NIH Public Access
Author Manuscript
Br J Ophthalmol. Author manuscript; available in PMC 2012 July 11.

Published in final edited form as:
Br J Ophthalmol. 2010 August ; 94(8): 1094–1099. doi:10.1136/bjo.2009.171892.

Nonsense mutation in *MERTK* causes autosomal recessive retinitis pigmentosa in a consanguineous Pakistani family

Amber Shahzadi[1], **S Amer Riazuddin**[1], **Shahbaz Ali**[1], **David Li**[2], **Shaheen N Khan**[1], **Tayyab Husnain**[1], **Javed Akram**[3], **Paul A Sieving**[2], **J Fielding Hejtmancik**[2], and **Sheikh Riazuddin**[1,3]

[1]National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

[2]Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA

[3]Allama Iqbal Medical College, University of Health Sciences, Lahore, Pakistan

Abstract

Background—Retinitis pigmentosa (RP) is one of the most common ophthalmic disorders affecting one in approximately 5000 people worldwide. A nuclear family was recruited from the Punjab province of Pakistan to study the genetic basis of autosomal recessive RP.

Methods—All affected individuals underwent a thorough ophthalmic examination and the disease was characterised based upon results for fundus photographs and electroretinogram recordings. Genomic DNA was extracted from peripheral leucocytes. Exclusion studies were performed with short tandem repeat (STR) markers flanking reported autosomal recessive RP loci. Haplotypes were constructed and results were statistically evaluated.

Results—The results of exclusion analyses suggested that family PKRP173 was linked to chromosome 2q harbouring mer tyrosine kinase protooncogene (*MERTK*), a gene previously associated with autosomal recessive RP. Additional STR markers refined the critical interval and placed it in a 13.4 cM (17 Mb) region flanked by D2S293 proximally and D2S347 distally. Significant logarithm of odds (LOD) scores of 3.2, 3.25 and 3.18 at θ=0 were obtained with markers D2S1896, D2S2269 and D2S160. Sequencing of the coding exons of *MERTK* identified a mutation, c.718G→T in exon 4, which results in a premature termination of p.E240X that segregates with the disease phenotype in the family.

Conclusion—Our results strongly suggest that the nonsense mutation in *MERTK*, leading to premature termination of the protein, is responsible for RP phenotype in the affected individuals of the Pakistani family.

INTRODUCTION

Retinitis pigmentosa (RP) is a group of retinopathies involving progressive photoreceptor degeneration.[1] Patients experience night blindness, constriction of peripheral visual field in

Correspondence to Dr Sheikh Riazuddin, National Centre of Excellence in Molecular Biology, 87 West Canal Bank Road, Lahore 53700, Pakistan; riaz@aimrc.org.

AS, SAR, JFH and SR contributed equally to this study.

Competing interests None.

Patient consent Obtained.

Ethics approval Institutional Review Board approval was obtained for this study from the Centre of Excellence in Molecular Biology (CEMB) (Lahore, Pakistan) and the National Eye Institute (Bethesda, Maryland, USA).

Provenance and peer review Not commissioned; externally peer reviewed.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

the early stages and complete vision loss at later stage due to melanin-containing bone spicule formation on the fundus. Vascular attenuation and waxy pallor of the optic disc are other characteristic features of RP.[2] Epidemiological studies estimate that 50–60% cases of non-syndromic familial RP are inherited in an autosomal recessive fashion.[3] To date 29 genes have been identified with mutations known to cause non syndromic autosomal recessive RP (http://www.sph.uth.tmc.edu/Retnet). Most of these genes are expressed in photoreceptors or retinal pigment epithelium and thus are directly involved in photo-transduction cascade or in photoreceptor and retinoid metabolism.[4]

