

ONLINE MUTATION REPORT

Mutation in *PITX2* is associated with ring dermoid of the cornea

K Xia, L Wu, X Liu, X Xi, D Liang, D Zheng, F Cai, Q Pan, Z Long, H Dai, Z Hu, B Tang, Z Zhang, J Xia

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Ring dermoid of the cornea (RDC, MIM180550) is an autosomal dominantly inherited syndrome characterised by bilateral annular limbal dermoids with corneal and conjunctival extension. The genetic basis of RDC is unknown. We report linkage of chromosome 4q24-q26 to RDC and identification of a missense mutation in *PITX2* in 17 disease affected individuals but not in eight genetically related normal individuals in a large Chinese family.

METHODS

A large Chinese family with 17 individuals affected by the RDC was identified (figs 1 and 2). All patients were diagnosed by the same physician (XHX). Informed written consent for blood sample collection was obtained from all participants.

Linkage analysis and genotyping were done essentially as previously described.¹ Genome-wide screening was carried out with 382 microsatellite markers covering all autosomal chromosomes, with an average interval of 10 cM (ABI PRISM™ linkage mapping set, version 2.0). Fine mapping was accomplished using fluorescent labelled primers from the Marshfield database. Alleles were analysed by GeneScan analysis, version 3.0 software, and Genotyper, version 2.1 software (ABI PRISM™ linkage analysis and haplotype

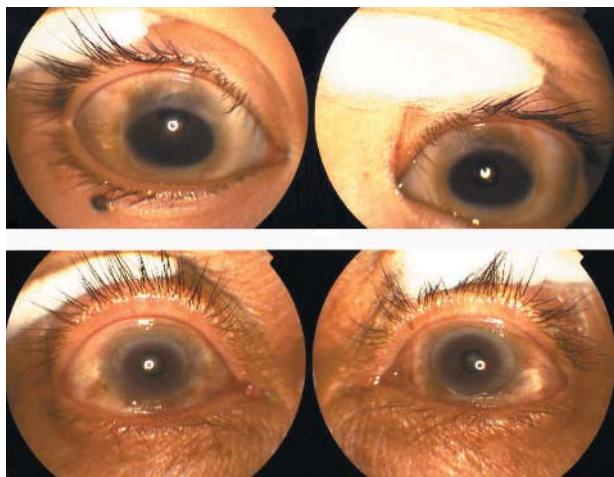


Figure 1 Eyes affected by ring dermoid of the cornea (RDC) in two patients, II-2 (upper panel) and IV-3 (lower panel). Yellow-white tumour-like apophyses are visible on the corneal border of both eyes. The apophyses are diffuse in the superficial layer of the cornea and conjunctiva. The corneal border is not clear and the diameter of the transparent region of cornea is diminished to about 7–8 mm. The upward shift of the right pupil of IV-3 is caused by cataract resection (the affected individual, IV-3, also has bilateral glaucoma and congenital cataracts in the right eye).

Key points

- The ring dermoid of the cornea (RDC, MIM180550) is an autosomal dominantly inherited syndrome characterised by bilateral annular limbal dermoids with corneal and conjunctival extension.
- We report linkage of a 15 cM interval on chromosome 4q24-q26 to RDC. A missense mutation in *PITX2* was found in 17 disease affected individuals but not in eight genetically related normal individuals in a large Chinese family, and in 157 normal unrelated individuals.
- Given that *PITX2* functions in eye development, these findings suggest mutations in *PITX2* as a potential cause of RDC.

construction). Two point linkage analysis was conducted using the MLINK program of the Linkage 5.1 package (Rockefeller University, New York, USA). The disease allele frequency was set at 0.0001 with the recombination fraction (θ) in male and female subjects considered equal. The penetrance frequency of the disease was assumed to be 0.99 with autosomal dominance. The most likely haplotype was constructed by the Cyrillic program (Electrotechnical Laboratory, Tokyo, Japan).

