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A Second Locus for Rieger Syndrome Maps to Chromosome 13q14

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Summary

Rieger syndrome is a genetically and phenotypically heterogeneous disorder typically characterized by malformations of the eyes, teeth, and umbilicus. The syndrome is inherited as an autosomal dominant trait and exhibits significant variable expressivity. One locus associated with this disorder has been mapped to 4q25. Using a large four-generation pedigree, we have identified a second locus for Rieger syndrome located on chromosome 13q14.

Introduction

Rieger syndrome is an autosomal dominant disorder of morphogenesis that results in abnormal development of the anterior segment of the eye, which can result in blindness from glaucoma in ~50% of affected individuals (Fitch and Kaback 1978). Systemic abnormalities have also been associated, including dental hypoplasia, failure of involution of periumbilical skin, and maxillary hypoplasia (Alkemade 1969; Judisch et al. 1979). Less commonly, hydrocephalus, hearing defects, cardiac and kidney abnormalities, and congenital hip anomalies have been described (Saba 1927; Delmarcelle et al. 1958; Breebaart 1966; Alkemade 1969; Wolkowicz et al. 1971). The anterior segment of the eye, the enamel of the teeth, and other structures associated with this syndrome are derived from embryonic neural crest (Shields 1983; Nakano and Nakamura 1985). Defective developmental processes involving neural crest may be the source of the phenotypic abnormalities observed in patients affected by this disorder.

One locus for Rieger syndrome has been mapped to 4q25 (RGS; MIM 180500). Deletions of this region were first recognized in two individuals demonstrating the characteristic features of the syndrome (Ligutic et al. 1981; Mitchell et al. 1981). Subsequently, linkage was demonstrated in three small pedigrees, with a highest combined lod score of 4.36 at 0% recombination at D4S193 (Murray et al. 1992). Linkage to 4q25 has also recently been observed in a pedigree affected by iris hypoplasia (Heon et al. 1995).

Genetic heterogeneity of Rieger syndrome has been suggested by descriptions of affected individuals with a variety of chromosomal abnormalities, including two reports of deletions of chromosome 13q14 (Akazawa et al. 1981; Stathacopoulos et al. 1987), a deletion of chromosome 10 (Hervé et al. 1984), a pericentric inversion of chromosome 6 (Heinemann et al. 1979), and an isochromosome of the long arm of 6 (Tabbara et al. 1973). One pedigree affected by Rieger syndrome does not demonstrate linkage to the 4q25 locus, providing further evidence for genetic heterogeneity (Legius et al. 1994).

Variable expressivity of the clinical features that define Rieger syndrome has been well established. Diverse combinations of clinical findings, both ocular and systemic, can be found among affected family members belonging to the same pedigree (Alkemade 1969). Moreover, an affected parent can produce affected offspring exhibiting a wide range of phenotypic abnormalities (Alkemade 1969; Fitch and Kaback 1978). Clinical or genetic evidence of anticipation has not been observed.

We have identified a four-generation pedigree affected by Rieger syndrome. Initial linkage studies did not show linkage to the previously defined locus on chromosome 4q25 and suggested that a distinct locus was responsible for the observed phenotypic abnormalities. In this report, we describe linkage studies designed to identify the locus responsible for the disease affecting this pedigree.

Methods

Pedigree Ascertainment

Twenty-seven (11 affected) members of the pedigree (fig. 1) were examined either by one of the authors or by a referring ophthalmologist. An individual was determined to be affected if one of the characteristic ocular findings associated with Rieger syndrome was observed. When indicated, medical records were obtained from