Mer tyrosine kinase protooncogene (*MERTK*) belongs to receptor tyrosine kinase family of cell surface receptors. The gene consist of 19 exons encoding a 999 amino acid protein expressed in retinal pigment epithelium (RPE).[5] *MERTK* was identified due to the widespread retinal degeneration resembling RP in a large family harbouring a partial deletion of the gene.[6] After its first association with arRP, three mutations, IVS10-2A→G, R651X and 2070delAGGAC, were identified in patients affected with arRP.[7] Subsequently three additional novel sequence variations, R722X, R844C and R865W, causing severe retinal dystrophy in a single patient, were reported.[5] Recently, a splice donor site mutation in intron 16 has been implicated in rod–cone dystrophy and arRP.[89] Altogether seven mutations in *MERTK* have been associated with arRP.

Here, we report a consanguineous Pakistani family diagnosed with arRP. Exclusion analyses localised the critical interval to chromosome 2q and bi-directional sequencing identified a nonsense mutation in *MERTK* that segregates with disease phenotype in the family. These results strongly suggest that a nonsense mutation in *MERTK* is responsible for the disease phenotype in this family.

MATERIALS AND METHODS

Seventy-five consanguineous Pakistani families with non-syndromic RP were recruited to participate in a collaborative study for identification of retinal disease causing loci between the National Centre of Excellence in Molecular Biology (NCEMB), Lahore, Pakistan and the National Eye Institute (NEI) Bethesda MD, USA. The family described in this study is from the Punjab province of Pakistan. A detailed medical history was obtained by interviewing family members. All of the ophthalmological examinations were completed at either the Layton Rahmatulla Benevolent Trust (LRBT) hospital or at NCEMB, Lahore, Pakistan. Fundus photographs were acquired by a camera manufactured by Fuji (Tokyo, Japan). Electroretinogram (ERG) responses were recorded using ERG equipment manufactured by LKC (Gaithersburg, Maryland, USA). Scotopic responses were recorded under dark-adapted conditions using a single bright flash stimulus; photopic responses were recorded under light-adapted conditions using a 30 Hz flicker stimulus. Blood samples were collected from affected and unaffected family members. DNA was extracted by a non-organic method as described previously.[10]

Genotype analysis

Multiplex PCR were completed in GeneAmp 9700 PCR System (Applied Biosystems, Foster City, California, USA). Briefly, each reaction was carried out in a 5 μl mixture containing 40 ng genomic DNA, various combinations of 10 μM fluorescently labelled primer pairs, 0.5 μl 10× PCR Buffer (Applied Biosystems), 1 mM dNTP mix, 2.5 mM $MgCl_2$ and 0.2 U of Taq DNA polymerase (Applied Biosystems). Initial denaturation was carried out for 5 min at 95°C, followed by 10 cycles of 15 s at 94°C, 15 s at 55°C and 30 s at 72°C and then 20 cycles of 15 s at 89°C, 15 s at 55°C and 30 s at 72°C. The final extension was performed for 10 min at 72°C and followed by a final hold at 4°C. PCR products from each DNA sample were pooled and mixed with a loading cocktail containing HD-400 size

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

standards (Applied Biosystems). The resulting PCR products were separated in an ABI3100 DNA sequencer and analysed by using GENESCAN 4.0 software package (Applied Biosystems).

Linkage analysis

Two point linkage analyses were performed using the FASTLINK version of MLINK from the LINKAGE Program Package.[1112] Maximum logarithm of odds (LOD) scores were calculated. using ILINK. arRP was analysed as a fully penetrant trait with an affected allele frequency of 0.001. The marker order and distances between the markers were obtained from the Marshfield database (http://research.marshfieldclinic.org/) and the National Center for Biotechnology Information chromosome 2 sequence maps (http://www.ncbi.nlm.nih.gov). For the initial genome scan equal allele frequencies were assumed, while for fine mapping allele frequencies were estimated from 96 unrelated and unaffected individuals from the Punjab province of Pakistan.

