# Rieger syndrome locus: a new reciprocal translocation t(4;12)(q25;q15) and a deletion del(4)(q25q27) both break between markers D4S2945 and D4S193

Rachel H Flomen, Patricia A Gorman, Radost Vatcheva, Juergen Groet, Ingeborg Barišić, Ivo Ligutić, Denise Sheer, Dean Nižetić

**Abstract**

**Rieger syndrome (RS) is an autosomal dominant disorder of morphogenesis characterised by malformation of the anterior segment of the eye, dental hypoplasia, and failure of the periumbilical skin to involute. RS has been mapped to the 4q25-q27 chromosomal segment by a series of cytogenetic studies as well as by genetic linkage to DNA markers. It was first localised to chromosome 4q based on an association with a constitutional deletion of 4q23-q27. In this paper we localise the proximal breakpoint of this deletion from the original patient, and we describe a new family with a de novo balanced reciprocal translocation t(4;12)(q25;q15) segregating with full RS in two generations. Using FISH and the P1 artificial chromosomes (PACs) as probes, we have physically localised both the deletion and the translocation breakpoints between genetic markers which are known to be strongly linked to RS. We have mapped both the proximal deletion breakpoint and the translocation breakpoint within a region between two groups of PACs bearing the markers D4S2945 (on the centromeric side) and D4S193 and D4S2940 (on the telomeric side). We believe that these recombinant bacterial clones derived directly from genomic DNA (not subcloned from YACs) will be valuable complementary tools in the efforts to clone the RS gene and to construct a full transcriptional and sequence ready map of this region.**

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**Centre for Applied Molecular Biology, School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK**
R H Flomen
J Groet
D Nižetić

**Human Cytogenetics Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK**
P A Gorman
R Vatcheva
D Sheer

**Department of Clinical Genetics, Children's Hospital Zagreb, Zagreb University Medical School, Klaićeva 16, 10 000 Zagreb, Croatia**
I Barišić
I Ligutić

Correspondence to:
Dr Nižetić.

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The Rieger syndrome (RS) is an autosomal dominant disorder of morphogenesis characterised by malformation of the anterior segment of the eye, dental hypoplasia, and failure of the periumbilical skin to involute.[1 2] Ocular features typically result in hypoplasia of the anterior iris stroma, corectopia, posterior embryotoxon, and strands running from the iris to the posterior surface of the cornea, inserting into the unusually prominent and anteriorly displaced Schwalbe line. Approximately one half of patients develop glaucoma, typically by young adulthood.[3] Dental features include microdontia and oligodontia, most commonly absence of maxillary incisor teeth. Other features often involve craniofacial malformations such as maxillary and midfacial hypoplasia. Classical RS was first localised to chromosome 4q[4] based on an association with a deletion 4q23-q27,[5] (also family A in this paper). Subsequently, other cases with fully developed features of RS have been reported associated with deletions[6–9] and translocations[10 11] in the same region. The association of reciprocal balanced translocations with RS,[10 11] and the finding of deletions in 4q21.1-4q25 and 4q25-q27 which are associated with craniofacial, skeletal, and other abnormalities, but no ocular Rieger anomaly,[12 13] suggest a very narrow DNA locus, most probably a single gene, to be responsible for the full features of RS. Murray *et al*[14] first described a genetic linkage of full RS with a set of markers in 4q26 including the epidermal growth factor (EGF) gene, distinguishing it from a disorder with identical ocular features to RS but no dental or umbilical changes, commonly referred to as Axenfeld-Rieger anomaly. The latter has subsequently been very convincingly excluded from linkage with markers on chromosome 4q in a three generation family.[15] However, a further implication of the locus at 4q25-q26 in the development of the iris was found by linkage of markers in this region to autosomal dominant iris hypoplasia[16] in which the ocular features are similar, but not identical, to RS. The authors suggested that this disorder might be allelic to RS.[16] Murray *et al*[10] subsequently excluded the EGF gene as a candidate for RS by showing the lack of disease segregating mutations using SSCP in a number of RS patients. This led to a currently continuing positional cloning effort in 4q25-q26 to isolate the putative RS gene using large YACs that span translocation breakpoints and subcloning them into cosmids.[17 18]

In this paper we localise the proximal breakpoint of the original deletion patient[5] used to localise RS to 4q, and we describe a new de novo balanced reciprocal translocation, t(4;12)(q25;q15), segregating with full RS in a three generation family. Using FISH with the P1 artificial chromosomes (PACs), we physically localise both the deletion and the translo-

cation breakpoints between genetic markers which are known to be strongly linked to RS.

