A variant form of Oguchi disease mapped to 13q34 associated with partial deletion of GRK1 gene

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Abstract

Purpose: To map the locus for a variant form of Oguchi disease in a Pakistani family and to identify the causative mutation.

Design: Ophthalmological observational report and molecular genetic analysis.

Participants: Family 61029 was ascertained in the Punjab province of Pakistan. It includes three 13- to 19-year-old patients with night blindness and 12 unaffected family members.

Methods: A complete ophthalmological examination including fundus photography and electroretinography (ERG) was performed on each family member. A genome-wide scan was performed using microsatellite markers at about 10cM intervals and by calculating two-point lod scores. PCR cycle dideoxynucleotide sequencing was used to screen candidate genes inside the linked region for mutations as well as to delineate the deletion. Multiplex PCR and long template PCR were used to detect deletions and to define the size of deletions.

Main outcome measures: Evaluation of fundus changes and ERG, lod score estimation and identification of a mutation in the GRK1 gene.

Results: All patients had night blindness since early childhood. Irregular coarse pigmentation was observed in the peripheral retina of each patient. The fundus appearance before and after 4 hours of dark adaptation is similar except that the peripheral retinal pigmentary changes were slightly less evident after extended dark adaptation. Minimal or no rod function with normal cone function on ERG recordings were detected in all three affected members. The rod showed slow recovery to nearly normal amplitude after 4 hours in the dark ERG in one individual but not in two other patients. A genome wide scan showed linkage only to D13S285. Fine mapping defined a region from D13S1315 to 13qter, with a lod score of 2.89 at $theta$ = 0 shown by D13S285 and 2.90 at $theta$ = 0 by the D13S261-D13S285-D13S1295-D13S293 haplotype. Analysis of the GRK1 gene, which is included in this interval, identified a c.827+623_883del mutation. This intragenic deletion cosegregates with the disease in the family and is only homozygous in affected individuals.

Conclusion: A variant form of Oguchi disease in a Pakistani family localizes to 13q34 and is caused by a novel deletion including exon 3 of the GRK1 gene. This not only demonstrates phenotypic variability of Oguchi disease resulting from GRK1 mutations but also is the first report in which both linkage analysis and identification of a mutation implicate GRK1 as a cause of Oguchi disease.

Introduction

Hereditary retinal diseases represent a broad range of retinal dysfunction and/or degeneration including retinitis pigmentosa (RP), cone or cone-rod dystrophy, Leber congenital amaurosis, congenital stationary night blindness (CSNB), color blindness, pathologic myopia, macular degeneration, retinoschisis, and chorioretinal atrophy. Over one hundred genes have been reported to be associated with these diseases (RetNet, http://www.sph.uth.tmc.edu/Retnet/).

RP is characterized clinically by night blindness, gradual constriction of the visual fields, typical fundus changes and finally loss of central vision[1]. The fundus changes include a waxy-disc appearance, attenuation of the retinal vessels especially the arteries, and bone-spicule pigmentation in the mid-peripheral retina. Such fundus changes may be very mild in early childhood. A small number of patients may have no pigmentation in the retina at all and other patients may have pigmentation involving the macula, especially in advanced stages of the disease. The rod ERG response is reduced or completely extinguished. The cone ERG response may be normal in early stages of the disease, but a less severe cone ERG involvement is usually observed and the cone ERG may become non-detectable in advanced cases. Some patients lose all vision around thirty years of age but others may preserve useful vision after fifty. Thus, RP represents a clinically and genetically heterogeneous group of retinal degenerations. RP may show autosomal dominant or X-linked inheritance, but is most frequently inherited as an autosomal recessive trait[2]. Autosomal recessive RP has been mapped to 21 loci and causative mutations have been identified in at least 17 genes (RetNet, http://www.sph.uth.tmc.edu/Retnet/). However, mutations in each gene identified so far have been responsible for arRP in less than 2—5% of families. Overall, causative mutations have been identified in a fraction of arRP families, probably around 30% [Steve Daiger, personal communication]. Additional loci responsible for the remaining families have yet to be identified.

CSNB refers to a group of diseases with obvious but stationary rod dysfunction. The fundus may be normal or have a distinctive appearance (e.g., Mizuo-Nakamura phenomenon in Oguchi disease, white dots in fundus albipunctatus, or typical changes in high myopia)[3]. Mizuo-Nakamura phenomenon is the typical sign of Oguchi disease where unique fundus changes, diffuse or patchy, silver-gray or golden-yellow metallic sheen with retinal vessels stand out in relief against the background, could be observed. A prolonged dark adaptation of three hours or more leads to disappearance of such changes. So far, mutations in RHO, GNAT1 or PDE6B have been associated with autosomal dominant CSNB, mutations in SAG, GRK1 or RDH5 have been associated with autosomal recessive CSNB, and mutations in CACNA1F and NYX have been associated with X-linked CSNB [3,4]. The recessive forms of CSNB, including Oguchi disease resulting from mutations in SAG or GRK1, as well as fundus albipunctatus, resulting from mutations in RDH5, have typical fundus changes.

The human GRK1 (G protein-coupled receptor kinase 1) gene, also called RHOK is located on chromosome 13q34 and consists of 7 exons encoding 563 amino acids[5,6]. It encodes rhodopsin kinase, which desensitizes rod photoreceptors to light, inactivating photoactivated rhodopsin in rod photoreceptors by phosphorylating it[5,7]. Mutations in GRK1 previously have been described in 3 unrelated cases with the Oguchi form of stationary night blindness[8,9]. However, the typical fundus and ERG changes described in those patients are not well manifested in the patients seen here.