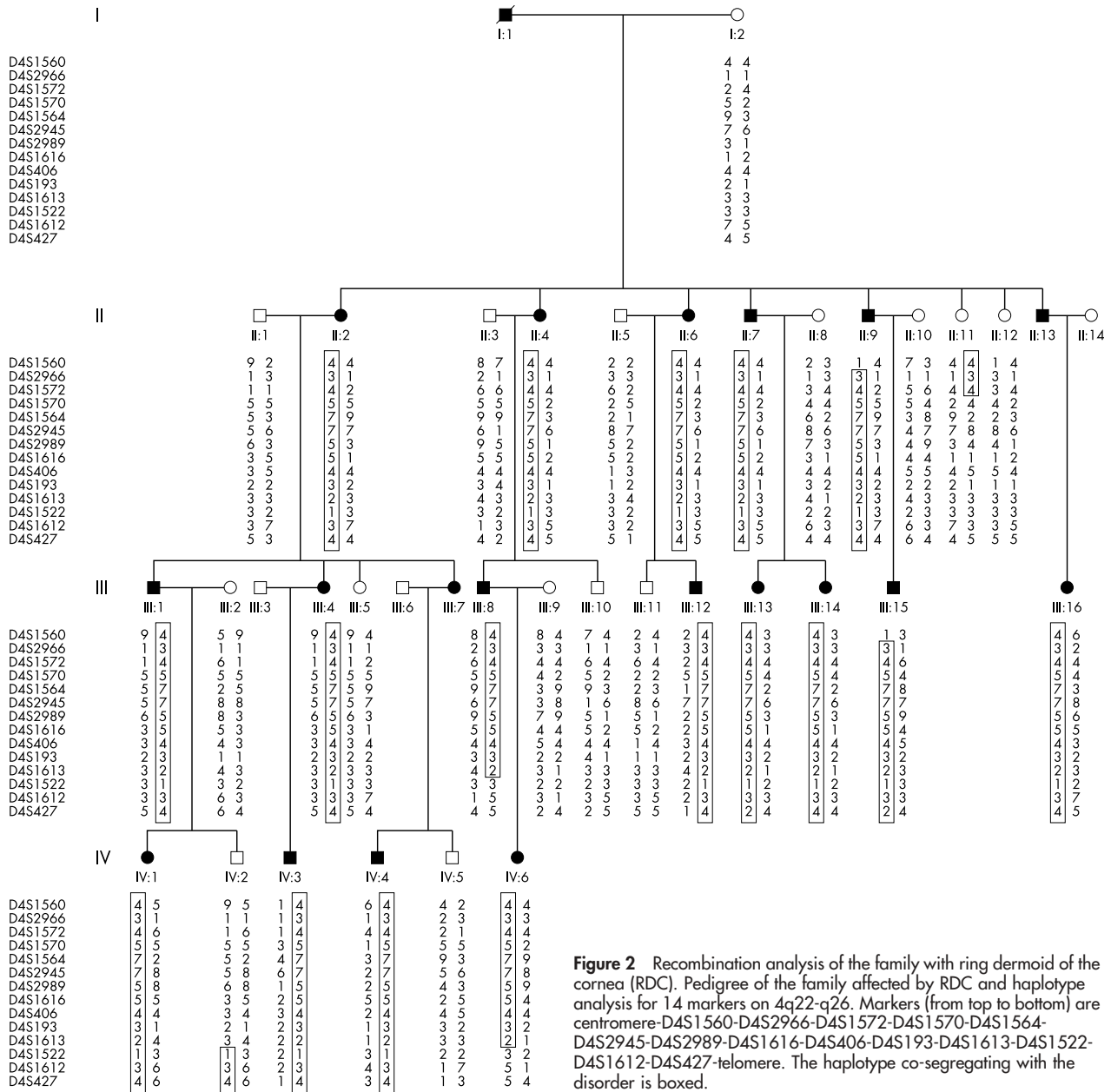
For mutation analysis, all exons and intron–exon boundaries of selected genes were amplified by polymerase chain reaction (PCR) using genomic DNA of the proband as template and primers designed according to the genomic sequences obtained from Genbank. Sequencing of the PCR products was automated (ABI3100 sequencer).

