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

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\textbf{Purpose:} The purpose of this paper is to map the locus for a variant form of Oguchi disease in a Pakistani family and to identify the causative mutation.

\textbf{Methods:} 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. 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 10 cM intervals, and two-point lod scores were calculated. Polymerase chain reaction (PCR) cycle dideoxynucleotide sequencing was used to screen candidate genes inside the linked region for mutations and to delineate the deletion. Multiplex PCR and long template PCR were used to detect deletions and to define the size of deletions. Evaluation of fundus changes and ERG, lod score estimation, and identification of a mutation in the \textit{GRK1} gene were carried out.

\textbf{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 h of dark adaptation was 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 h 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 θ=0 shown by D13S261-D13S285-D13S1295-D13S293 haplotype. Analysis of the \textit{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. This mutation was not detected in 96 controls.

\textbf{Conclusions:} 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 h 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 (i.e., a variant form of Oguchi disease but unlikely to be retinitis pigmentosa). The disease in the Pakistani family localizes to 13q34 and is caused by a novel deletion including Exon 3 of the \textit{GRK1} gene.

Hereditary retinal diseases represent a broad range of retinal dysfunction and 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 choroidoretinal atrophy. Over one hundred genes have been reported to be associated with these diseases (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 undetectable 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, including most sporadic cases [2]. Autosomal recessive RP has been mapped to 21 loci and causative mutations have been identified in at least 17 genes (RetNet). However, mutations in each gene identified so far have been responsible for autosomal recessive retinitis pigmentosa (arRP) in less than 2-5% of families. Overall, causative mutations have been identified in a fraction of arRP families, probably around 30% (Dr. Stephen Daiger, University of Texas, Houston, TX, 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., the Mizuo-Nakamura phenomenon in Oguchi disease, white dots in fundus albipunctatus, or typical changes in high myopia) [3]. The 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, can be observed. A prolonged dark adaptation of 3 h or more leads to disappearance of such changes. So far, mutations in RHO, GRK1, and PDE6B have been associated with autosomal dominant CSNB, mutations in SAG, GRK1, and 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, and fundus albipunctatus, resulting from mutations in RDH5, have typical fundus changes.

The human GRK1 (G protein-coupled receptor kinase 1) gene, also called RHO, is located on chromosome 13q34 and consists of seven exons encoding 563 amino acids [5,6]. It encodes rhodopsin kinase, which desensitizes rod photoreceptors to light, inactivating photoreactivated 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 characterized a consanguineous Pakistani family with individuals affected by nightblindness and retinal pigmentary changes and mapped the disease in this family to chromosome 13q34. Sequencing of GRK1 identified a novel deletion encompassing 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 Center of Excellence in Molecular Biology (CEMB), Lahore, Pakistan and the National Eye Institute (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 standards of International Society for Clinical Electrophysiology of Vision (ISCEV) [10] on selected affected members of this family, using commercial ERG equipment (LKC Inc., Gaithersberg, MD). Stimulus intensities were -25 dB for scotopic and 0 dB 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 min dark-adaptation or 4 h 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 5 min for photopic conditions, and 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 Lyton Rehmatullah Benevolent Trust (LRBT) Hospital/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 LRBT Hospital, Lahore, Pakistan. This project was approved by the IRBs of the NEI, Bethesda, MD and the CEMB, Lahore, Pakistan.

Genotyping: Genomic DNA was prepared from white blood cells as previously described [11]. Genotyping for all

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participating family members was performed using 5’-fluorescein labeled microsatellite markers. A genome-wide scan was conducted using panels 1 to 27 of the ABI PRISM linkage Mapping Set Version 2, which includes 382 markers spaced at intervals of about 10 cM. PCR was conducted with an initial denaturing step of 94 °C for 8 min, followed by 10 cycles of amplification at 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s; then 20 cycles at 89 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; and finally an elongation step at 72 °C for 10 min. After mixing with GENESCAN™ 400 HD ROX™ standards (ABI, Foster City, CA) and deionized formamide, PCR products were denatured at 95 °C for 5 min and then immediately placed on ice for 5 min. Amplified DNA fragments were separated on Long Ranger sequencing gels (Cambrex Bio Science, Rockland, ME) 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 (version 5.1, available from the Ott laboratory) [12,13]. 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 chromosomes 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 seven coding exons of GRK1 and their adjacent intronic 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 and GRK1 consensus sequences from the NCBI human genome database were imported into the SeqManII program of the Lasergene