In this study we clinically characterize a consanguineous Pakistani family with individuals affected by nightblindness and retinal pigmentary changes and map the disease in this family to chromosome 13q34. Sequencing of GRK1 identifies a novel deletion including exon 3.

Methods

Family and Clinical Data

Family 61029, containing one consanguineous marriage, was ascertained from Punjab province of Pakistan as part of a collaborative project between the CEMB, Lahore, Pakistan and the NEI to identify genetic cause of eye diseases. Three affected and 12 unaffected persons spanning three generations participated in this study. Electroretinogram (ERG) responses were recorded consistent with ISCEV standards on selected affected members of this family, using commercial ERG equipment (LKC Inc., Gaithersberg, MD). Stimulus intensities were -25dB for scotopic and 0dB for photopic 30 Hz flicker. Scotopic responses to single bright-flash stimuli were recorded after dark-adapting the subjects in a room without lighting. Scotopic ERGs were recorded under two conditions, of either 30 minutes dark-adaptation or four hours dark-adaptation. Two affected subjects were recorded under both conditions. Responses under these scotopic conditions originate primarily from rod activity but also contain a small cone component. Subjects were then light-adapted for five minutes for photopic conditions; responses to the 30 Hz flicker stimulus reflect cone system activity nearly exclusively. Ophthalmological examination was performed by Dr. Z.A. Qazi, Dr. M. Amer and Ms Fareeha Zulfiqar at the LRBT/CEMB, Lahore, Pakistan. Informed consent was obtained from all participating individuals, conforming to the tenets of the Declaration of Helsinki. Clinical examinations were carried out at the Lyton Rehmatullah Benevolent Trust hospital, Lahore, Pakistan. This project was approved by the IRBs of the National Eye Institute, Bethesda, MD and the Centre of Excellence in Molecular Biology, Lahore, Pakistan.

Genotyping

Genomic DNA was prepared from white blood cells as previously described[10]. Genotyping for all participating family members was performed using 5'-fluorescein labeled microsatellite markers. A genome-wide scan was carried out using panels 1 to 27 of the ABI PRISM linkage Mapping Set Version 2, which includes 382 markers spaced at intervals of about 10cM. PCR was conducted with an initial denaturing step of 94°C for 8min, followed by 10 cycles of amplification

at 94degC 15s, 55degC 15s, and 72degC 30s; then 20 cycles at 89degC 15s, 55degC 15s, 72degC 30s; and finally an elongation step at 72degC for 10 minutes. After mixing with GENESCAN$^TM^$ 400HD {ROX$^TM^$} standards (ABI) and deionized formamide, PCR products were denatured at 95degC for 5minutes and then immediately placed on ice for 5 minutes. Amplified DNA fragments were separated on Long Ranger sequencing gels (Cambrex) on an ABI 377 DNA sequencer. Genotyping data were collected by using GeneScan Analysis 3.0 and analyzed using Genotyper 2.5 (ABI).

Linkage Analysis

Two-point linkage analysis was performed using the MLINK program of the FASTLINK implementation of the LINKAGE program package[11,12]. CSNB in the family was analyzed as a fully penetrant autosomal recessive trait with a disease allele frequency of 0.0001. For fine mapping, the markers were arranged according to the National Center for Biotechnology Information (NCBI). Haplotypes were generated using the Cyrillic 2.1 program and confirmed by inspection. Marker allele frequencies were arbitrarily set as equal for the genome wide scan and fine mapping. Haplotype frequencies were calculated by genotyping 156 control chromosome from the same ethnic population for haplotype linkage analysis.

Mutation Screening of Candidate Genes

Nine pairs of primers (table 1) were used to amplify the 7 coding exons of GRK1 and their adjacent intron sequences. The amplicons were sequenced with the ABI BigDye Terminator cycle sequencing kit v3.1, according to the manufacturer's instructions, on an ABI 3100 Genetic Analyzer. Sequencing results from affected and unaffected individuals as well as GRK1 consensus sequences from NCBI human genome database were imported into the SeqManII program of the Lasergene package (DNASTAR) and then aligned to identify variations.

Deletion Detection

The inability to amplify exon 3 in affected individuals but not in unaffected family members or controls suggested the possibility of a homozygous deletion of exon 3, especially in view of the consanguinity in this family. Multiplex PCR was used to verify the deletion by combining two pairs of primers, one in exon 3 and other from a 5' (1b) or 3' (6b) exon. In order to define the boundaries of the deletion, a group of additional primers between exon 2 and exon 4 were designed to amplify junctional fragments harboring the deletion. Primers used in deletion detection are listed in table 1(D1, D2, D5, and E3R) and illustrated in figure 4. For primers D1-D5, PCR was conducted at 94°C for 8min, followed by 10 cycles of amplification at 94degC 30s, 68degC 30s, and 72degC 3min; then 30 cycles at 94degC 30s, 65degC 30s, 72degC 3min; and a final incubation at 72degC for 10 minutes. For primer D1-E3R, PCR was conducted at 94degC for 8min, followed by 10 cycles of amplification at 94degC 30s, 68degC 30s, and 72degC 2min; then 30 cycles at 94degC 30s, 65degC 30s, 72degC 2min; and a final incubation at 72degC for 10 minutes. For primers D2-D5, PCR was conducted at 94degC for 8min, followed by 10 cycles of amplification at 94degC 30s, 68degC 30s, and 72degC 30s; then 30 cycles at 94degC 30s, 65degC 30s, 72degC 30s; and a final incubation at 72degC for 10 minutes. PCR products of two patients and a carrier were compared with that of a control sample. PCR fragments harboring deletions were sequenced and analyzed as described above.