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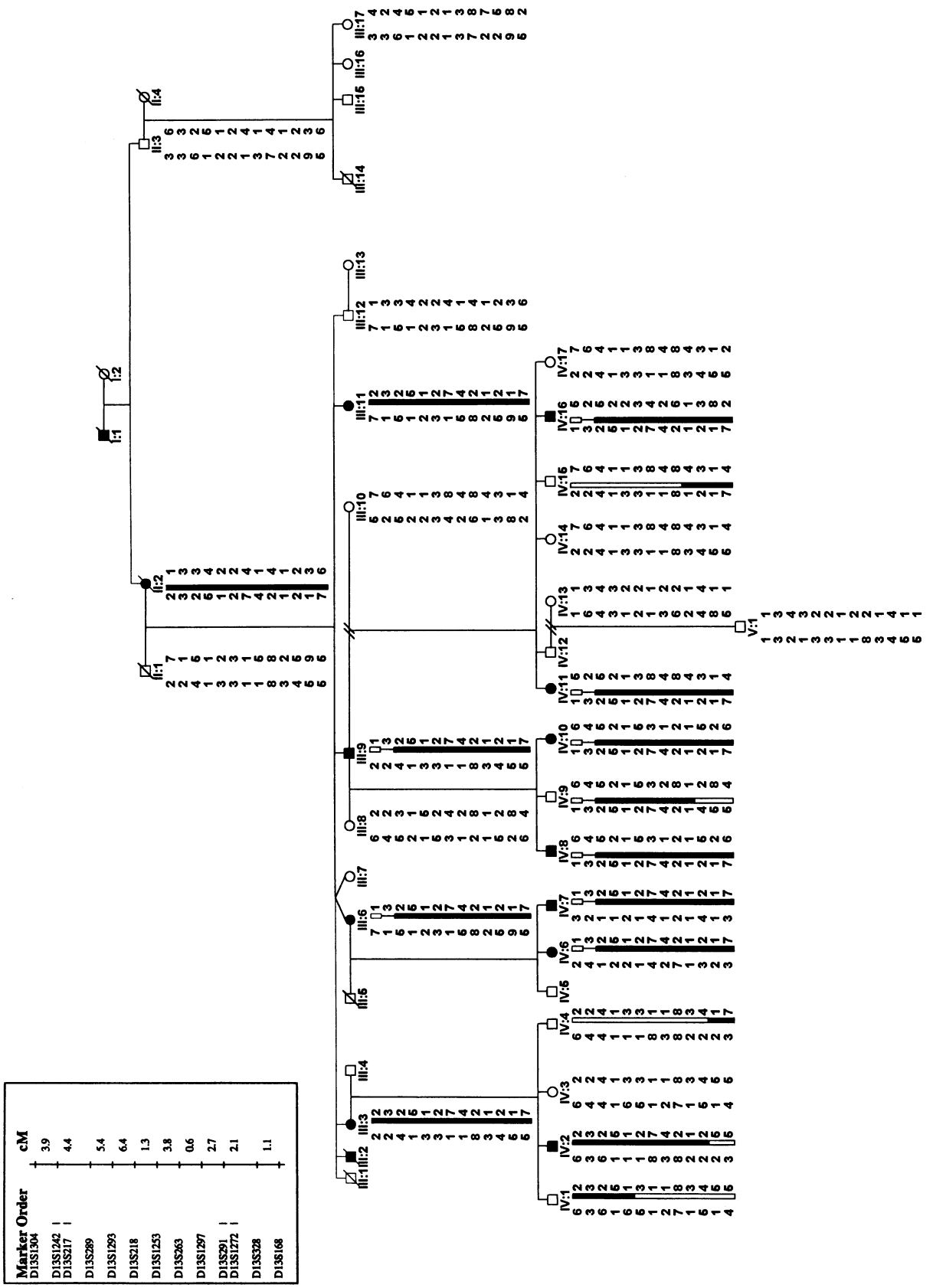

Figure 1 Affected individuals, shown as blackened circles (females) or blackened squares (males). Deceased individuals are indicated with slashes. Haplotypes are presented to illustrate the segregation of the parental chromosomes.

Table 1

Clinical Features of Affected Individuals

Individual	Ocular Anomalies	Systemic Anomalies
III-3	Posterior embryotoxon; iris hypoplasia; glaucoma (mild)	None
III-6	Posterior embryotoxon	Premature loss of teeth
III-9	Posterior embryotoxon; iris adhesions; iris hypoplasia; glaucoma (mild)	Premature loss of teeth Hearing loss
III-11	Posterior embryotoxon; iris adhesions; iris hypoplasia; corectopia; glaucoma (severe)	Premature loss of teeth Hearing loss; congenital hip malformation
IV-2	Posterior embryotoxon; iris adhesions; iris hypoplasia; glaucoma (severe)	Premature loss of teeth Hearing loss; congenital hip malformation; cryptorchism
IV-6	Posterior embryotoxon; iris adhesions	Oligodontia; microdontia; fetal lobulations of the kidney
IV-7	Posterior embryotoxon; glaucoma (mild)	Premature loss of teeth
IV-8	Posterior embryotoxon; iris adhesions; iris hypoplasia; glaucoma (severe)	Maxillary hypoplasia Broad nasal root
IV-10	Posterior embryotoxon; iris hypoplasia; glaucoma (severe)	Hydrocephalus; broad nasal root
IV-11	Posterior embryotoxon; iris adhesions; iris hypoplasia; glaucoma (severe)	Premature loss of teeth Hearing defects; congenital tricuspid valve anomaly
IV-16	Posterior embryotoxon; iris adhesions; glaucoma (severe)	Premature loss of teeth Hearing defects