RESULTS

In this large Chinese family, 21 of 36 genetically linked individuals were affected by RDC.² Patients showed yellow-white tumour-like apophyses (2–3 mm high and 3–5 mm wide) on the corneal border of both eyes (fig 1). The apophyses were clinically detectable at birth and progressively impaired the patients' vision with aging. Some affected individuals also had glaucoma (II-2, III-4, and IV-3), unilateral cataracts (IV-3), or involuntary oscillation of the eyes (IV-3). The only clinical manifestation in the affected individuals were in the eyes. Consistent with previously reported autosomal dominantly inherited patterns of RDC, affected cases were found in both male and female descendants of each of four generations.

The linkage analysis was carried out with samples collected from 17 affected individuals, eight genetically related normal

Abbreviations: RDC, ring dermoid of the cornea



siblings, and seven genetically unrelated family members. Results showed a maximum two point lod score of 3.91 ($\theta = 0$), 2.33 ($\theta = 0$), and 1.87 ($\theta = 1$) for D4S1572, D4S406, and D4S402, respectively. The results establish a linkage of RDC to chromosome 4q. Fine mapping using 12 microsatellite markers around D4S1572 and D4S406 identified a maximum two point lod score of 6.72 ($\theta = 0$) for D4S2989. The lod scores for the neighbouring markers D4S2945 and D4S161 were 5.04 and 5.01 ($\theta = 0$), respectively (table 1). Haplotype analysis for 14 markers on chromosome 4q showed that all affected family members were carriers of the risk haplotype (fig 2). Recombination between D4S1560 and D4S2966 was detected in affected individuals II-9 and III-15. Another recombination between D4S1613 and D4S1522 was found in affected individuals III-8 and IV-6. Most normal members of the family who were examined did not carry the haplotype. However, the genetically related normal individual II-11 carried a partial haplotype with a recombination between D4S1572 and D4S1570, suggesting

that the candidate risk gene is located distal to D4S1572. Moreover, a genetically related normal individual, IV-2, carried the haplotype with a recombination between D4S1613 and D4S1522, where a recombination was detected in III-8 and IV-6. The results indicate that the candidate risk gene is located at the proximal side of D4S1522 (fig 2). Together, the haplotype between D4S1572 and D4S1522 co-segregates with the disease in this family. Thus a linkage of the RDC locus to a 15 cM interval between D4S1572 and D4S1522 was established.

The genomic interval between D4S1572 and D4S1522 contains 65 known genes and 56 reference genes. Three potential candidate genes for RDC were chosen for further mutation examination, based on both their tissue specific expression and their roles in regulating cell proliferation, differentiation, and migration that may play an important part in RDC pathogenesis.² These include *IDAX* (NM 025212),³ *TM4SF9* (NM005723),⁴ and *PITX2* (NM 153427).⁵ Mutation analysis showed no mutations in *IDAX* or *TM4SF9*