Results

Clinical Findings

Although initially referred as a family with arRP, the disease in family members clinically appears to a variant of Oguchi disease without the typical Mizuo-Nakamura phenomenon. The presenting symptom in all three affected individuals is night blindness, apparent by early childhood. Visual

acuity remains 20/20 for affected individuals #11, #13, and #15 at the ages of 19, 16, and 13 years old respectively. The peripheral retinas of all three affected patients show irregular course pigmentation but not gross pigmentary clumping (Figure 1), and a normal caliber of retinal blood vessels, both of which point away from frank retinal structural degeneration.

The fundus appearance before and after 4 hours of dark adaptation is similar except that the peripheral retinal pigmentary changes are slightly less evident after the extended dark adaptation (Figure 1). This could be a variant of the Mizuo-Nakamura phenomenon. The ERG of affected subject #11 (age 19 years) shows slow recovery to nearly normal amplitude after 4 hours in the dark but not after only 30 minutes (Figure 2), and the cone ERG amplitude is essentially normal. This is consistent with a structurally normal retina but with physiologically delayed rod dark-adaptation. Two other younger affected subjects #13 and #15 show minimal or no rod function even after 4 hours dark-adaptation whereas their cone 30 Hz flicker response amplitudes are normal. While reduced rod responses can indicate rod loss, the essentially normal cone ERG amplitudes suggest structural preservation of the retina with dysfunction rather than degeneration.

Molecular Genetic Analysis

The disease locus in this family are excluded from currently known arRP loci as listed in RetNet by examination of affected individuals for homozygosity in closely flanking markers (data not shown). In a genome-wide scan of chromosomes 1 through 22 by two-point linkage analysis, D13S285 yields a lod score of 2.83 with no other marker giving a lod score greater than 1.5. Fine mapping further confirms that this locus lies in an 11.7cM region flanked proximally by D13S1315 and extending distally to 13qter on chromosome 13q34 (Figure 3, table 2). All 4 microsatellite markers examined inside this region generate positive lod scores when analyzed with allele frequencies calculated from 156 control chromosomes. D13S285 shows the highest lod score, 2.89 at $theta$=0, suggestive of linkage to this region.

Haplotypes of markers in this region support the linkage results (Figure 3). Recombination at D13S1315 in individual 13 with an additional recombination event at D13S1265 in individual 11 set the proximal boundary for the linked region. No distal marker shows obligate recombination with the disease, setting the distal boundary of the linked region at the telomeric end of the long arm. This region contains the rhodopsin kinase gene (GRK1), mutations in which have been detected in patients with Oguchi disease[8]. Linkage analysis using the haplotype generated by 4 linked markers (D13S261, D13S285, D13S1295, and D13S293), gives a lod score of 2.90 at $theta$=0 (Table 2), close to the theoretical maximum lod score which could be generated in this type of family.

Sequencing of the 7 exons of the GRK1 gene discloses no sequence changes, but PCR of DNA from affected individuals using primers for exon 3 produces no products. Multiplex PCR of the exon 3 primers together with primers for a proximal or distal exon included as a positive control reveals a deletion involving exon 3 (Figure 4). Long range PCR shows that the deletion extended for 1.1kb. Subsequent sequence analysis of the junctional fragment demonstrates that the deletion starts at the 623rd bp of intron 2 and extends through the 56th bp of exon 3, spanning an 1118bp sequence composed of 1062bp of intron 2 and 56bp of exon 3 (Figures 4 and 5). Thus, the mutation is c.827+623_883del (reference sequence, NM_002929).

Genetic Information Analysis

This deletion is predicted to result in skipping of exon 3 in the GRK1 transcript, producing a prematurely terminated protein ending in 5 novel amino acids, p.Gln277fsX6 with the most likely outcome being nonsense mediated decay of the mutant transcript. Alternately, a cryptic splice acceptor site in the remaining part of exon 3 might be activated through removal of the normal site by this deletion[13]. To test this hypothesis, potential sites in the remaining portion of exon 3 were analyzed for their corresponding information contents using the Automated Splice Site Analysis server at https://splice.cmh.edu[13]. This analysis predicts two potential cryptic sites in

the remaining part of exon 3 at c.893 (2.4 bits) and c.900 (4.7 bits). While the 5' ends of both cryptic sites overlap the deletion, the novel junction sequences from IVS2 do not significantly alter the strengths of these sites (c.893: 2.4 -> 2.4 bits; c.900: 4.7 -> 5.2 bits). In any case, these pre-existing sites are respectively 8.8 bits and 6 bits weaker than the natural exon 3 acceptor site, corresponding to at least a 64 fold decrease (2$^8.8^$ or 2$^6^$) in predicted affinity for these sites[13]. Activation of the c.893 cryptic acceptor site would result in an in-frame deletion of 22 amino acids from the catalytic kinase domain (p.Tyr277_Thr298del), while activation of c.900 cryptic acceptor site would result in a frameshift mutation (p.Ser277fsX85). Thus, neither of these splice sites would be expected to produce a functional protein even if their products were transcribed and translated.

Discussion

The retinal disease in the family described here is a variant form of Oguchi disease. Oguchi disease is a rare autosomal recessive CSNB with unique fundus changes called Mizuo-Nakamura phenomenon. Patients usually claim improvement of light sensitivity when they adapt to a dark environment and a minimal or non-recordable ERG becomes normal after 3-4 hours of dark adaptation. Oguchi disease has been reported to be associated with mutations in SAG and GRK1[3,8,14]. The retinal disease in the family reported here has several features differing from typical Oguchi disease, including an atypical Mizuo-Nakamura phenomenon and a non-recordable rod ERG even after 4 hours of dark adaptation. Normal visual acuity, normal caliber of retinal blood vessels, and normal cone response on ERG recording suggest retinal dysfunction rather than degeneration. It is clear that the "stationary night blindness" conditions (CSNB-NYX and fundus albipunctatus) can both have RPE pigmentary changes. It is hard to explain why two younger affected subjects still showed minimal or non-recordable rod function even after 4 hours dark adaptation.