## Materials and methods

### PAC LIBRARY SCREENING

The P1 artificial chromosome (PAC) library, RPCI-1, made from partially digested whole human genomic DNA,[19] cloned in the *Bam*HI site of the HI HpCYPAC1 vector and plated on DH10B strain, was used. The library was screened by PCR through the HGMP-UK Resource Centre (Hinxton Hall, Cambridgeshire). For the STS primer sequences and PCR conditions, information given in the Genome Data Base (GDB, Johns Hopkins, Baltimore) was precisely followed.

### ISOLATION AND ANALYSIS OF PAC CLONES

PAC clones were grown for 14 hours in 15 ml of superbroth (32 g tryptone, 20 g yeast extract, 5 g NaCl, 5 ml 1N NaOH per litre) with 30 mg/ml Kanamycin. DNA was isolated using a modified alkaline lysis method.[20] After DNA precipitation, phenol and chloroform/isoamyl-alcohol (24:1) extractions were performed followed by a final ethanol precipitation.

### FLUORESCENCE IN SITU HYBRIDISATION (FISH)

Peripheral blood cultures were established from each patient. Harvesting of cells, DAPI staining, and preparation of slides were performed according to standard procedures.[21] PAC DNA was labelled with biotin-11-dATP (Gibco BRL) (for green signals) or with digoxygenin-11-dUTP (Boehringer Mannheim) (for red signals). Approximately 0.1 μg of each labelled PAC DNA sample was mixed with 2-4 μg of Cot1 DNA (Gibco BRL), precipitated, denatured, allowed to preanneal, and then applied to a denatured slide and hybridised overnight. Chromosome specific paints and centromere probes for chromosome 4 and 12 were biotin (Cambio) and digoxygenin (Oncor) labelled and hybridised to slides, using manufacturers' instructions. Slides were washed and biotin/digoxygenin presence was detected using avidin-FITC/antidigoxygenin-rhodamine, yielding green and red signals, respectively. Chromosome images were captured using a Zeiss Axioskop microscope equipped with a charge coupled device (CCD) (Photometrics) connected to an Apple Powermac 8100 computer. Separate images of probe signals and DAPI banding patterns were pseudocoloured and merged using SmartCapture software (Vysis Inc, Chicago, IL, USA). The presented results were derived from at least 20 separate and clearly readable metaphases in each experiment.

### CELL LINES

Results were obtained from peripheral blood lymphocytes of the family members, but EBV immortalised cell lines for the patients II.1 in family A and II.1 and III.1 in family B (fig 1) are available from the ECACC (European Collection of Cell lines), Centre for Applied Microbiology, CAMR, Porton Down, Salisbury SP4 0JG, UK.