Mutation screening

Primer pairs for individual exons were designed using the primer3 program (http://primer3.sourceforge.net/). The primer sequences and annealing temperatures are available upon request. Amplifications were performed in 25 μl reaction containing 50 ng of genomic DNA, 400 nM, of each primer, 250 μM dNTPs, 2.5 mM $MgCl_2$ and 0.2 U Taq DNA polymerase in the standard PCR buffer provided by the manufacturer (Applied Biosystems). PCR amplification consisted of a denaturation step at 96°C for 5 min, followed by 40 cycles, each consisting of 96°C for 45 s followed by 57°C (or primer set specific annealing temperature) for 45 s and at 72°C for 1 min. PCR products were analysed on 2% agarose gel, precipitated and purified by ethanol precipitation. The PCR primers for each exon were used for bidirectional sequencing using Big Dye Terminator Ready reaction mix according to manufacturer instructions (Applied Biosystems). Sequencing products were re-suspended in 10 μl of formamide (Applied Biosystems) and denatured at 95°C for 5 min. Sequencing was performed on an ABI PRISM 3100 Automated sequencer (Applied Biosystems). Sequencing results were assembled with ABI PRISM sequencing analysis software version 3.7 and analysed using SeqScape software (Applied Biosystems).

RESULTS

PKRP173 was recruited from the Punjab province of Pakistan. A detailed medical and family history was obtained by interviewing family members. Three affected and one unaffected individual underwent detailed ophthalmic examination. According to medical records available to us, all affected individuals started experiencing visual difficulties during the first decade of their life; at the time of examination vision was reduced only to perception of light (table 1). Fundus examination revealed marked vascular attenuation, and bony spicules in peripheral regions of the retina combined with atrophic maculopathy with pigment dispersion (figure 1). ERG recordings at 0 dB (scotopic) showed no rod and cone response. Isolated cone responses measured at 30 Hz flicker (photopic) were absent (figure 2), illustrating an advanced stage of retinopathy encompassing both rod and cone cells of the retina. Retinal attributes and ERG recording in the unaffected individual 12 were completely normal (figures 1 and 2)

Exclusion studies were performed with closely linked short tandem repeat (STR) markers for 23 reported arRP loci (data not shown). Haplotypes were constructed to investigate homozygous regions shared by all the affected individuals of the family. PKRP173 was found linked to markers D2S1896, D2S2269 and D2S160 at chromosome 2q14.1 flanking *MERTK* (figure 3). All the affected individuals were homozygous for D2S1896, D2S2269

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and D2S160. Additional STR markers D2S2264, D2S293 and D2S347 were designed to define the proximal and distal boundaries. As shown in figure 3, there is proximal recombination in affected individual 18 at D2S293. Similarly, there is a distal recombination in affected individuals 10, 11 and 18 at D2S347. Taken together, this places the disease interval in the 13.4 cM (17 Mb) region flanked by markers D2S293 proximally and D2S347 distally. These results were statistically evaluated by investigating the probabilities of linkage to markers on chromosome 2q; two point LOD scores of 3.2, 3.25 and 3.18 at $\theta=0$ were obtained with markers D2S1896, D2S2269 and D2S160 (table 2).

The critical interval harbours *MERTK*, a gene previously associated with arRP. All coding exons and exon–intron boundaries were sequenced to identify any and all sequence variations present in the affected individuals. We identified six sequence variations including two missense variations (Arg466Lys and Ile587Val), a non-synonymous variation (Ser627Ser), two non-coding intronic variations (IVS6-46G→A, IVS15-11C→A), and most importantly a homozygous c.718G→T substitution in exon 4. All affecteds were homozygous, whereas unaffected individuals were heterozygous carriers for this change (figure 4a). This mutation introduces a premature termination at amino acid 240 and was not present in 96 ethnically matched control samples.

