Ring 22 duplication/deletion mosaicism: clinical, cytogenetic, and molecular characterisation

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Abstract
A patient with several features consistent with duplication of 22q11.2 (cat eye syndrome or CES) was found to be mosaic for a dicentric double ring chromosome 22 on postnatal karyotyping of peripheral blood. The initial karyotype was 46,XX,r(22)(p12q13) [46]/46,XX, dic r(22)(p12q13; p12q13)[4]. The amount of material duplicated in the dic r(22) was determined to include and extend beyond the CES critical region into 22q13.3. However, karyotyping of lymphocytes and fibroblasts, at 27 and 13 months of age respectively, showed no dic r(22) present in any of the cells examined. We suggest that the CES features in this patient, and potentially in other ring cases with CES phenotypic features, might result from a high level of mosaicism for a dic r(22) during early fetal development. Usually this unstable dic r(22) is subsequently lost from most cells.


Keywords: chromosome 22; duplication; deletion; cat eye syndrome

D duplication of 22q11.2, known as cat eye syndrome (CES), is a rare human condition that derives its name from its association with ocular coloboma, involving the iris or retina or both. Other common phenotypic features include anophthalmia (with or without fistula), heart defects (most often total anomalous pulmonary venous return), urogenital defects, preauricular skin tags and pits, facial dysmorphism (usually hypertelorism and downward slanting palpebral fissures), and mild to moderate mental retardation. This syndrome is extremely variable both in frequency and severity of these features, including ocular coloboma. The most common cytogenetic finding is the presence of a supernumerary bisatellited chromosome 22.2,4 The CES chromosome is an isodicentric composed of the short arm and proximal long arm of chromosome 22 (iddic 22pter-22q11.2), resulting in the presence of four copies of this region. However, features of CES have also been seen in a small number of patients with other chromosome 22q abnormalities, including interstitial tandem duplication of 22q11.24 and various chromosomal translocations involving proximal 22q.3,5 A family with two cases of CES associated with a supernumerary chromosome was described by Buhler et al.6 The marker chromosome in these patients was later identified as the product of a 3:1 non-disjunction of a balanced carrier with a t(10;22) (q26.2;q12.1).7 Thus, the patients had only three copies of the CES region. Two cases of 22q11.2 interstitial duplication with features of CES confirm that three copies of the CES region can produce a CES phenotype.8 A child with a supernumerary double r(22) and all major features of CES has also been reported.9 However, this proband's phenotypically normal father and grandfather each had a single supernumerary r(22), indicating that having three copies of the region does not necessarily produce the CES phenotype.

We report an unusual patient with some phenotypic features of CES and a neonatal peripheral blood karyotype of 46,XX,r(22)(p12q13) [46]/46,XX, dic r(22)(p12q13; p12q13)[4]. The patient is deleted for part of 22q13.3 in all cells, but in cells with the dic r(22) there are also three copies of 22q11.2-22q13.3. We suggest that a high level of cells with the dic r(22) during development might account for the CES features seen in this patient.

Case report
An infant female with a birth weight of 3580 g, length 51 cm, and head circumference 35 cm (all 75th centile for gestational age) was born to healthy, non-consanguineous parents after an uncomplicated term pregnancy. The couple had one normal daughter and a history of one first trimester spontaneous abortion. The 37 year old mother had two normal sons and a first trimester spontaneous abortion from a previous marriage. There was no family history of chromosomal aberrations, birth defects, or mental retardation. The proband (fig 1) presented with several clinical features of CES: right iris coloboma, bilateral chloriorretinal colobomata with optic nerve involvement, a right preauricular pit, broad nasal bridge, a small anterior fontanelle, and a renal defect. The patient showed additional clinical features that have not been associated with CES. There were bilateral linear ear lobe creases, a small oral alveolar cleft, aberrant palmar creases, generalised hypotonia that interfered with feeding, thin ribs, and spina bifida occulta of C6. She developed hypoglycaemia, hyperbilirubinaemia, and a transient supplemental oxygen requirement. Bacterial and viral cultures of cerebrospinal fluid and bacterial cultures of blood and urine were negative. Head ultrasound was unremarkable. MRI of the central nervous system at 4 months of age showed normal brain anatomy with a possible delay of myelination of the motor cortex. A
A horseshoe kidney and a small subcapsular hepatic cyst were noted by abdominal ultrasonography. Oesophageal pH study, endoscopy, and upper GI contrast study were normal. Because of poor feeding, a gastrostomy tube was placed for nutritional supplementation.

