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.

Keywords: Rieger eye anomaly; interstitial deletion (4)(q25→q27)

Ligutic et al and Shiang et al 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 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). Recently, Semina et al 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 antihelical
Haplotype insufficiency causing the Rieger malformation

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 (digoxegenised, 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.

COMPLEMENTSARY EXAMINATIONS
Hearing tests including reflex tympanography disclosed normal hearing. Cardiac examination showed a small perimembranous 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 presumably 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,XX,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 digoxegenised 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. 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 Herwigh Rieger (1899-1991) described a complex eye
malformation, named it dysgenesis mesoderma-
lis iridis et corneae, and recognised its autosomal
dominant transmission. 1 The characteristic fea-
tures 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 microph-
thalmia. Furthermore, the following extracor-
elar findings are common in Rieger syndrome: olio-
dontia, microdontia, abnormal tooth shape,
small mandible, retracted upper lip, broad nose
with flattened bridge, telecanthus, eye muscle
hypoplasia, and lack of reduction of the perium-
bilical skin fold. Mental development is normal,
borderline normal, or mildly subnormal except
for occasional cases with more severe mental
deficiency. In the 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 4p23, 10p13,
13q14q14, 17p11.1p11.3, 20p11, 21q22, and
duplications of 2p21-p25 and 16q. 2 The only
aberration repeatedly detected was an interstitial
deletion of 4q including, in all cases, the segments
4q25-q27. In individual cases, the breakpoints
were defined by the authors as follows: presump-
tively 4q14–4q28 3 (the determination of break-
points in this case is the least confident as this
deletion is associated with a de novo pericentric
inversion of chromosome 4), 4q23–q27, 4q24–q32, 4q25–q27. 4, 5 On recent
re-examination, the breakpoints in the case of
Ligutic et al 6 were corrected to 4q25–q27. 7
The observation that other patients with del(4)(q25–q27) do not show the Rieger eye
malformation 8, 9 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. 10 The observation
of distal 4q deletions of similar segments in sev-
eral 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 syn-
drome in a dominant fashion, which resulted in
close linkage (lod score of 2.72 at 2 cm) to
markers at 4q25/26. 9
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. 8 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 Berghe 1 and
Vaux et al. 11 However, our patient is, to the best
of our knowledge, the first whose chromosome
aberration did not occur de novo, but was inher-
itated from a father carrying a balanced insertional
translocation of 4q25–q27 into 6q26. On
detailed ophthalmological investigation, the fa-
ther did not show any signs of the Rieger
syndrome, nor did he exhibit abnormalities of
the nose, teeth, umbilicus, or other organs occa-
sionally affected in Rieger syndrome patients.
Although in individual patients proximal break-
points were found proximal to 4q25 (4q23 in the
case of Ligutic et al. 6 and 4q24 in the case of
Shiang et al. 12) and distal to 4q27 (4q32 in the
case of Fryns and Broustet 13), it is theoretically
possible that one of the breakpoints was incor-
rectly determined. Thus, it cannot fully be
excluded that the Rieger syndrome in the de-
cleted cases was the result of gene disruption
(through one of the breakpoints) and not haplo-
type 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 find-
ing deletion of microsatellite markers flanking
the RIEG locus, makes it very likely that the
Rieger syndrome is caused by haplotype insuf-
fiency. 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 dele-
tion in probands with aniridia as a component of
the WAGR syndrome. 14

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