internists, pediatricians, and dentists, after informed consent by the patient.

Linkage Analysis

Peripheral blood samples were obtained from 27 members of the pedigree. DNA was purified from a lymphocyte pellet according to standard procedures. Microsatellites were selected from the genome at 10–20-cM intervals and amplified and scored as described by Wiggs et al. (1994). Two-point linkage analysis was performed on an IBM PC–compatible computer using the MLINK feature of the LINKAGE program 5.1 (Lathrop and Lalouel 1984). For the linkage calculations, Rieger syndrome was modeled as an autosomal dominant trait with a mutation rate assumed to be 1×10^{-6}. Recombination frequencies were assumed to be equal between males and females. Because of the variable expressivity of the disease phenotype, lod-score calculations were performed at penetrances of 1.0 and 0.9. Published marker-allele frequencies were used for these calculations. Haplotype analysis was used to determine the minimal candidate region for location of the Rieger gene.

Results

Each of the 11 affected individuals examined demonstrated at least one of the typical findings of anterior-segment dysgenesis that characterizes the ocular component of the Rieger syndrome. Nine of the affected individuals also showed evidence of glaucoma defined by elevation of intraocular pressure and deterioration of the optic nerve. The severity of the glaucoma ranged from mild (treatment requiring only medications) to severe (significant blindness, requiring surgical treatment) (see table 1). The ocular findings varied in severity from mild dysgenesis of the anterior segment (posterior embryotoxon and iris hypoplasia) to severe dysgenesis of the anterior segment and angle of the eye (iris adhesions, corectopia, and severe glaucoma). In addition to the ocular features, all of the affected individuals, except III-3, also demonstrated one or more systemic abnormalities, including dental and hearing defects, mild craniofacial dysmorphism, hydrocephalus, cryptorchism, fetal lobulations of the kidney, a congenital heart defect, and congenital hip abnormalities (table 1). Individual III-3 has significant ocular abnormalities, including iris hypoplasia and glaucoma. She has an affected son who exhibits ocular and systemic abnormalities, indicating the variable expressivity of this condition. Seven of the affected individuals had dental abnormalities evidenced by loss of teeth early in life (10–20 years) secondary to the decay caused by the dental hypoplasia and abnormal tooth enamel characteristic of this disease. Redundancy of the periumbilical skin has been described as a common feature of the Rieger syndrome, however none of the affected members of this pedigree were found to have this abnormality. One child of an affected individual (II-2) died in infancy and was said to have had an abnormal navel, but this could not be documented by medical records.

Genetic linkage studies were initially performed using microsatellite markers known to map to the previously described locus for Rieger syndrome on chromosome 4q25 (Murray et al. 1992; Heon et al. 1995). A 36-cM region was effectively excluded by this analysis, including the markers linked to iris hypoplasia ($Z_{MAX} = 3.70$ at D4S1616) and Rieger syndrome ($Z_{MAX} = 4.36$ at D4S193) (table 2). D4S193 and the gene for epidermal growth factor (EGF) are located in the interval between

D4S1570 and D4S430 (Heon et al. 1995). These results indicated that our pedigree was not linked to 4q25 and that a second gene must be responsible for the observed phenotype. We proceeded to screen the human genome by using microsatellite markers at 10–20-cM intervals, focusing initially on those regions of the genome where chromosomal abnormalities associated with Rieger syndrome had previously been described. Significant linkage was found for markers mapping to 13q14. This region is included in both deletions of chromosome 13 previously reported to be associated with Rieger syndrome (Akazawa et al. 1981; Stathacopoulos et al. 1987) The peak Z_{MAX} was found for marker D13S1253 (Z_{MAX} = 4.64 at θ = 0, penetrance = .9; Z_{MAX} = 3.93 at θ = .01, penetrance = 1.0) (table 3).