The variant form of Oguchi disease, inherited as autosomal recessive trait, was mapped to chromosome 13q34 and a novel c.827+623_883del mutation was identified in the GRK1 gene, which lies in this region. A genome-wide scan shows linkage only to the GRK1 region. In addition, characteristics of the mutation and the protein which the mutant gene encodes, as well as previous functional studies of GRK1[15-17] all suggest that this mutation is the cause of the retinal disease in this family. To our knowledge, this is the first retinal disease mapped to the GRK1 locus by linkage analysis, and although the lod score is suggestive it approaches the theoretical maximum obtainable in this small family. The c.827+623_883del variation in GRK1 identified in the family is predicted to result in skipping of exon 3, resulting in synthesis of p.Gln277fsX6, a nonfunctional mutant since the catalytic domain (extending from residues 187-457)[5] would be absent from the mutant protein. However, since the mutant transcript encodes a protein truncated in an internal exon, the most likely outcome would be nonsense mediated decay resulting in minimal or absent mRNA and protein.

Three distinct mutations in GRK1 have been identified in three unrelated cases with Oguchi disease, all of European descent[8]. Two cases have the same homozygous deletion encompassing exon 5 and one case is a compound heterozygote of a missense mutation (c.1139T>A, p.Val380Asp) and a frameshift mutation (c.1607_1610delCGGA, p. Val537fsX543). These 3 null mutations encode proteins with absent or minimal catalytic activity as demonstrated by *in vitro* functional studies[15,16]. Although no mutations of the GRK1 gene have been found in screening 311 unrelated patients with either dominant or recessive RP, heterozygous partial deletions of the GRK1 gene, which might not be uncommon based on this study and a previous report[8], might be missed in routine PCR-SSCP or direct sequencing analysis.

GRK1 is a photoreceptor specific G protein-coupled receptor kinase that is required to inactivate photoactivated rhodopsin. It is localized in rods, cones and pinealocytes in humans[6,7]. Although thus far, mutations in GRK1 have not been associated with RP in humans, mice lacking GRK1 are extremely sensitive to light damage, and retinal degeneration can be induced easily by either

bright light or low-intensity cyclic room light[17,18]. This suggests that mutations in GRK1 might potentially cause retinal degenerative change in humans, especially if they are environmentally exposed to intense light. Consistent with this, mutations in such genes as PDE6B[19,20], RHO[21-23], and SAG[14,24] can cause either retinitis pigmentosa or stationary night blindness. Both GRK1 and SAG participate in the rhodopsin deactivation process and regulate the transducin-dependent apoptotic pathway induced by low light[18].

In summary, a variant form of Oguchi disease in a Pakistani family maps to chromosome 13q34 and is associated with a novel c.827+623_883del mutation in GRK1. To our knowledge, this is the first instance in which association of retinal disease with GRK1 mutation is supported by linkage data. The results also indicate phenotypic variability of retinal diseases caused by GRK1, since previous mutations in this gene have been described in association with Oguchi disease. Expressing the mutant mRNA and analyzing the mutant protein in vitro may help to elucidate the mechanism causing the phenotypic variability.

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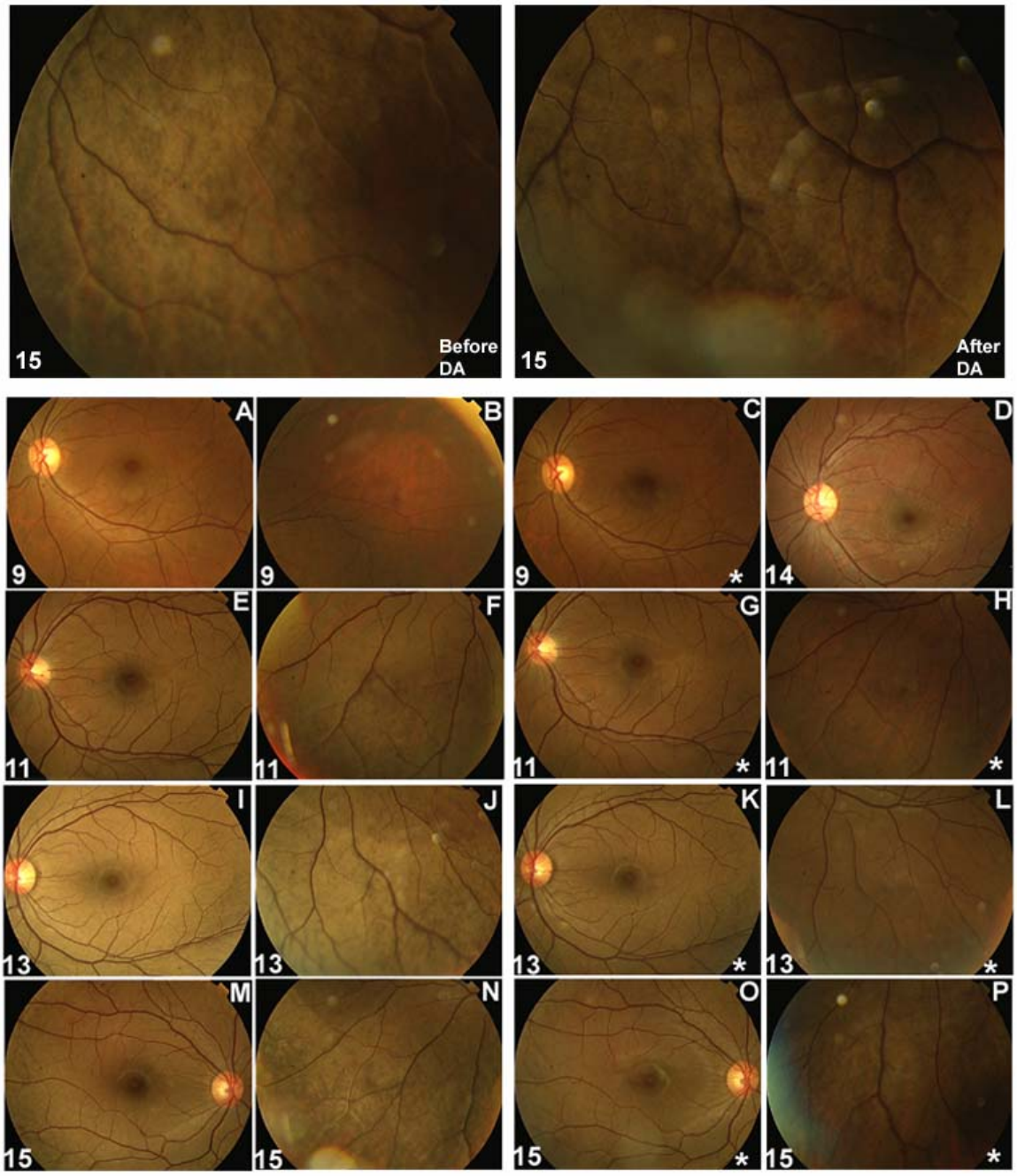