The postnatal course was significant for chronic interstitial pulmonary opacification and recurrent upper respiratory infections with chronic purulent rhinitis and otitis media, persistent mild tachypnoea, wheezing, and râles. Bronchoscopy performed at 1 year of age showed an anteriorly displaced larynx, mild distal tracheomalacia, and left bronchomalacia. There were purulent secretions and mucosal inflammation throughout the lower airways.

Biopsy of the bronchial mucosa showed normal cilia ultrastructure by electron microscopy. Serum levels of immunoglobins G, A, and M and IgG subclasses were normal for age. White blood cell arylsulphatase A assay and quantitative plasma amino acid assay were normal.

At 13 months of age she was noted to have mildly asymmetrical soft tissue growth of her extremities, the right larger than the left. Abdominal and renal ultrasonography studies were unchanged. Ophthalmological examination at 16 months showed searching nystagmus and profound visual impairment with slight fixation preference with the right eye. Interpupillary distance measured 5.0 cm (80th centile) and inner canthal distance was 3.2 cm (>97th centile) at the age of 18 months. Brainstem
auditory evoked response testing showed normal bilateral hearing sensitivity. At 18 months she remains hypotonic and globally developmentally delayed, but with normal somatic growth with gastrostomy feedings. The growth asymmetry has persisted.

Methods
Karyotypes were done using the standard G banding protocol. Fluorescence in situ hybridisation (FISH) was performed according to Oncor protocols. FISH studies used the commercially available Oncor probes for detection of DiGeorge syndrome, consisting of D22S75, which maps to the DiGeorge syndrome critical region, and D22S39, which maps to the terminal region of chromosome 22.

DNA from the patient and both parents was obtained from peripheral blood samples and from the patient’s fibroblasts. Restriction fragment length polymorphisms were used to determine the extent of the patient’s deletion. The probes used in the DNA studies are from 22q12 and 22q13 and have been previously described by Dumanski et al.9 The informative loci were D22S22, using a 1.9 kb HindIII fragment which yields TaqI alleles of 1.9 kb and 1.8 kb, plus a 2.2 kb constant band, and D22S95, a 2.0 kb HindIII fragment which gives EcoRV alleles of 20 kb and 15 kb. Probes for D22S91, PDGF (pSM-1), and D22S97 were uninformative.

Results
Cytogenetic findings indicated that the patient lacked the expected supernumerary CES chromosome. Initial karyotyping of blood cells obtained at 2 days of age showed that the patient was mosaic for a single ring chromosome 22 and a dicentric double ring 22 chromosome that appeared to consist of two copies of the single ring 22: 46,XX,r(22)(p12q13)[46]/46,XX,dic r(22)(p12q13;p12q13)[4] (fig 2). FISH analysis confirmed the presence of a deletion on the r(22) (fig 3). D22S39, which maps to 22q13.3, was present only on the single normal copy of chromosome 22. Cytogenetic analysis of skin fibroblasts at 12 months of age indicated a different mosaic pattern, with a single r(22) in 46 cells and monosomy 22 in 11 cells, while no dic r(22) cells were observed: 46,XX,r(22)(p12q13)[46]/45,XX,−22[11]. A second peripheral blood sample at 27 months confirmed the absence of the dic r(22) in all 175 metaphase spreads examined. These findings are consistent with ring chromosome instability. Parental chromosomes were normal.