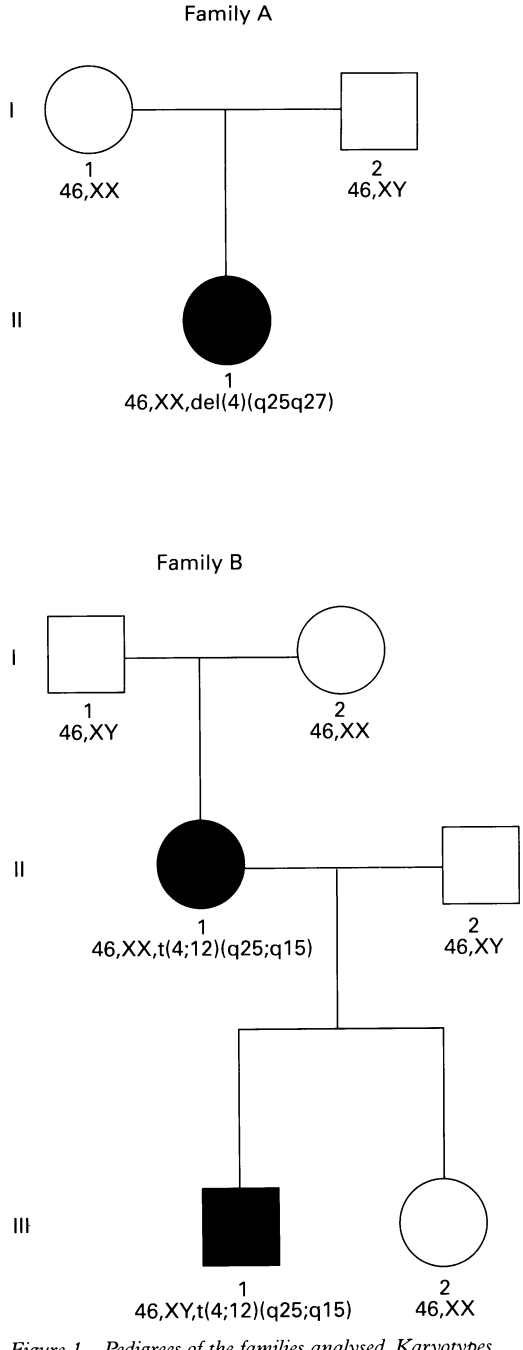


*Figure 1 Pedigrees of the families analysed. Karyotypes were obtained from fresh peripheral lymphocytes using standard and DAPI banding.*

## Results

### RS FAMILIES AND THEIR KARYOTYPES

Two unrelated families from Croatia were analysed (fig 1). Family A has a case of a de novo deletion of 4q23-27, as described previously[5] using G banding. The patient (II.1 in fig 1) had microcornea, corectopia, hypoplastic iris stroma, strands connecting the hypoplastic iris to the posterior surface of the cornea, and glaucoma. Dental malformations included three deciduous and seven permanent teeth missing, and hypoplastic and malpositioned other teeth. There were no umbilical skin stigmata recorded. Other features included dyscrania, hypertelorism, and dysplastic ears. The parents had no clinical or cytogenetic abnormalities. Chromosomal analysis from peripheral blood lymphocytes with DAPI banding in

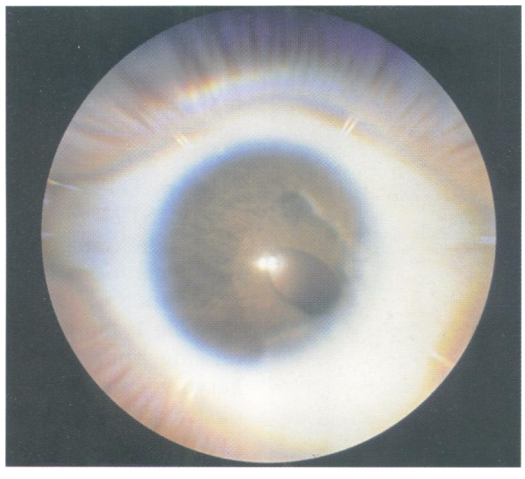

*Figure 2 The eye of patient II.1 from family B. Corectopia of the pupil, coloboma and hypoplasia of the iris, and apparent dysplasia of the anterior chamber are visible. Biomicroscopy showed numerous tissue threads between the posterior surface of the cornea and the hypoplastic iris. The signs are typical of Rieger eye anomaly, which with dental and other symptoms led to the diagnosis of Rieger syndrome.*

subject II.1 showed a deletion del(4)(q25q27) in one homologue of chromosome 4 (not shown).

Family B included a patient (II.1) described 22 years ago in the hospital archives[22] as a 9 year old girl, with microcornea, iridocorneal strands, corectopia, and posterior embryotoxon in one eye (fig 2), hypoplasia and coloboma of the iris, and glaucoma, as well as oligodontia and maxillary hypoplasia. In the girl's father (I.1) the two lower central incisors were missing, but he was otherwise normal. No ocular, dental, or other changes had been found in a wider three generation search in both parental families of I.1 and I.2.[22] At present, patient II.1 is married and has given birth to two children, a normal girl and a boy (III.1) with RS, with identical ocular and similar dental changes to the mother, plus failure of involution of the umbilical skin. All members of this family had normal karyotypes, as reported by morphology and G banding.[22] On analysing the chromosomes from peripheral blood lymphocytes with DAPI banding (fig 3a), we found in subjects II.1 and III.1 a balanced reciprocal translocation t(4;12)(q25;q15), which was also confirmed by chromosome 4 and 12 painting (fig 3b).