Figure 1.Fundus photos before and after dark adaptation. Fundus photographs from two carriers (No. 9 and 14) and three patients (No. 11, 13, and 15) taken under ordinary light and after 4 hours of dark adaptation (DA). The number on lower left corner represents the individual identification number as shown in pedigree on figure 3. Photos with star (*) on lower right corner were taken immediately after 4 hours of dark adaptation.

Generalized carpet-like retinal degeneration on the peripheral retina before (top left) and after (top right) 4 hours of dark adaptation. Such peripheral retinal changes present in all three affected individuals. Silver-gray or golden-yellow metallic sheen of fundus appearance and retinal vessels standing out in relief against the radiant background were not obvious. There are no obvious changes of color after 4 hours of dark adaptation in affected individuals No. 11 and 15.

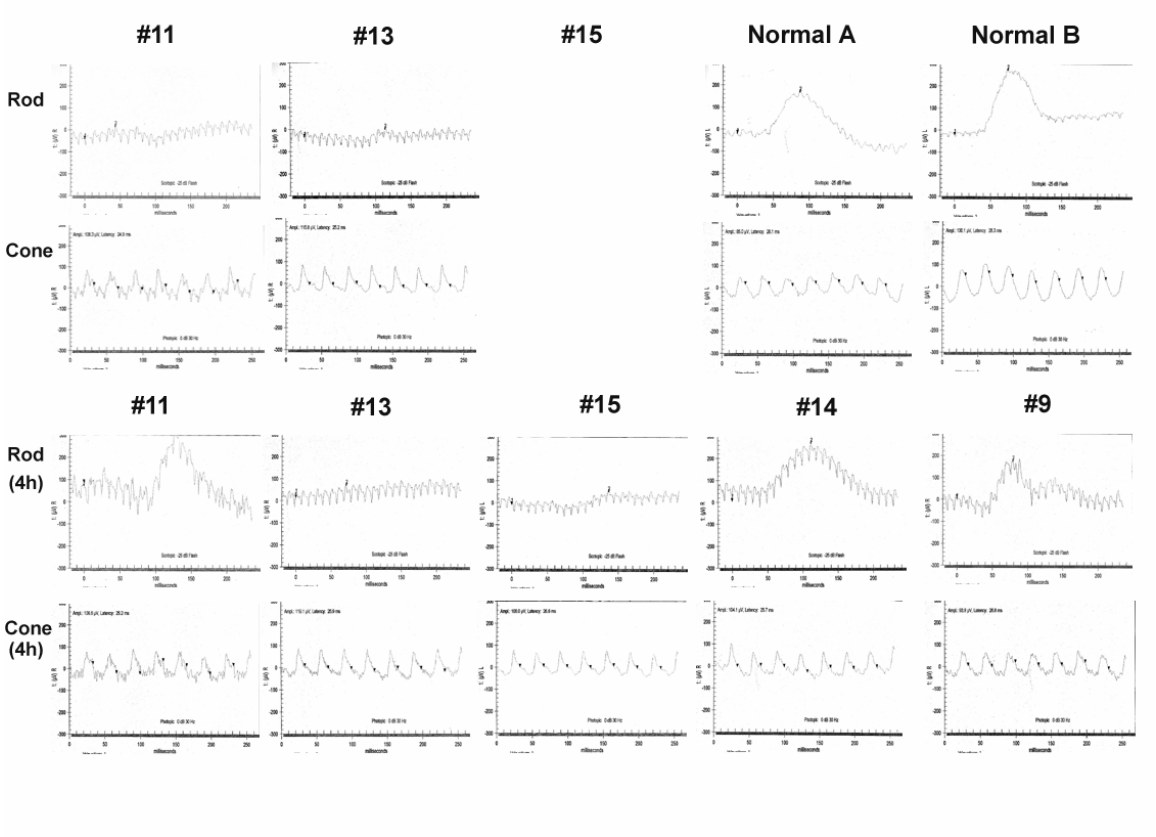

Figure 2. ERG recording under different conditions. ERGs recorded under standard conditions of 30 minutes dark-adaptation for affected family members #11 and #13, and for two normal controls (upper two rows) and after 4 hours of dark adaptation for three affected members (#11, 13, 15) and two carriers (#9, 14). A non-recordable ERG b-wave becomes normal after 4 hours of dark adaptation was observed in a patient (#11) but not in other two affected subjects (#13, 15). Cone 30 Hz flicker responses had essentially normal amplitudes for all three patients.

