Short reports

Multiple congenital anomalies including the Rieger eye malformation in a boy with interstitial deletion of (4)(q25→q27) secondary to a balanced insertion in his normal father: evidence for haplotype insufficiency causing the Rieger malformation

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Abstract

A 7 year old boy with minor facial anomalies, the Rieger eye malformation, reduced vision, genital anomalies, and severe mental retardation had deletion of the segment 4q24→q26. His phenotypically normal father had a balanced insertion of that segment into the distal long arm of chromosome 6: 46,XY,ins(6;4)(q26;q24q26). Microsatellite loci flanking the RIEG gene on 4q25 were deleted giving indirect evidence of deletion of this locus. This finding and the normal ocular findings in the insertion carrier father show that haplotype insufficiency can cause the Rieger eye malformation.

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Ligutic *et al*¹ and Shiang *et al*² each reported one patient with interstitial deletion of segments in 4q overlapping 4q24q27 who, in addition to variable major and minor anomalies, both had the Rieger eye malformation and Murray *et al*³ conducted a linkage study of three families with members affected with autosomal dominant Rieger syndrome. They showed evidence of linkage of the disease with 4q markers close to the epidermal growth factor gene locus. Subsequently, two other cases of interstitial deletion of 4q featuring, among other findings, the Rieger eye malformation were reported, narrowing the deleted segment to (4)(q25→q27).^{4,5} Recently, Semina *et al*⁶ cloned the "Rieger gene" (RIEG) and identified it as a member of the homeobox gene family.

We describe here a further patient with multiple congenital anomalies including the Rieger eye malformation and an interstitial 4q deletion. However, unlike the previous reports, the deletion in our patient was inherited from his healthy father who carried a balanced interstitial (4;6) translocation.

Case report

Both parents were young (the father aged 24 and the mother 20 years at his birth), healthy, non-consanguineous, and have normal vision. The proband is the product of the mother's first pregnancy. She subsequently had a spontaneous abortion, at about 10 weeks of gestation.

The proband, a boy, was born at term following an uneventful pregnancy apart from a short period of bleeding in the seventh month. Birth weight was 2900 g (10th centile) and length 50 cm (close to the 50th centile). The delivering obstetrician noticed a "very short umbilical cord", but no measurements were taken. No other abnormalities were noted during the postnatal days in hospital.

At 4 weeks, ectopic pupils were noticed. Reduced vision was found during the first year of life, and by the age of 1 year, motor and mental retardation became evident. With age, behavioural problems appeared and became more and more severe, with aggression, temper tantrums, and biting, especially aimed at the parents.

He was able to sit without support at 7 months, to walk at 17 months, and he spoke single words at 4 years but with no further progress in speech thereafter. At the age of 3 years, he was operated on for phimosis and right inguinal hernia. On clinical examination at 5 years 9 months, his height was 107 cm (3rd centile), weight was 18.3 kg (25th centile), and OFC was 53 cm (50th-75th centile).

CLINICAL EVALUATION AT 7 YEARS OF AGE

The following minor abnormalities were noticed: a prominent occiput, red fuzzy hair (the parents both have dark brown hair, but one maternal cousin also has red hair; the father has curly hair), a prominent, narrow forehead, downward slanting palpebral fissures, ocular findings as described below, slightly beaked nose with rounded tip, small teeth of normal morphology, narrow palate, dysplastic ears of normal size, hypoplasia of the middle antherical

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Figure 1 Left: chromosomes 4 and 6 from a GTG banded metaphase of the father (upper row) and the proband (lower row). Breakpoints indicating sites of interstitial translocation are on the normal chromosome 4 (father and proband) and the rearranged chromosome 6 (father). Right: metaphase of the proband's father painted with a library from chromosome 4 (biotinylated, green) and a library from chromosome 6 (digoxigeninised, red). Note insertion of a small segment of chromosome 4 (green) into the distal long arm of one chromosome 6 (red).

segment, prominent and fleshy helix and posterior rotation of the auricle, prominent umbilicus, shawl scrotum, scars from right inguinal herniorrhaphy, and normal hands and feet. He almost always walks on his toes although he is able to stand on flat feet.

Ophthalmological examination showed alternate divergent squint, bilateral microcornea and probable microphthalmia, hypoplastic anterior chamber, greyish, hypoplastic irides, and normal lens and retina. According to the parents, central vision is good, but the visual fields are narrow.

COMPLEMENTARY EXAMINATIONS

Hearing tests including reflex tympanography disclosed normal hearing. Cardiac examination showed a small perimembraneous VSD (1 mm) and a conduction defect (WPW syndrome). An EEG showed no evidence of epilepsy. On magnetic resonance imaging, multiple small lesions were seen in both parieto-occipital regions.

CYTOGENETIC EXAMINATION AND RESULTS

GTG banded metaphases from a lymphocyte culture of the proband showed a tiny interstitial deletion in distal 4q: band 4q25 and presum-

ably smaller parts of one or both of bands 4q24 and 4q26 were missing. Investigation of the parents disclosed a normal maternal karyotype; the father's karyotype, however, contained the same deleted chromosome as the proband. The segment missing in 4q was incorporated into the distal long arm of chromosome 6, presumably in band 6q26. Thus, the proband's karyotype is 46,XY,der(4)ins(6;4)(q26;q24q26)pat (fig 1, left). No other family members could be investigated. FISH examination of the paternal chromosome preparations using a biotinylated library of chromosome 4 and a digoxigeninised library of chromosome 6 (from Laurence Livermore Laboratory, USA) confirmed the balanced insertion and showed that it was non-reciprocal (fig 1, right). No cell line is available from the proband or his father.

