Molecular characterisation of a ring chromosome 22 in a patient with severe language delay: a contribution to the refinement of the subtelomeric 22q deletion syndrome

P De Mas, N Chassaing, Y Chaix, M-C Vincent, S Julia, G Bourrouillou, P Calvas, E Bieth

The common clinical features reported in ring chromosome 22 cases include overall developmental delay with severe speech disability, growth retardation with frequently associated microcephaly, hypotonia, and dysmorphic traits, such as epicanthus, normally placed but large and dysplastic ears, long eyelashes with full eyebrows, and occasionally high arched palate, dental malocclusion, and mild hypertelorism. Second and third toe syndactyly, unsteady gait, hyperactivity, aggressive behaviour, autistic disorders, and seizures or abnormal EEG have also been reported. The recently described subtelomeric 22q deletion syndrome showed overlapping clinical features with a generalised developmental delay, particularly severe in the area of expressive speech. The phenotype is usually more severe in cases of ring chromosome 22 compared with subtelomeric 22q deletions. The phenotypic differences, particularly the growth retardation with microcephaly and the severe mental delay, could be the result of a larger deletion size in ring chromosome 22 cases. We report the molecular cytogenetic analysis of a ring chromosome 22 in a young boy investigated for global developmental delay, particularly severe in the area of expressive speech. Cytogenetic analysis

Standard and high resolution R and G banding chromosomal analyses were performed on metaphase preparations obtained from peripheral blood lymphocytes of the proband and his parents.

Figure 1 Face of patient. Note broad nasal bridge and long eyelashes.
Fluorescence in situ hybridisation (FISH)

Five fluorescent 22q13 specific probes were used: two commercially available probes, D22S39 (Appligene Oncor Inc., Illkirch) and D22S1726 (Amplitetch Cytocell Inc, Compiègne) and three BAC recombinants containing the markers D22S45, D22S55, and ARSA.

Metaphase spreads were prepared from peripheral blood lymphocyte cultures of the proband and his parents. Commercial probes were hybridised according to the manufacturer's instructions. BACs were biotinylated by nick translation (Roche Inc, Mannheim) and subsequently hybridised overnight after preannealing with Cot-1 DNA (Vysis, Inc) using standard protocols. For these probes, signal amplification was performed using fluorescein labelled avidin and anti-avidin antibodies (Oncor). Chromosomes were counterstained with propidium iodide (Sigma) or 4',6-diamidino-2-phenylindole (DAPI) (Sigma). Preparations were examined using a Zeiss Axioplan 2 epifluorescence microscope with a triple band pass filter.

RESULTS

Cytogenetic analysis

Examination of 50 metaphases from the proband showed, in all cells, 46 chromosomes with a ring chromosome 22. His karyotype was designated 46,XY,r(22). Parental karyotypes were normal.

Molecular cytogenetic study

To characterise a putative 22qter deletion in the proband, we performed FISH experiments using a set of markers specific for five 22q13 loci (table 1). A hybridisation signal was present on both chromosomes 22 with the D22S45, D22S39, and D22S55 probes. On the other hand, the signal was absent from the ring chromosome 22 with the D22S1726 and ARSA probes and observed only on its normal homologue (fig 2).

These results were highly suggestive of a small 22q deletion distal to D22S55 on the ring chromosome 22.

Table 1  Molecular data from simple subtelomeric 22q deletion studies and evaluation of the deleted segment size (bold). Loci are listed in order from most proximal to most distal [STS map on www.ncbi.nih.gov, September 2001]

<table>
<thead>
<tr>
<th>Loci</th>
<th>Nesslinger et al16</th>
<th>Flint et al16</th>
<th>Precht et al13</th>
<th>Schröder et al15</th>
<th>Our case</th>
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Δ, deleted; NΔ, not deleted; –, not done; U, uninformative.

Figure 2  FISH analysis of the proband. (Left) D22S55 fluorescence signals on normal and ring chromosome 22. (Right) D22S1726 fluorescence signal on only normal chromosome 22 (arrow, ring chromosome 22).
DISCUSSION
Severe speech delay is one of the main clinical feature observed in cases of ring chromosome 22 and 22q13 cytogenetically apparent and cryptic deletions.1−3−10 Cyto genetic anomalies involving the terminal 22q arm may be associated with a severe phenotype, especially in the case of ring chromosome 22, where it includes profound mental delay, growth retardation, and microcephaly. On the other hand, most 22qter cryptic deletions show a less severe phenotype with milder mental retardation and few dysmorphic traits, while the severe speech impairment remains constant. Among the 10 22qter cryptic deletions reported so far,9−10 22−25 the eight with clinical features described9−10 show the constancy of the language impairment. The other clinical features, dysmorphic traits, anomalies of the extremities, and autistic and behavioural disorders, are inconsistent and shared by both ring chromosome 22 and 22qter deletions. The more severe phenotype in cases of ring chromosome 22 could be because of a larger deletion size. Hence, it is worth noting that in our proband mild mental retardation and normal growth and head circumference are associated with a cryptic 22qter deletion. It is tempting to assign this relatively mild phenotype to larger deletion size. Hence, it is worth noting that in our

ACKNOWLEDGEMENTS
The first two authors contributed equally to this work. The authors are grateful to Mrs Kristina Schröder for providing the BAC recombinants.

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J Med Genet 2002 39: e17
doi: 10.1136/jmg.39.4.e17

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