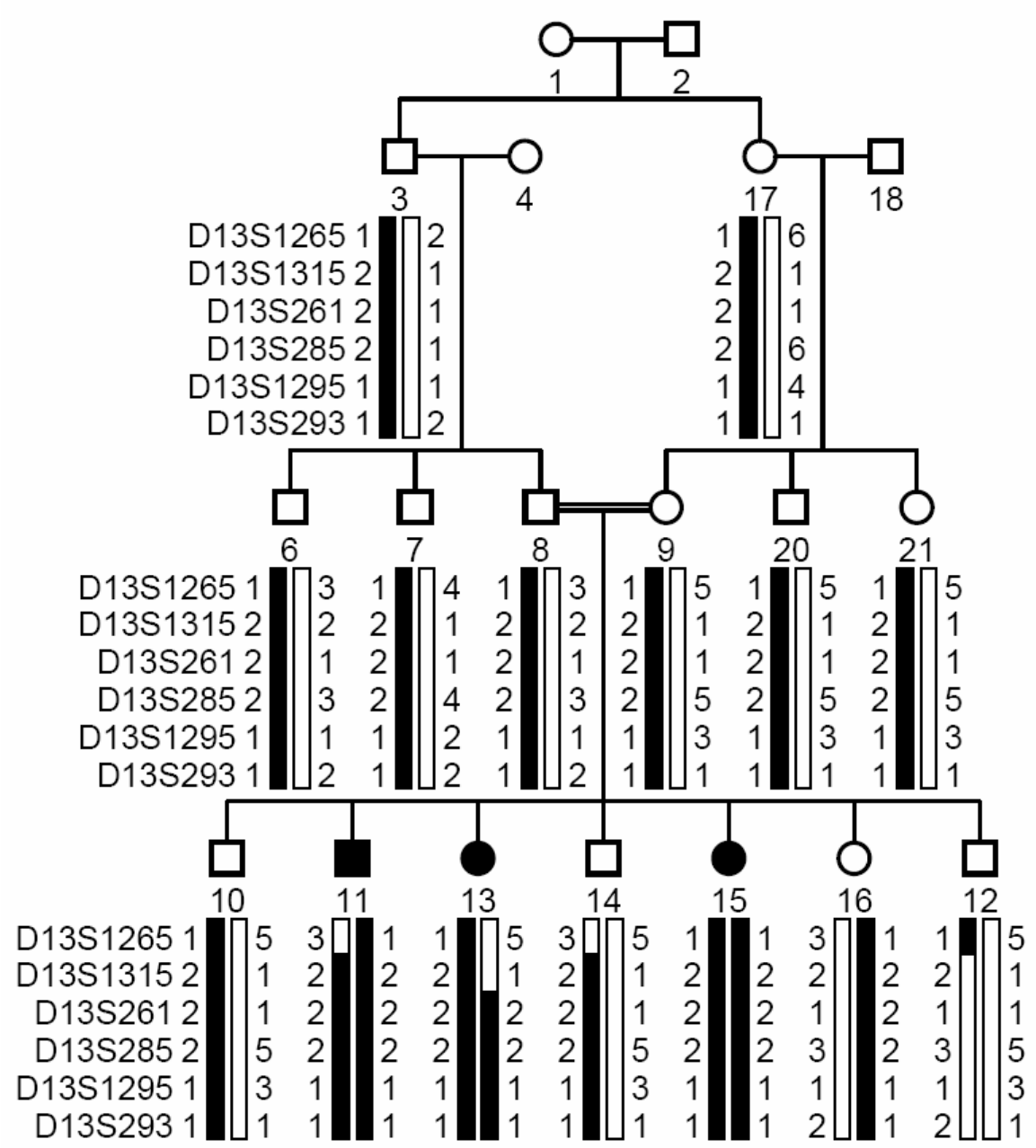

Figure 3. Family pedigree and haplotype diagram. Squares and circles filled with black indicate individuals affected with retinal degeneration. Black bars represent the disease allele inherited from common ancestor. All affected individuals are homozygous for haplotype consisted of D13S261-D13S285-D13S1295-D13S293.