MOLECULAR INVESTIGATIONS

The family was genotyped with markers proximal to the RIEG locus (D4S406 and D4S427 at 4q24-q25) and markers telomeric to that locus (D4S194 and D4S175 at 4q25-q26). The genetic distance between the microsatellites is 11 cM.³ Between 200 and 500 ng of genomic DNA, extracted from peripheral blood, were used for PCR reactions. Conventional conditions were used with a Perkin-Elmer 9600, the reactions were carried out for a total of 35 cycles, and the annealing temperature was 56°C. The reaction products were separated on a polyacrylamide/urea gel and visualised by silver staining. The two microsatellites proximal to the RIEG locus (D4S406 and D4S427) and one of the two microsatellites distal to RIEG (D4S194) showed only maternal inheritance in the proband, thus confirming paternal deletion and indirectly showing evidence of deletion of RIEG. D4S175 showed normal biparental inheritance (fig 2).

Discussion

In 1935, the Austrian ophthalmologist Herwig Rieger (1899-1991) described a complex eye

Figure 2 Results of microsatellite marker analysis for markers D4S175 (left) and D4S427 (right) in the proband and his parents showing biparental inheritance of D4S175, but lack of the paternal D4S427 allele in the proband.

malformation, named it dysgenesis mesodermalis iridis et corneae, and recognised its autosomal dominant transmission.⁷ The characteristic features of the Rieger syndrome include hypoplasia of the anterior chamber and the mesodermal layer of the iris as major findings and frequent occurrence of secondary glaucoma, corneal clouding, cataract, iris coloboma, and microphthalmia. Furthermore, the following extraocular findings are common in Rieger syndrome: oligodontia, microdontia, abnormal tooth shape, small mandible, retracted upper lip, broad nose with flattened bridge, telecanthus, eye muscle hypoplasia, and lack of reduction of the periumbilical skin fold.⁸ Mental development is normal, borderline normal, or mildly subnormal except for occasional cases with more severe mental deficiency. In a proportion of the latter group, which frequently occurs with further anomalies not common to the dominantly inherited Rieger syndrome and with a tendency to sporadic occurrence, various chromosome aberrations were found, including deletions of 6p23, 10p13, 13q14q14, 17p11.1p11.3, 20p11, 21q22, and duplications of 2p21-p25 and 16q.⁹ The only aberration repeatedly detected was an interstitial deletion of 4q including, in all cases, the segment 4q25→q27. In individual cases, the breakpoints were defined by the authors as follows: presumably 4q14→q28¹⁰ (the determination of breakpoints in this case is the least confident as this deletion is associated with a de novo pericentric inversion of chromosome 4), 4q23→q27,¹ 4q24→q32,² and 4q25→q27.^{4,5} On recent re-examination, the breakpoints in the case of Ligutic *et al*¹ were corrected to 4q25→q27.¹¹ The observation that other patients with del(4)(q25→q27) do not show the Rieger eye malformation^{12,13} can be explained by a proximal breakpoint distal to the Rieger gene in these cases and thus is in agreement with the map location of this gene at 4q25.⁶ The observation of distal 4q deletions of similar segments in several patients with the Rieger eye malformation prompted a linkage study using DNA from three larger families (16 meioses altogether) with multiple members affected with Rieger syndrome in a dominant fashion, which resulted in close linkage (lod score of 2.72 at 2 cM) to markers at 4q25/26.³

Recently, a collaborative study on two patients with balanced translocations at 4q26 and Rieger syndrome resulted in the cloning and sequencing of a gene, mutations of which were found in a number of patients with familial Rieger syndrome, whereas unaffected family members did not show the mutation.⁶ The authors characterised the gene as a novel homeobox gene and named it "RIEG".

The chromosome aberration in our family is not new; it is similar to the deletions in at least the patients of Fryns and van den Berghe⁴ and Vaux *et al*.⁵ However, our patient is, to the best of our knowledge, the first whose chromosome aberration did not occur de novo, but was inherited from a father carrying a balanced insertional translocation of 4q25→q27 into 6q26. On detailed ophthalmological investigation, the father did not show any signs of the Rieger syndrome, nor did he exhibit abnormalities of

the nose, teeth, umbilicus, or other organs occasionally affected in Rieger syndrome patients. Although in individual patients proximal breakpoints were defined proximal to 4q25 (4q23 in the case of Ligutic *et al*¹ and 4q24 in the case of Shiang *et al*²) and distal to 4q27 (4q32 in the case of Serville and Broustet¹⁰), it is theoretically possible that one of the breakpoints was incorrectly determined. Thus, it cannot fully be excluded that the Rieger syndrome in the deleted cases was the result of gene disruption (through one of the breakpoints) and not haplotype insufficiency. However, if gene disruption caused the Rieger syndrome phenotype in the deleted cases, the balanced father of our proband should also show the Rieger syndrome, as incomplete penetrance is not known in this syndrome. The fact that his eyes are completely normal and the indirect evidence, through finding deletion of microsatellite markers flanking the RIEG locus, makes it very likely that the Rieger syndrome is caused by haplotype insufficiency. The only alternative (and extremely unlikely) explanation would be that our proband, in addition to a deletion of 4q, had a de novo mutation in the second allele of the Rieger gene which would have occurred in the oocyte. Similar evidence for haplotype insufficiency was reported, for example, for the PAX6 gene through demonstration of familial unbalanced insertional translocations leading to 11p13 deletion in probands with aniridia as a component of the WAGR syndrome.¹⁴

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