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**Figure 1.** Diagrams for identification of GRK1 deletion. A: Absence of Exon 3 PCR products in three patients was found by multiplex PCR using primers for Exon 1b and Exon 3 as well as Exon 6b and Exon 3 (data not shown for the latter). The first lane in the gel image is the size standards of 100 bp ladder where the heavy band indicates 500 bp. Other lanes represent the results from the nearest individuals immediately above that lane. B: The location of the deletion was narrowed down by various combinations of different primers situated between Exon 2 and Exon 4. Ctr represents normal controls. The results showed that affected individuals 11 and 15 have a homozygous deletion. Unaffected individual 14 has a heterozygous deletion so that he is a carrier. Absence of normal PCR products and addition of a smaller band were found in individuals 11, 14, and 15 by using primer pairs D1-D5 and D1-E3R. This indicates a partial deletion of at least one allele between primers D1 and E3R. The lack of PCR products in individuals 11 and 15 with primers D2-D5 implies a homozygous deletion involving D2 in these patients. The presence of a 1.5 kb band in individual 14 and in a normal control subject (ctr) for primers D2-D5 indicates that individual 14 also has a normal allele apart from an allele with a deletion. Hence, individual 14 is an unaffected heterozygous carrier. C: The relative genomic position of the deletion is illustrated together with the primers used to identify the bounds of the deletion (not to scale).
Figure 2. Fundus photographs before and after dark adaptation. Fundus photographs from two carriers (numbers 9 and 14) and three patients (numbers 11, 13, and 15) taken under ordinary light and after 4 h of dark adaptation (DA). The number on lower left corner represents the individual identification number as shown in pedigree on Figure 4. Photos with an asterisk (*) on the lower right corner were taken immediately after 4 h of dark adaptation. Generalized carpet-like retinal degeneration on the peripheral retina before (top left) and after (top right) 4 h of dark adaptation. Such peripheral retinal changes presented in all three affected individuals. A silver-gray or golden-yellow metallic sheen of the fundus or retinal vessels standing out in relief against a radiant background was not obvious in any of the patients. There were no obvious changes of color after 4 h of dark adaptation in affected individuals number 11 or 15.
package (DNASTAR Inc., Madison, WI) 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 this 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 1. For primers D1-D5, PCR was conducted at 94 °C for 8 min, followed by 10 cycles of amplification at 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 3 min; then 30 cycles at 94 °C for 30 s, 65 °C 30 s, 72 °C 3 min; and a final incubation at 72 °C for 10 min. For primer D1-E3R, PCR was conducted at 94 °C for 8 min, followed by 10 cycles of amplification at 94 °C for 30 s, 68 °C for 30 s, and 72 °C 2 min; then 30 cycles at 94 °C for 30 s, 65 °C for 30 s, 72 °C for 2 min; and a final incubation at 72 °C for 10 min. For primers D2-D5, PCR was conducted at 94 °C for 8 min, followed by 10 cycles of amplification at 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 3 min; then 30 cycles at 94 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s; and a final incubation at 72 °C for 10 min. 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 appeared to be 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 number 11, number 13, and number 15 at the ages of 19, 16, and 13 years, respectively. The peripheral retinas of all three affected patients showed irregular course pigmentation but not gross pigmentary clumping (Figure 2), 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 h of dark adaptation was similar except that the peripheral retinal pigmentary changes were slightly less evident after the extended dark

Figure 3. ERG recording under different conditions. ERGs were recorded under standard conditions of 30 min dark-adaptation for affected family members number 11 and 13, and two normal controls (upper two rows) and after 4 h of dark adaptation for three affected members (number 11, 13, 15) and two carriers (number 9, 14). A non-recordable ERG b-wave becoming normal after 4 h of dark adaptation was observed in a patient (number 11) but not in other two affected subjects (number 13, 15). Cone 30 Hz flicker responses had essentially normal amplitudes for all three patients.
adaptation (Figure 2). This could be a variant of the Mizuo-Nakamura phenomenon. The ERG of affected subject number 11 (age 19 years) showed slow recovery to nearly normal amplitude after 4 h in the dark but not after only 30 min (Figure 3), and the cone ERG amplitude was essentially normal. This is consistent with a structurally normal retina but with physiologically delayed rod dark-adaptation. Two other younger affected subjects, numbers 13 and 15 showed minimal or no rod function even after 4 h dark-adaptation whereas their cone 30 Hz flicker response amplitudes were normal. While reduced rod responses can indicate rod loss, the essentially normal cone ERG amplitudes suggested structural preservation of the retina with dysfunction rather than degeneration.