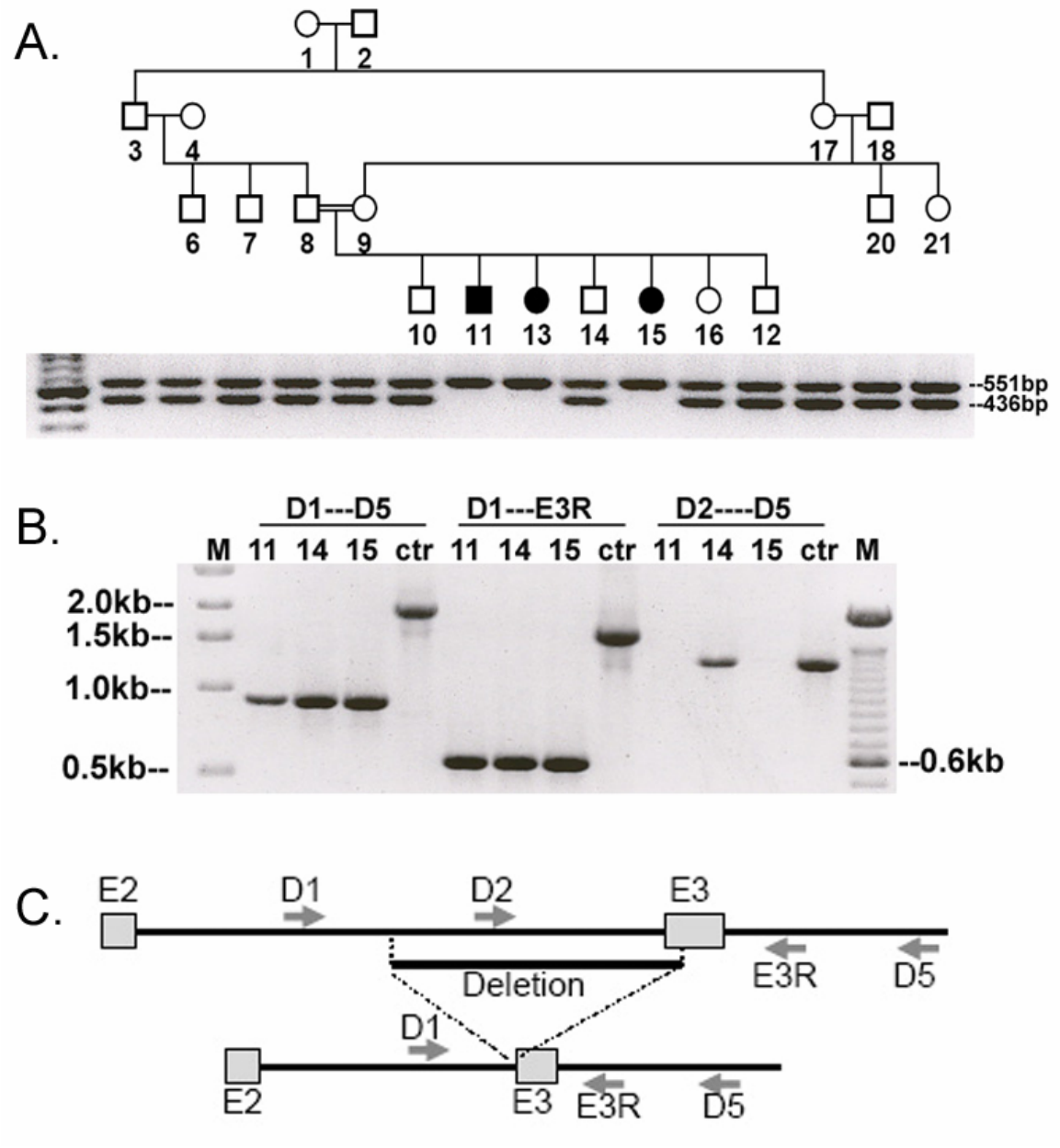

Figure 4. Diagrams show the identification of GRK1 gene partial deletion in the family. A. Absence of exon 3 PCR products in 3 patients was found by multiplex PCR using primers for exon 1b and exon3 as well as exon 6b and exon 3 (data not shown for the later). B. The location of the deletion was narrowed down by various combinations of different primers situated between exon 2 and exon 4. Absence of normal PCR products with presence of a smaller band was found in individuals 11, 14 and 15 by using primer pairs D1-D5 as well as D1-E3R, which indicates a

partial deletion of at least one allele between primers D1 and E3R. Lack of PCR products in individuals 11 and 15 for primers D2-D5 implies a homozygous deletion involving D2 in these patients. Presence of a ~1.5kb band in individual 14 as well as control for primers D2-D5 discloses that individual 14 also had a normal allele apart from an allele with deletion. C. Relative genomic position of the deletion together with primers for identifying the deletion (not to scale).