DISCUSSION

Here, we report association of arRP phenotype in a consanguineous Pakistani family with *MERTK*, a gene previously associated with arRP. Ophthalmological examination of affected individuals revealed classical features of RP, including arteriolar attenuation, pigmentation on peripheral and mid peripheral regions of retina and optic disc pallor. Exclusion analyses indentified chromosome 2q as the critical interval harbouring *MERTK* and subsequent sequential analyses identified a nonsense mutation segregating with disease phenotype in PKRP173. Linkage to chromosome 2q14.1,presence of a nonsense mutation in gene previously associated with arRP, segregation of the mutation with the disease phenotype in the family, along with absence of the variation in ethnically matched controls strongly suggest that this mutation is responsible for RP phenotype in PKRP173.

To date seven mutations in *MERTK* causing arRP have been described for Caucasian, mid-eastern and Spanish families.[5,7,8] Of particular interest is a single base deletion, c.2214delT, causing frame shift and leading to a premature after-addition of 31 novel amino acids.[13] The clinical evaluation includes bull's eye maculopathy and absence of pigmentation for individuals homozygous for the deletion mutation.[13] In our study the pathogenic mutation would be expected to be degraded by a nonsense-mediated decay (NMD) mechanism, leading to complete ablation of the mutant mRNA. If even somehow the transcript evades NMD, the protein thus synthesised would lack functionally important domains, namely fibronectin and tyrosine kinase domains (figure 4b). The more severe phenotype in affected individuals of PKRP173 comprising of extensive pigmentation in peripheral and mid-peripheral region of retina coupled with macular atrophy might be due to the relatively older age of examination.

In conclusion, a nonsense mutation in *MERTK* has been identified as a molecular defect responsible for RP phenotype in a consanguineous Pakistani family. To the best of our knowledge this is the first report of mutation in immunoglobulin domain of *MERTK*.This and other pathogenic mutations thus identified will help us better understand the pathphysiology of RP at a molecular level. Further, in addition to genetic counselling and early diagnosis of affected individuals, improved understanding of the mutations that lead to arRP will help in development of better therapeutic procedures.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Acknowledgments

The authors are grateful to all members for their participation in this study. We are also thankful to members of the staff of Layton Rehmatullah Benevolent Trust (LRBT) hospital for clinical evaluation of affected individuals.

Funding This study was supported, in part by Higher Education Commission and Ministry of Science and Technology Islamabad, Pakistan.

REFERENCES

1. Bird AC. Retinal photoreceptor dystrophies LI. Edward Jackson Memorial Lecture. Am J Ophthalmol. 1995; 119:543–62. [PubMed: 7733180]
2. Yeung KY, Baum L, Chan WM, et al. Molecular diagnostics for retinitis pigmentosa YEUNG2001Q. Clin Chim Acta. 2001; 313:209–15. [PubMed: 11694261]
3. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet. 2006; 368:1795–809. [PubMed: 17113430]
4. Hims MM, Diager SP, Inglehearn CF. Retinitis pigmentosa: genes, proteins and prospects. Dev Ophthalmol. 2003; 37:109–25. [PubMed: 12876833]
5. McHenry CL, Liu Y, Feng W, et al. MERTK arginine-844-cysteine in a patient with severe rod—cone dystrophy: loss of mutant protein functions in transfected cells. Invest Ophthalmol Vis Sci. 2004; 45:1456–63. [PubMed: 15111602]
6. D'Cruz PM, Yasumura D, Weir J, et al. Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. Hum Mol Genet. 2000; 9:645–51. [PubMed: 10699188]
7. Gal A, Li Y, Thompson DA, et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. Nat Genet. 2000; 26:270–1. [PubMed: 11062461]
8. Ebermann I, Walger M, Scholl HP, et al. Truncating mutation of the DFNB59 gene causes cochlear hearing impairment and central vestibular dysfunction. Hum Mutat. 2007; 28:571–7. [PubMed: 17301963]
9. Brea-Fernandez AJ, Pomares E, Brion MJ, et al. Novel splice donor site mutation in MERTK gene associated with retinitis pigmentosa. Br J Ophthalmol. 2008; 92:1419–23. [PubMed: 18815424]
10. Kaul H, Riazuddin SA, Yasmeen A, et al. A new locus for autosomal recessive congenital cataract identified in a Pakistani family. Mol Vis. 2010; 16:240–45. [PubMed: 20161816]
11. Lathrop GM, Lalouel JM. Easy calculations of LOD scores and genetic risks on small computers 509. Am J Hum Genet. 1984; 36:460–5. [PubMed: 6585139]
12. Schaffer AA, Gupta SK, Shriram K, et al. Avoiding recomputation in genetic linkage analysis 1669. Hum Hered. 1994; 44:225–37. [PubMed: 8056435]
13. Tschernutter M, Jenkins SA, Waseem NH, et al. Clinical characterisation of a family with retinal dystrophy caused by mutation in the Mertk gene. Br J Ophthalmol. 2006; 90:718–23. [PubMed: 16714263]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