Molecular genetic analysis: The disease locus in this family were 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 yielded a lod score of 2.83 with no other marker giving a lod score greater than 1.5. Fine mapping further confirmed that this locus lays in an 11.7 cM region flanked proximally by D13S1315 and extending distally to 13qter on chromosome 13q34 (Figure 4, Table 2). All four microsatellite markers examined inside this region generated positive lod scores when analyzed with allele frequencies calculated from 156 control chromosomes. D13S285 showed the highest lod score, 2.89 at θ=0, suggestive of linkage to this region.

The haplotypes of markers in this region supported the linkage results (Figure 4). 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 showed obligate recombination with the disease, setting the distal boundary of the linked region at the telomeric end of the long arm of chromosome 13. 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 four linked markers (D13S261, D13S285, D13S1295, and D13S293), gave a lod score of 2.90 at θ=0 (Table 2), close to the theoretical maximum lod score that could be generated in this type of family.

Sequencing of the seven 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 1). Long range PCR shows that the deletion extended for 1.1 kb. Subsequent sequence analysis of the junctional fragment demonstrates that the deletion starts at position 623 in Intron 2 and extends through the position 56 of Exon 3, spanning an 1118 bp sequence composed of 1062 bp of Intron 2 and 56 bp of Exon 3 (Figure 1, Figure 5). Thus, the mutation is c.827+623_883del (using NM_002929 as the reference sequence).

**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 five novel amino acids, p.Gln277fsX6 with the most likely outcome being nonsense mediated decay [14] 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 [15]. 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 Children’s Mercy Hospital [15,16]. 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 (unchanged); c.900: 4.7 ->5.2 bits, which strengthens this site by about 1.4 fold). In any case, these pre-existing sites are, The asterisk represents the disease allele (2=111 bp) for D13S285, which was detected in four of 154 (0.026) control chromosomes. The double asterisks denote haplotype 2211 of the D13S261, D13S285, D13S1295, and D13S293 region. The incidence of one allele in 156 control chromosomes accounts for the haplotype allele frequency of 0.0064.

![Figure 5. Sequence of the GRK1 mutation. Results of direct cycle sequencing are illustrated for the smaller band resulting from primers D1-E3R shown in Figure 1. Bi-directional sequencing revealed a homozygous 1118 bp deletion in individuals 11, 13, and 15 involving 1062 bp from Intron 2 and 56 bp from Exon 3. A,B: An alignment of a patient sequence (B) is shown with the 5' breakpoint normal sequence (A). The red bases in A as well as in F are generated by the SeqMan program and highlighted the unmatched or mismatched bases. C-E: Comparison of the 5' breakpoint sequence (C), patient sequence (D), and 3' breakpoint sequence (E) is depicted. Vertical lines indicate identical bases between two adjacent sequences and asterisks represent different bases between two adjacent sequences. F,G: An alignment of a patient sequence (G) with the 3' breakpoint normal sequence (F) is indicated.](http://www.molvis.org/molvis/v11/a117/)
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 or 2^6) in predicted affinity for these sites [15]. 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 phenomena. 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 h of dark adaptation. Oguchi disease has been reported to be associated with mutations in SAG and GRK1 [3,8,17]. 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 h 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 h 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, the protein which the mutant gene encodes, and previous functional studies of GRK1 [18-20] 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.

Overall, it seems highly likely that exon 3 is skipped. In Rogan et al. [15], we found that weak splice sites with low RI values were more susceptible to complete inactivation than strong sites (we define as Ri <Rsequence). Cryptic sites are more likely to be activated in those instances where the natural splice site is weak. Small changes in information at strong sites are more likely to be benign (ΔRI ≥1-2 bits), unless there are neighboring cryptic sites of similar strength (which would be activated by such mutations). However, a large decrease (about 5 bits) in the strength of a strong site is predicted to affect the affinity by 32 fold, so the relative weakness of the mutant splice site would be expected to significantly affect splice site recognition. The predictive accuracy of this approach has been validated using approximately 1300 mutations, although not all of these mutations were splicing mutations [16]. Specific examples of this approach can be found at Rogan’s lab site and Children’s Mercy Hospital.

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 three null mutations encode proteins with absent or minimal catalytic activity as demonstrated by in vitro functional studies [18,19]. 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 [20,21]. 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 [22,23], RHO [24-26], and SAG [17,27] 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 [21].

In summary, a variant form of Oguchi disease in a Pakistani family mapped to chromosome 13q34 and was 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 indicated 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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