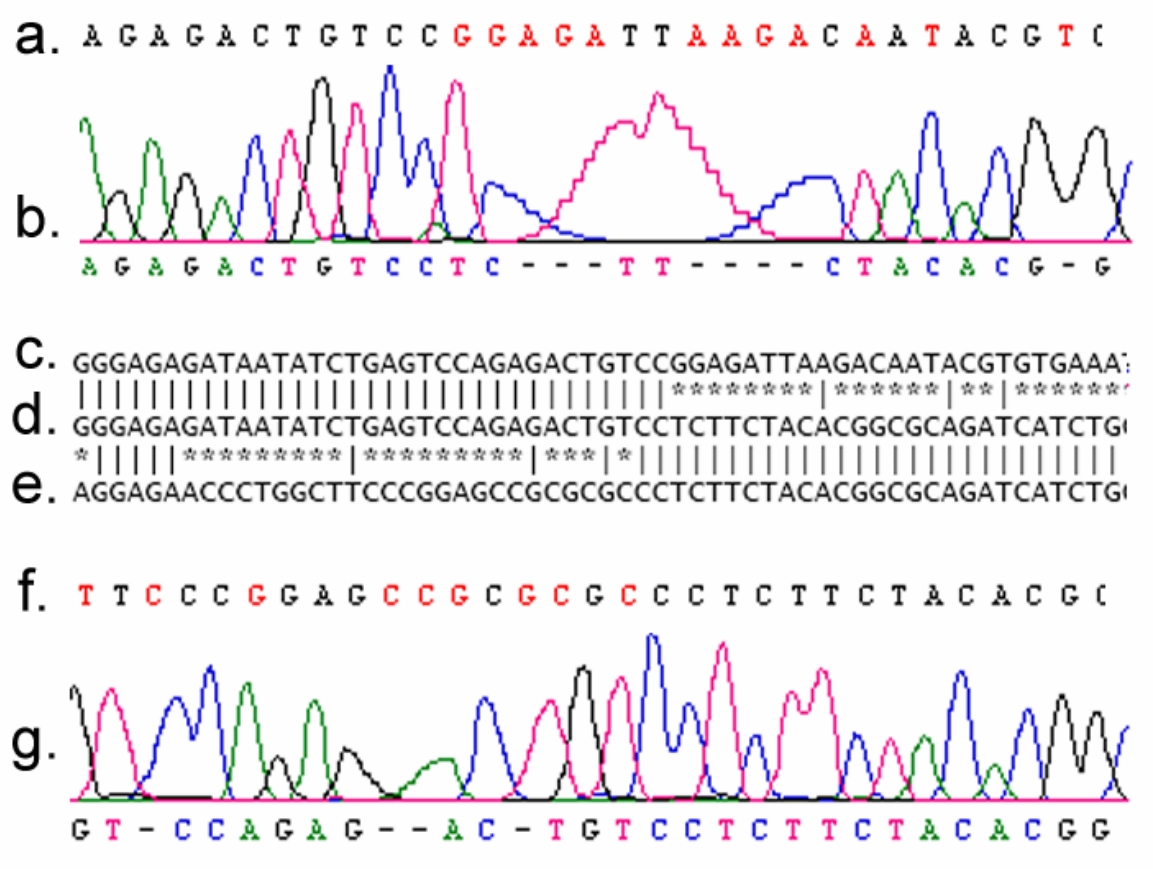

Figure 5. Sequence of the GRK1 mutation. Result of direct cyclic sequencing of the smaller band resulted from primers D1-E3R shown in figure 4. Bidirectional sequencing revealed a homozygous 1118bp deletion in individuals 11, 13 and 15 involving 1062bp intron 2 and 56bp exon 3. a and b: Align of patient sequence (b) with 5' breakpoint normal sequence (a). c, d, and e: Comparison of 5'breakpoint sequence (c), patient sequence (d), and 3'breakpoint sequence (e). Vertical lines indicate identical bases between two adjacent sequences and asterisk represent different bases between two adjacent sequences. f and g: Align of patient sequence (g) with 3' breakpoint normal sequence (f).

Table 1. Primers for sequencing and deletion detection

Name	sequence	Tm	PCR length
RHOK-E1AF	5'-CAGGCCAAGGGCAGCAGTCAGG-3'		
RHOK-E1AR	5'-TTCGCCACTATCCCCTCATCCA-3'	61.8	497 bp
RHOK-E1BF	5'-CGGCCCTGGAGCTCTGGAAAGA-3'		
RHOK-E1BR	5'-TAAGGGCCCCGGAGCCGACAGT-3'	63.5	551 bp
RHOK-E2F	5'-TGCGACTGCTCCGTGGCTGTG-3'		
RHOK-E2R	5'-AGCGAGAAGGGGATTGTGATGG-3'	60.6	394 bp
RHOK-E3F	5'-TGGCCTTCGGGTGTCCTCTGC-3'		
RHOK-E3R	5'-ATGAACCCCCACCGTCCACAC-3'	62	436 bp
RHOK-E4F	5'-CGCCTGTCCTGTGCAGCCAG-3'		
RHOK-E4R	5'-GTAACCCATGCACGGAGCCT-3'	63	about 150 bp
RHOK-E5F	5'-GCATCCCCAGAGCATCAGTCC-3'		
RHOK-E5R	5'-CCACCCCAGGGCCCACAGAACAA-3'	61	339 bp
RHOK-E6AF	5'-GAGGCGGGTCTGGCAGGGCTAAG-3'		
RHOK-E6AR	5'-GGGCCTTGCTGGGGGCAGAG-3'	63.6	387 bp
RHOK-E6BF	5'-TTCTGCGAGGCGCTGCTGGAGA-3'		
RHOK-E6BR	5'-GCGCACATAGTATCTTCCGCATCC-3'	62.7	524 bp
RHOK-E7F	5'-CCATGGGGGAGGGGGCTTTTTG-3'		
RHOK-E7R	5'-GGAGATGGCGTGATGGGGACCTG-3'	62.8	519 bp
RHOK-D1	5'-GTGGTGCTGTGGGGGACGTGTGATGC-3'		
RHOK-D2	5'-AGGCGGAGGGGAGGGGACATTGGGAAGT-3'		
RHOK-D5	5'-TGACGGTGGAGGGCGCAGGGTGTAG-3'		

Table 2. Two-point linkage results for makers around the RP locus on chromosome 13q34

	Position		Lod score at θ =								
Markers	cM	Mb	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z_{max}	θma
D13S1265	101.70	?	-100.00	-1.22	-0.01	0.35	0.48	0.38	0.21	0.48	0.1
D13S1315	105.20	108.04	-100.00	-0.67	-0.08	0.08	0.11	0.06	0.01	0.12	0.1
D13S261	112.70	109.72	2.48	2.43	2.20	1.92	1.34	0.78	0.30	2.48	0.0
D13S285	112.80	110.74	2.83	2.77	2.55	2.26	1.68	1.11	0.56	2.83	0.0
D13S1295	112.90	111.04	1.36	1.33	1.22	1.09	0.82	0.56	0.29	1.36	0.0
D13S293	117.50	112.23	0.76	0.74	0.65	0.55	0.35	0.17	0.05	0.76	0.0
*D13S285			2.89	2.83	2.60	2.31	1.72	1.13	0.56	2.89	0.0
#Haplotype			2.90	2.85	2.62	2.32	1.73	1.14	0.56	2.90	0.0

* Disease allele (2=111bp) for D13S285 was detected in 4/154 (0.026) control chromosomes.

Haplotype 2211 for D13S261, D13s285, d13s1295,d13s293. This haplotype allele frequency

accounts for 1/156 (0.0064) control chromosomes.