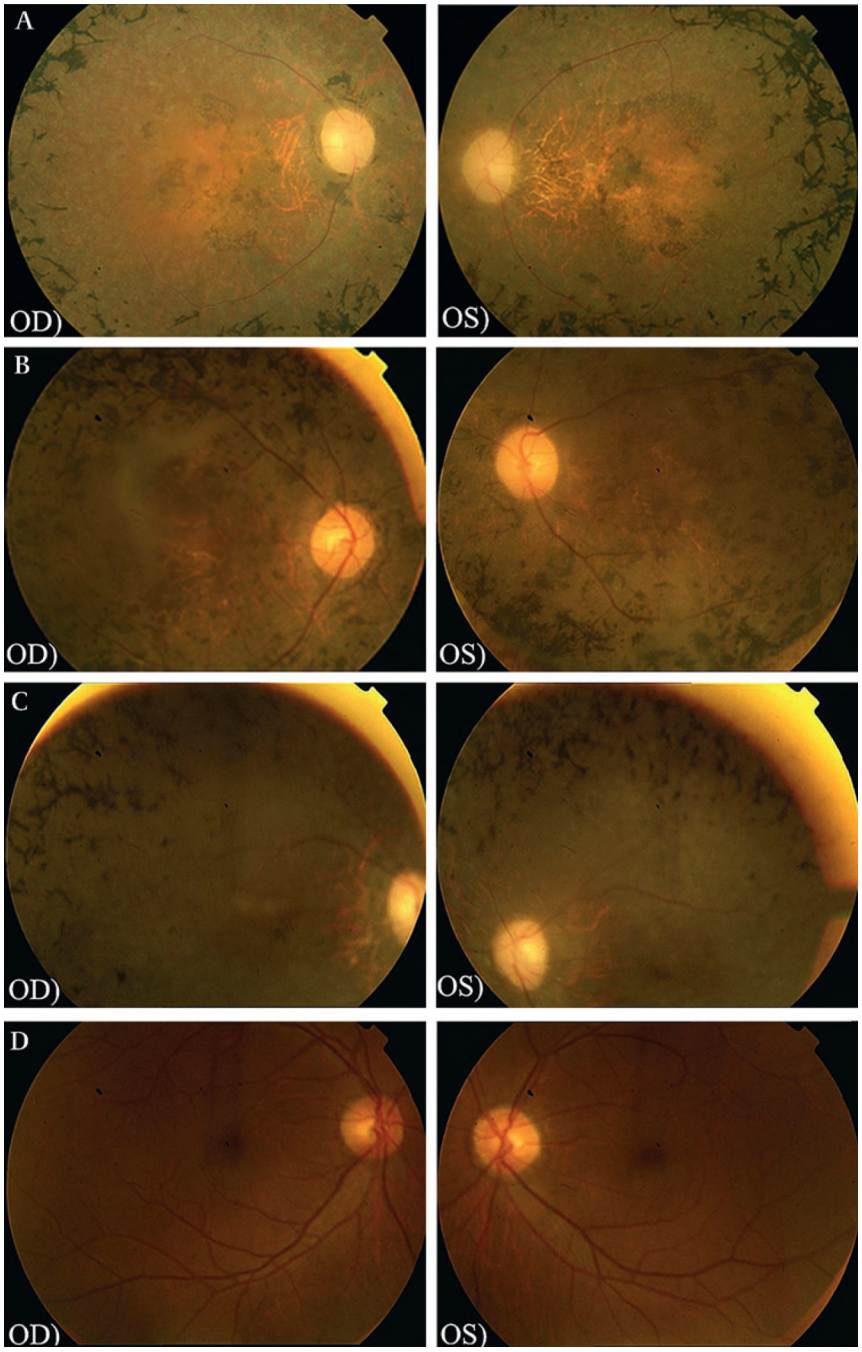

Figure 1.
Fundus photographs of members of family PKRP173. Fundus demonstrating several features associated with retinitis pigmentosa (RP) including a waxy pallor of the optic disc, attenuated arterioles, atrophy of retinal pigment epithelium (RPE) and peripheral bone spicules. Notably, macular atrophy with pigment clumping in peripheral regions is present. (A) Affected individual 18; (B) affected individual 9; (C) affected individual 10; (D) unaffected individual 12. OD, right eye; OS, left eye.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