### FISH MAPPING USING PACS

In order to localise the breakpoints on the chromosome 4 map, we initially used genetic markers previously reported to have a strong linkage to RS. The markers D4S193 and D4S1616 gave the highest lod scores for linkage with RS[14] and no recombinants with these markers have been found in either RS[14] or autosomal dominant iris hypoplasia.[16] Other markers, D4S2940 and D4S2945, were also used because they were reported to flank the RS linked markers at 0.7 and 0.0 cM distance, respectively,[23] and to share YACs sized at a little over 1000 kb with them.[24]

Using the above markers, we screened the whole human genomic PAC library of five genomic equivalents[19] by PCR. Several PACs were identified (not all shown). Table 1 lists the PCR results obtained on selected YACs and PACs bearing markers linked to RS. Three PACs were used as probes for FISH: 121I20 bearing the marker D4S2945 and 2H4 or 316D15 both bearing markers D4S193 and D4S2940 (table 1). The FISH analysis of both translocation patients in family B (II.1 and III.1) showed the following result (fig 3c): the normal chromosome 4 gave a mixed colour overlapping signal in 4q25, in addition to the centromere signal, when PACs 121I20 (red) and 2H4 (green) were cohybridised with the chromosomes 4 and 12 centromere probes (red and green, respectively). The der(4) chromosome showed only the red signal, whereas the der(12) showed only the green signal, in addition to the centromeric signals. Normal chromosome 12 showed only the centromere probe signal. In all other members of family B (including I.1 who had two teeth missing), two colour FISH using the same pair of PACs showed that both PACs were on the same locus in both homologous chromosomes 4 (not shown).

These results map the translocation breakpoint to 4q25, most probably between markers D4S2945 on the centromeric side and the group of markers D4S193 and D4S2940 on the telomeric side.

In the deletion patient II.1 from family A (fig 3d), two colour FISH using the PACs 121I20 (D4S2945) and 316D15 (D4S2940, D4S193) showed an overlapping mixed colour signal on the normal chromosome 4, whereas only the signal from 121I20 was present on the homologue with the deletion in 4q25. This was also found when 2H4 was used instead of 316D15 (not shown). All PACs examined were present in two copies in both parents in family 1 (not shown). This maps the proximal breakpoint of this deletion to 4q25, probably between markers D4S2945 on the centromeric side and D4S193 or D4S2940 on the telomeric side. This finding confirms and refines the initial positioning of this deletion (4q23-q27[5]) to del(4)(q25q27), which agrees with the result using DAPI banding on the peripheral blood lymphocytes of this same person (not shown).

## Discussion

Rieger syndrome is an autosomal dominant disorder linked to the 4q25-q27 chromosomal segment by a series of cytogenetic abnormalities as well as by genetic linkage to DNA markers.[5-16] The only reported gene near the RS region is EGF,[14] which has been excluded as the causative gene for RS.[10] A series of 4q deletions have been described which result in RS,[5-9] including the one further analysed in this paper. An autosomal dominant disorder can arise either from a mutant allele on one of the homologues disturbing the normal protein function (for example, by competing with the normal protein), or by mere lack of one of the homologues (haploinsufficiency). It has recently been shown that an insertional translocation involving the 4q25-q27 segment in a normal father resulted in an RS child, with the child inheriting the same segment in one