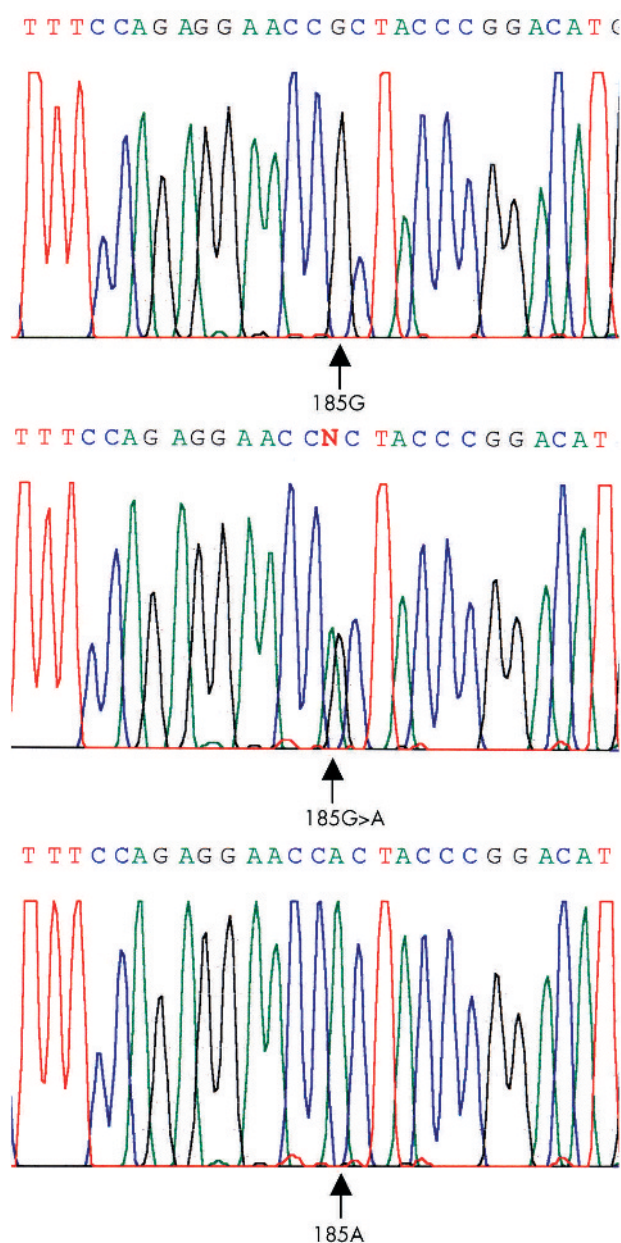


Figure 3 Partial sequence chromatographs of *PITX2*. Normal *PITX2* 185G (upper panel), the heterozygous missense mutation 185G→A identified in all patients except patient III-12 (middle panel), and a homozygous mutation 185G→A identified in patient III-12 (lower panel) are shown.

of the proband. A heterozygous mutation of guanine to adenine (185G→A) was detected in *PITX2* of the proband (fig 3). Further sequence analysis showed a perfect segregation of this mutation with the disease in all 17 affected individuals in the family. Interestingly, affected individual III-12 showed a homozygous mutation. The mother of III-12 is an affected person harbouring a heterozygous G185A mutation, while the father is normal, with no mutation at nucleotide 185. Genetic analysis confirms that the father is the biological father (not shown). It is likely that III-12 carries a mutation inherited from the mother and a de novo mutation at the same position. No mutation of *PITX2* was detected in eight genetically related normal individuals, seven genetically unrelated individuals in the family, and 150 ethnically appropriate normal controls. The results

indicate that the sequence change observed is not a common polymorphism.

COMMENT

PITX2, a downstream target of wnt/ β -catenin pathway, encodes a homeodomain transcription factor required for normal development of multiple organs, including eye, heart, and pituitary.⁶⁻¹³ Mutations in *PITX2* are associated with multiple dominantly inherited diseases related to malfunction of the eyes, including Rieger syndrome,⁵ iridogoniodysgenesis,¹⁴ iris hypoplasia,¹⁵ and Peter's anomaly.¹⁶ This study suggests that mutation in *PITX2* is linked to another eye disease. The *PITX2* G185A mutation found in RDC patients is a novel disease associated mutation resulting in a substitution of arginine by histidine at amino acid 62 (R62H) located in the conserved DNA binding homeodomain (fig 4). The mutation probably results in changes in its transcriptional activity, as with other disease associated mutations identified in this gene.¹⁷⁻²¹ Identification of this novel mutation in *PITX2* may reveal the molecular mechanism underlying the RDC pathology.