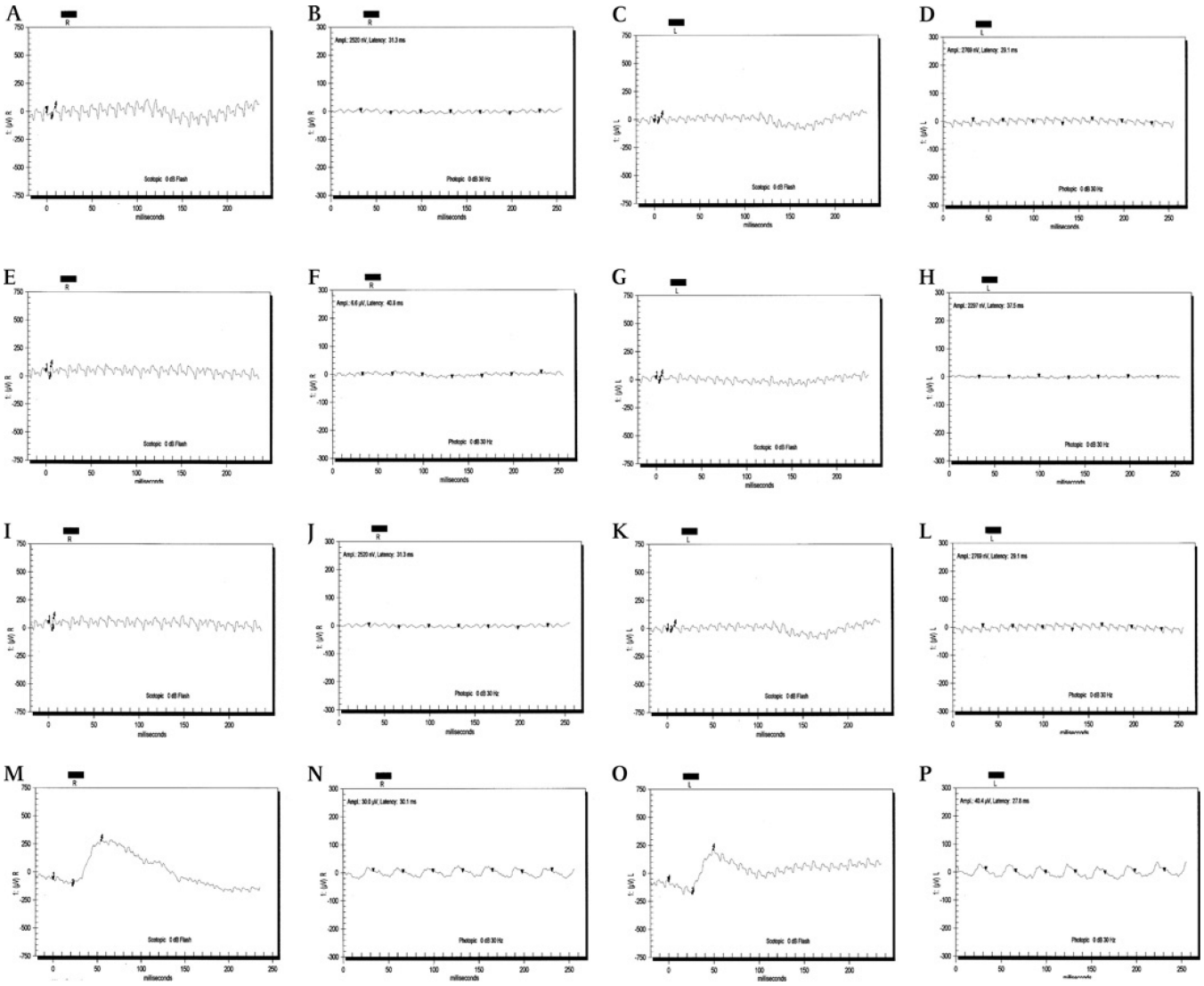

Figure 2.

Electroretinogram responses of members of family PKRP173. ERG recordings show no rod and cone response for affected individuals. Isolated cone responses measured at 30 Hz flicker are absent too, illustrating an advanced stage of retinopathy encompassing both rod and cone cells of the retina. Individual 18: (A) Right eye (OD) combined rod and cone response; (B) OD cone response; (C) Left eye (OS) combined rod and cone response; (D) OS cone response. Individual 9: (E) OD combined rod and cone response; (F) OD cone response; (G) OS combined rod and cone response; (H) OS cone response. Individual 10: (I) OD combined rod and cone response; (J) OD cone response; (K) OS combined rod and cone response; (L) OS cone response. Individual 12: (M) OD combined rod and cone response; (N) OD cone response; (O) OS combined rod and cone response; (P) OS cone response.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