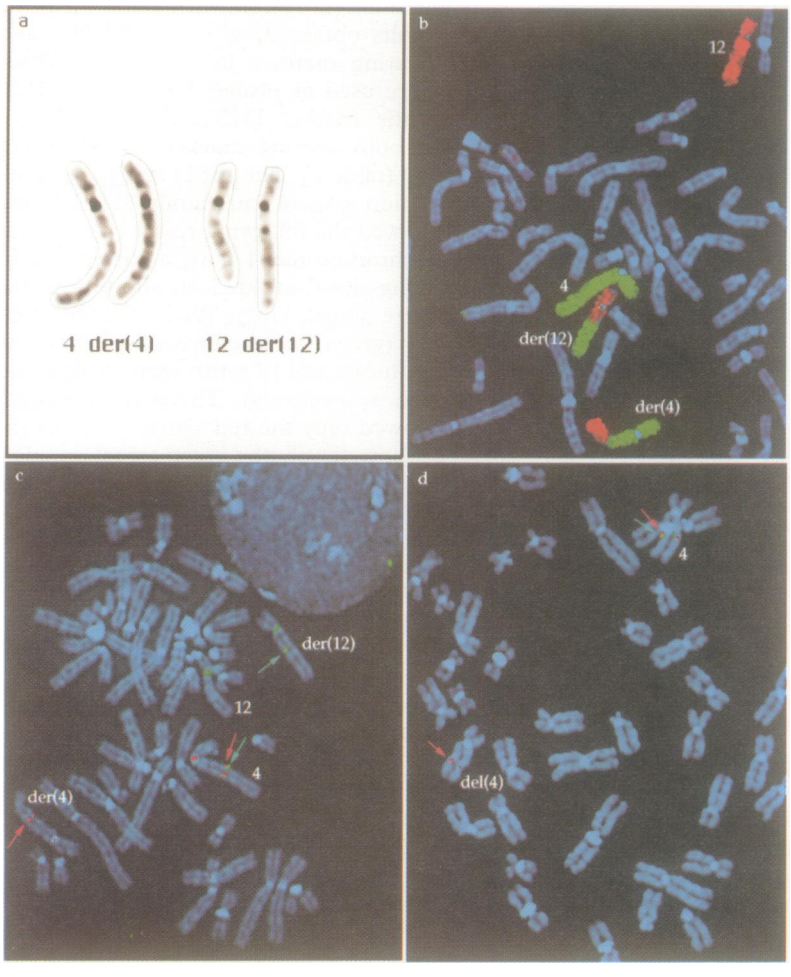


*Figure 3 DAPI banding (a) and dual colour FISH (b, c, d) on slides from peripheral blood lymphocyte metaphases using biotin or digoxygenin labelled DNA probes whose signals were detected using avidin-FITC or antidigoxygenin-rhodamine, yielding green and red signals, respectively. (a) Partial karyotype of patient III.1 (family B) with DAPI banding; (b) partial metaphase from the same patient paint hybridised to chromosome 4 paint (green) and chromosome 12 paint (red); (c) metaphase from patient III.1 (family B) hybridised to the chromosome 4 centromere probe and PAC 121I20 (both red) and chromosome 12 centromere probe and PAC 2H4 (both green); (d) partial metaphase from patient II.1 from family A, hybridised to PACs 121I20 (red) and 316D15 (green).*

*Table 1 The STS-marker content of the YACs and PACs in the wider RS locus as verified by PCR analysis. Plus symbols indicate the presence of the PCR product identical in size to the PCR product when human genomic DNA was used as template. Minus symbols indicate absence of any PCR products in a particular marker-clone combination. The three PACs used for FISH experiments (fig 3) are included. The PCR primers and conditions were as listed in the Genome Data Base (GDB, Johns Hopkins, Baltimore). The length of the YACs was taken from reference 24*

| *Clone name (kb)* | *EGF* | *D4S2945* | *D4S193* | *D4S2940* | *D4S1616* |
|---|---|---|---|---|---|
| YAC 743d2 (1710) | + | + | + | + | − |
| YAC 765c7 (1750) | + | + | + | + | − |
| YAC 946d8 (1770) | − | − | + | + | + |
| PAC 121I20 | − | + | − | − | − |
| PAC 316D15 | − | − | + | + | − |
| PAC 2H4 | − | − | + | + | − |

copy.[25] This indicates that RS can develop as a haploinsufficiency. Such an observation suggests that reciprocal translocations segregating with RS are much better tools to pinpoint the location of the RS gene than deletions, since their breakpoints probably have to disrupt the DNA very near or at the RS gene, whereas it appears that the deletion breakpoints do not necessarily have to. Such translocations are very rare, and only two have so far been mapped in detail.[17 18] In this paper we map a new translocation breakpoint within a region bordered by two groups of PACs bearing the markers D4S2945 (on the centromeric side) and D4S193 and D4S2940 (on the telomeric side). The translocation segregates with RS in two generations. In the generation before the occurrence of the translocation, the grandfather (fig 1, family B, I.1) had two incisor teeth missing, but no other signs of RS. Although his karyotype showed no translocation, this clinical observation is curious and justifies a closer look in future at the status of the RS gene in this person's genome. As a corollary to this work, the pairs of PACs shown as FISH probes (fig 3) could also be potentially used for prenatal diagnosis on interphase nuclei in both reported families.

One other study has mapped two different reciprocal translocation breakpoints to a 200 kb interval defined by fragmentation YACs.[18] The mapping interval is likely to be very similar to the one presented in this paper. The comparison of the exact physical locations of the few translocation breakpoints mapped by others (using YACs) and the one described in this paper may precisely define the borders of the DNA fragment carrying the gene. Since YAC clones are known to suffer from frequent deletions and rearrangements, which in many cases eliminate gene coding sequences,[26 27] we believe that PACs or cosmids derived directly from genomic DNA (not subcloned from YACs) will be valuable complementary tools in the efforts to clone the RS gene and to construct a full transcriptional and sequence ready map of this region. Work is currently under way by our group to bridge the interval between the two groups of PACs and markers presented in this report. This will enable the breakpoint to be precisely mapped and cloned.

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