Affected individuals shared a haplotype for markers spanning the region (fig. 1). Critical recombination events in affected individuals occurred between markers D13S1242 and D13S217 and D13S1272 and D13S328, defining a 26-cM critical interval extending from marker D13S1242 to D13S328 (Dib et al. 1996). Several unaffected individuals (IV-1, IV-4, and IV-15) demonstrate recombination events that could significantly narrow the critical region. However, because of the decreased penetrance of this condition, one or more of these individuals could still have this affected allele. To eliminate the potential ambiguity, the critical region has been defined by recombination events seen in individuals expressing the disease phenotype. One member of this pedigree (IV-9) has the affected haplotype throughout the critical region but does not show definite evidence of this disease. He is currently 16 years old and was not able to cooperate with a comprehensive ocular examination. He had an extra set of teeth in infancy but otherwise has not had any known medical or dental abnormalities. For the purposes of linkage analysis, he was scored as unaffected.

Discussion

We have mapped a second gene responsible for the Rieger developmental syndrome to chromosome 13q14.

Table 2

Pairwise Linkage Data

Marker and Penetrance	Z at θ = 0	.01	.05	.1	.2	.3	.4
D4S414:							
1.0	−∞	−6.72	−3.33	−1.96	−.77	−.23	−.01
.9	−9.36	−4.55	−2.52	−1.55	−.63	−.19	−.01
D4S1560:							
1.0	−∞	−4.90	−2.23	−1.19	−.35	−.05	.03
.9	−7.73	−4.00	−1.90	−1.04	−.32	−.05	.02
D4S1572:							
1.0	−∞	−6.50	−3.73	2.55	−1.36	−.68	−.25
.9	−13.13	−5.56	−3.34	−2.32	−1.26	−.64	−.24
D4S1570:							
1.0	0	0	0	0	0	0	0
.9	0	−.01	−.01	−.01	−.01	0	0
D4S1564:							
1.0	−∞	−9.23	−4.51	−2.64	−1.04	−.36	−.08
.9	−15.50	−7.15	−3.78	−2.30	−.98	−.36	−.09
D4S1616:							
1.0	−∞	−10.33	−5.57	−3.63	−1.83	−.91	−.41
.9	−15.49	−9.00	−5.17	−3.42	−1.75	−.88	−.36
D4S1611:							
1.0	−∞	−11.69	6.23	−3.99	−1.94	−.94	−.36
.9	−9.51	−8.04	−5.10	−3.40	−1.73	−.87	−.34
D4S402:							
1.0	−∞	−13.22	−7.07	−4.55	−2.24	−1.10	−.43
.9	−15.48	−9.31	−5.88	−3.94	−2.03	−1.02	−.41
D4S430:							
1.0	−∞	−8.42	−4.35	−2.71	−1.25	−.58	−.22
.9	−13.57	−7.24	−3.96	−2.53	−1.22	−.68	−.22
D4S1615:							
1.0	−∞	−3.90	−2.38	−1.66	−.93	−.50	−.21
.9	−2.04	−1.93	−1.56	−1.22	−.75	−.42	−.18