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Authors' affiliations

K Xia, L Wu, X Liu, D Liang, D Zheng, F Cai, Q Pan, Z Long, H Dai, Z Hu, Z Zhang, J Xia, National Laboratory of Medical Genetics of China, Central South University, Changsha, Hunan, China
X Xi, Xiangya 2nd Hospital, Central South University
B Tang, Xiangya Hospital, Central South University

Conflicts of interest: none declared

Correspondence to: Dr K Xia, National Laboratory of Medical Genetics of China, Central South University, Changsha, Hunan, China; nlmglcy@xysm.net

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Table 1 Two point LOD scores between the disease gene and 14 markers of chromosome 4q22-q26

Locus	Genetic distance†	LOD SCORE AT $\theta = *$					Z_{max}	θ_{max}
		0.0	0.1	0.2	0.3	0.4		
D4S1560	104.75	-2.50	1.25	1.17	0.86	0.45	1.25	0.1
D4S2966	106.89	3.41	2.87	2.26	1.57	0.81	3.41	0
D4S1572	107.95	3.91	3.41	2.72	1.88	0.93	3.91	0
D4S1570	109.02	3.58	2.98	2.31	1.56	0.73	3.58	0
D4S1564	112.62	6.07	5.04	3.90	2.66	1.30	6.07	0
D4S2945	116.37	5.04	4.21	3.30	2.27	1.11	5.04	0
D4S2989	117.06	6.72	5.64	4.44	3.09	1.55	6.72	0
D4S1616	117.06	5.01	4.33	3.47	2.43	1.21	5.01	0
D4S406	117.06	2.33	1.91	1.45	0.95	0.39	2.33	0
D4S193	117.06	6.07	5.08	3.97	2.74	1.35	6.07	0
D4S1613	121.61	6.63	5.57	4.38	3.05	1.53	6.63	0.1
D4S1522	123.13	0.64	3.93	3.31	2.38	1.21	3.93	0.1
D4S1612	124.45	-3.79	1.85	1.59	1.14	0.60	1.85	0.1
D4S402	124.45	-1.97	1.87	1.63	1.19	0.63	1.87	0.1
D4S427	124.45	-3.69	1.40	1.19	0.76	0.32	1.40	0.1

*LOD scores were calculated under an autosomal dominant mode of inheritance and a penetrance of 100%.

†Sex averaged genetic distance from the next marker in centimorgans according to the Genethon human genetic linkage map (1996).

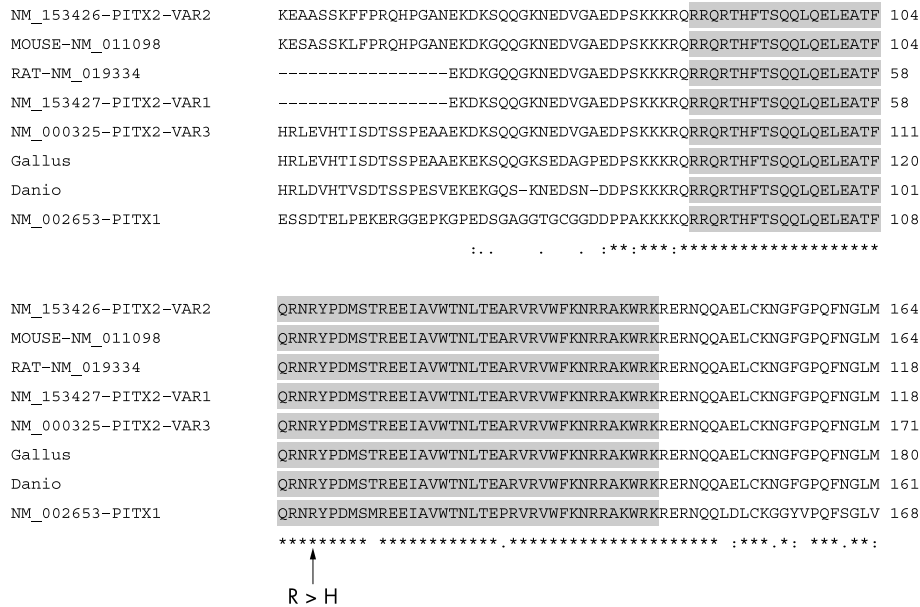


Figure 4 Sequence comparison of the homeodomain of human *PITX2* and several proteins related to the homeodomain containing protein bicoid. The missense mutation R68H in a conserved amino acid detected in patients with ring dermoid of the cornea is indicated.

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