed through the use of the Cytocell kit. In fact, the Ysis Kit was able to detect the transposition of 20q to the 22q telomere only. However, among the many cases studied with both kits none was described with a three telomere rearrangement, documenting the rarity of the event independently of the kit used.

The subtelomeric regions of human chromosomes are unusually dynamic and structurally complex regions of the human genome.<sup>43-44</sup> These regions contain large blocks of sequences, most of which are polymorphic in copy number, sequence, and location among individual subjects. The sequences that are repeated near the telomeres of many, but not all, chromosomes form a large transition zone, which exhibits unusual polymorphism suggesting that duplications or losses have occurred during recent human evolution.<sup>33</sup> The extensive homology created by these duplications provides opportunities for mispairing of chromosome ends at meiosis. The increased meiotic recombination of these regions has a role in diversifying gene families; the cost we have to pay for this is the occurrence of disease causing rearrangements (reviewed in Mefford and Trask<sup>33</sup>).

Most the telomeric rearrangements in which the size of the imbalance has been defined showed a size larger than 1 Mb.<sup>44</sup> Smaller rearrangements have also been described and found

both in association with phenotypic abnormalities<sup>14</sup> and as benign variants.<sup>8</sup> The definition of the gene content of the 6p and 20q duplication regions in our patient indeed suggests that this complex rearrangement could be just a genomic polymorphism. The finding of two cryptic telomeric rearrangements detected in a cohort of 150 control subjects suggested that submicroscopic telomeric abnormalities are not an uncommon finding in the general population. Similarly, subtelomeric FISH analysis in over 2000 specimens tested at Genzyme Genetics showed that among the 4.4% of specimens with an abnormal subtelomere result, 1.1% of probands have subtelomeric rearrangements that have been inherited from a parent reported to be phenotypically normal (J E Takacs, Genzyme Genetics, personal communication to the European Society of Human Genetics Congress, 2003). These rearrangements are therefore possibly normal variants with no clinical significance.

## GENERAL CONCLUSIONS

We showed that the cryptic complex rearrangement we studied is identical at the molecular level in the proband affected by coloboma and VUR and in his normal father. Unfortunately, the family did not agree to a molecular cytogenetic investigation on the paternal grandparents. According to the current genome maps, the trisomic 6p and 20q segments do not contain genes. This finding excludes the possibility that imprinting may cause the phenotypic difference in the proband and in his father (the paternally imprinted gene *GNAS1* on 20q is not duplicated and lies 57170 kb from the telomere, too far from the breakpoint for a possible position effect). We conclude that the rearrangement could represent a genomic polymorphism and that the proband's malformations could be the result of the mutation of some unknown gene.

## Authors' affiliations

**M C Bonaglia, R Giorda, A Cavallini, R Borgatti**, IRCCS Eugenio Medea, Bosisio Parini (Lecco), Italy

**M Rocchi**, Dipartimento di Anatomia Patologica e di Genetica, Sezione di Genetica, Bari, Italy

**T Pramparo O Zuffardi**, Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy

**O Zuffardi**, IRCCS Policlinico San Matteo, Pavia, Italy

Correspondence to: Dr M C Bonaglia, IRCCS Eugenio Medea, Via Don Luigi Monza 20, 23842 Bosisio Parini (Lecco), Italy; bonaglia@bp.lnf.it

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