Table 3

Pairwise Linkage Data

Marker and Penetrance	Z at θ = 0	.01	.05	.1	.2	.3	.4
D13S1304:							
1.0	−∞	−3.24	−.676	.227	.787	.784	.500
.9	−5.14	−2.09	−.311	.382	.811	.771	.484
D13S1242:							
1.0	−∞	−1.80	.134	.809	1.17	1.05	.651
.9	−.038	.317	.891	1.17	1.26	1.05	.630
D13S217:							
1.0	−∞	−1.01	.971	1.62	1.83	1.47	.797
.9	.908	1.26	1.80	2.01	1.91	1.45	.770
D13S289:							
1.0	−∞	1.54	2.34	2.54	2.33	1.74	.911
.9	2.81	2.83	2.85	2.76	2.34	1.69	.867
D13S1293:							
1.0	−∞	.995	2.13	2.37	2.16	1.59	.820
.9	2.74	2.75	2.73	2.62	2.18	1.54	.773
D13S218:							
1.0	−∞	2.24	2.73	2.75	2.39	1.80	1.00
.9	3.01	3.00	2.92	2.76	2.30	1.70	.937
D13S1253:							
1.0	−∞	3.93	4.25	4.07	3.31	2.32	1.17
.9	4.64	4.59	4.36	4.01	3.17	2.19	1.09
D13S263:							
1.0	−∞	3.93	4.25	4.07	3.31	2.32	1.17
.9	4.64	4.59	4.36	4.00	3.17	2.18	1.09
D13S1297:							
1.0	−∞	3.93	4.25	4.07	3.31	2.32	1.17
.9	4.64	4.59	4.36	4.00	3.17	2.18	1.09
D13S291:							
1.0	−∞	.340	1.49	1.77	1.70	1.30	.732
.9	2.07	2.08	2.10	2.04	1.74	1.29	.709
D13S1272:							
1.0	−∞	1.84	2.46	2.57	2.32	1.77	.998
.9	2.67	2.69	2.70	2.61	2.23	1.67	.928
D13S328:							
1.0	−∞	−.290	1.47	1.93	1.90	1.41	.710
.9	−2.58	1.41	2.04	2.15	1.90	1.36	.675
D13S168:							
1.0	−∞	−.062	1.70	2.16	2.11	1.59	.822
.9	−2.36	1.64	2.26	2.37	2.10	1.52	.770

This same region has been reported to be deleted in two patients affected by this syndrome. The clinical features of the deletion patients are similar to this pedigree and to other pedigrees affected by the syndrome.

Rieger syndrome is likely to be the result of a defective developmental process involving the neural crest ectoderm. It is interesting that the ocular findings in Rieger syndrome overlap to some extent with those observed in patients affected by aniridia caused by mutations in the Pax 6 gene. A gene analogous to Pax 6 in the rat has been shown to be important for normal migration of neural crest cells during early development. The result that genes mapped to two distinct loci (4q25 and 13q14) can be independently responsible for a similar phenotype suggests that these genes, and possibly the Pax 6 gene, may participate in a common developmental pathway.

Of the many medical problems affecting patients with Rieger syndrome, perhaps the most devastating is blindness from glaucoma. Glaucoma is a severe eye disease that is the result of retinal ganglion cell loss usually associated with increased intraocular pressure. Although it is likely that the rise in intraocular pressure is caused by an alteration of the outflow of aqueous humor from the eye, the actual anatomical or physiological processes that result in the disease are unknown. Approximately

50% of patients affected by Rieger syndrome develop glaucoma (Shields 1983). A direct correlation between the severity of the anterior segment dysgenesis and the incidence of glaucoma has not been observed. It is likely that the form of glaucoma affecting patients with Rieger syndrome is the result of abnormal development of structures in the eye that are intimately involved in the normal processes of aqueous-humor outflow. The identification and characterization of the gene responsible for this syndrome should provide important new insight into the ocular anatomy and physiology that is altered to cause glaucoma in patients affected by this disease.

The numerous congenital anomalies and the variable expressivity of the phenotype suggest that a gene responsible for Rieger syndrome plays a role in the regulation of certain developmental processes. It is interesting to note that a homologue to the *Drosophila melanogaster fork head* gene, which may function as a developmental regulatory protein, has been mapped to 13q14. This gene was initially identified as a product associated with a t(2;13)(q35;q14) translocation found in an alveolar rhabdomyosarcoma (Galili et al. 1993). Further analysis demonstrated the gene contained the conserved 109–amino acid motif that has been identified in forkhead proteins found in many different organisms, including *Drosophila* (Baumgartner et al. 1987; Côtè et al. 1987), *Caenorhabditis elegans* (Miller et al. 1993), zebrafish (Strähle et al. 1993), rat (Clevidence et al. 1993), and mice (Kaestner et al. 1994). Forkhead proteins function as regulators of transcription and appear to be developmentally controlled (Häcker et al. 1992). In particular, in mice, forkhead genes *HNF3*alpha and *HNF3*beta and two related genes *mf1* and *mf2* are involved in the establishment of germ layers during gastrulation (Sasaki and Hogan 1993). Because of the potential role this protein may have in developmental processes, it emerges as an excellent candidate gene for Rieger syndrome. Analysis of this gene for mutations in patients affected by the syndrome is currently in progress.

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