The extent of the deletion on the ring 22, and thus the extent of the duplication on the dic r(22), was determined by examining RFLPs of the patient and both parents (fig 4). The proband was heterozygous for locus D22S95, identifying the upper limit of the deletion. The proband was hemizygous for locus D22S22, inheriting a 1.9 kb band from her mother but no allele from her father, setting the lower limit of the deletion.

Discussion
With the initial clinical recognition of phenotypic features of CES, the finding of a r(22) with 46 chromosomes was unexpected. The presence of a r(22) is usually associated with a phenotype that is difficult to define clinically.10 The only consistent observation with r(22) is
moderate to severe mental retardation. Other features frequently reported include muscular hypotonia, poor coordination (unsteady gait), hypertonia, microcephaly, and non-specific dysmorphic features. The variability of this phenotype is thought to be the result of the instability of the r(22) rather than the extent of the deletion at 22q13.3. Loss of 22q13.3 is also seen as a simple terminal deletion. The phenotype associated with the 22q13.3 deletion syndrome is developmental delay, normal to accelerated growth, severe delay in expressive speech, hypotonia, and mild dysmorphic features. Neither the r(22) nor the 22q13.3 deletion syndromes are associated with major malformations. Although the hypotonia seen in our patient can be explained by the presence of a r(22) deletion, the malformations reminiscent of CES cannot. It is noteworthy that the respiratory problems similar to those seen in our patient have also been reported in previous r(22) cases.

The presence of the dic r(22) would lead to duplication of the region of chromosome 22 that is present on the ring chromosome. Since there would be three copies of the CES region in such cells, in high enough numbers this could be compatible with features of CES. Although the dic r(22) was present in a minority (4/50) of the prenatal peripheral blood lymphocytes, it may have been present at a much higher frequency early in development and then subsequently lost by most cells. A review of published cases of r(22) shows several rare cases with a single feature of CES, most frequently colobomata. Although no dic r(22)s were reported in these cases, it is conceivable that these subjects may also have had dic r(22)s in some cells during early fetal development.

Alternatively, the dic r(22) in our patient may be present perinatally owing to dynamic somatic mosaicism. This refers to the instability of the ring causing continuous production of further chromosomal abnormalities, such as dicentric double rings. Such cells do not survive because of their more severe genetic imbalance, and are lost rapidly at later cell divisions. However, dicentric double ring chromosomes can be present in a high proportion of a person’s cells. McGinniss et al describe a child with a dic r(21) present in 82% of lymphocytes (patient 11). Since no fetal karyotype of our patient is available, it is not conceivable that these subjects may also have had dic r(22)s in some cells during early fetal development.

To measure the extent of the duplication in the cells with dic r(22), we mapped the corresponding deletion with RFLPs. The deletion breakpoint maps within the 25.9 cM region of deletion breakpoints in the 22q13.3 deletion syndrome. Therefore the duplication in the dic r(22) cells extends from the chromosome 22 centromere to within 22q13.3. This duplication is considerably larger than the CES region. However, larger duplications of chromosome 22 can show CES features. For instance, a patient with duplication of 22q11-q12 displayed numerous CES features, including bilateral pauricular pits, a cardiac defect (total anomalous pulmonary venous return), absence of one kidney, various genital anomalies, downward slanting palpebral fissures, hypertelorism, and developmental delay.

We suggest that mosaicism for a duplication of this region can be associated with features of CES. Although our patient has a deletion and is mosaic for a duplication in only a minority of cells, it is the feature of the duplication that appears to predominate. Thus if the duplication is responsible for the phenotype, the dic r(22) must have been more prevalent during early embryogenesis when malformations are established. The overall phenotype of this patient was therefore not reflected in the predominant mosaic pattern of her postnatal karyotype. Therefore, the formation of transient dicentric double ring chromosomes during development may confound phenotype/karyotype correlations.

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