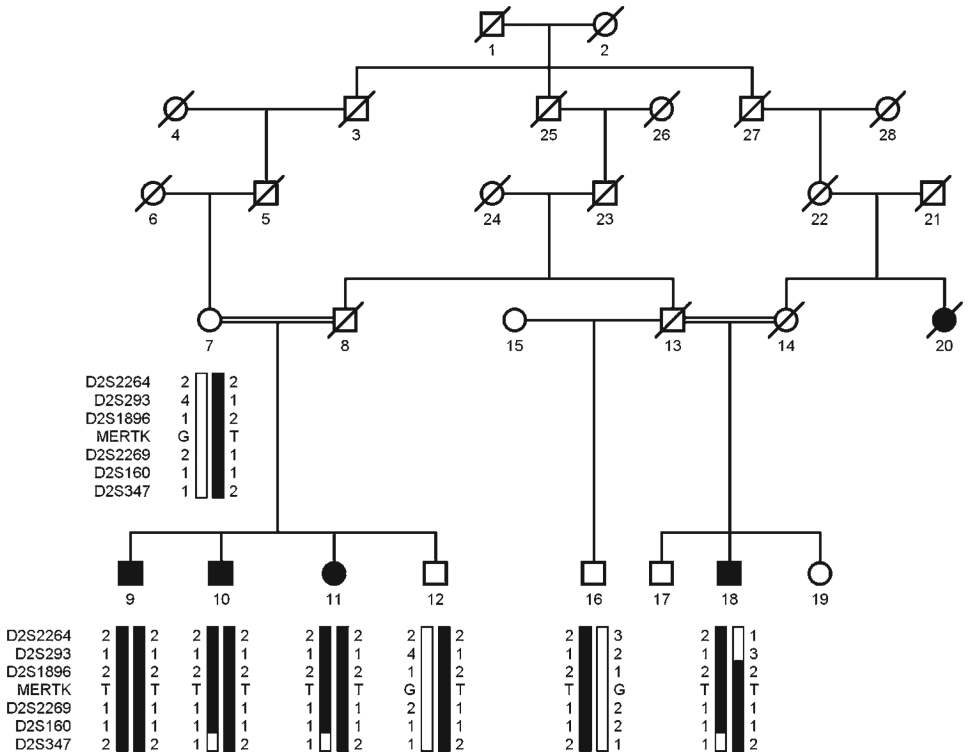

Figure 3.

Pedigree drawing and haplotype of chromosome 2q markers of family PKRP173. Squares are males, circles are females, filled symbols are affected individuals, double line between individuals indicates consanguinity and diagonal line through a symbol is deceased family member. The haplotypes of six adjacent chromosome 2q microsatellite markers are shown with alleles forming the risk haplotype are shaded black, and alleles not co-segregating with retinitis pigmentosa (RP) are shown in white.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

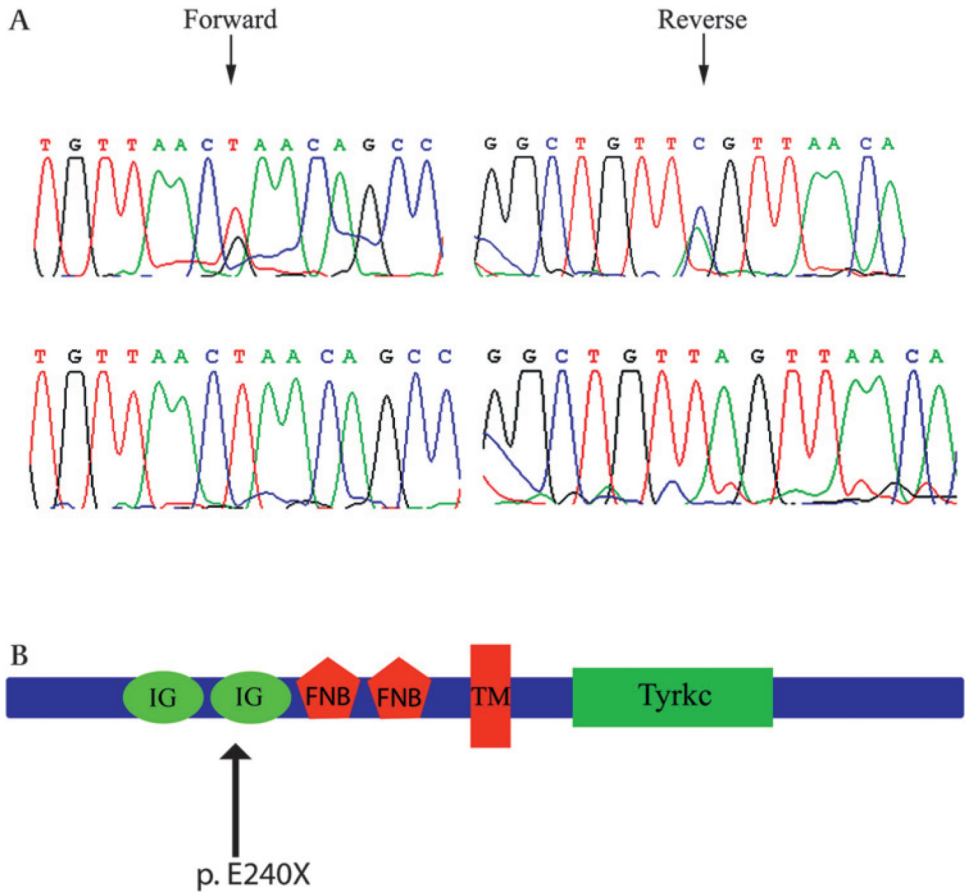

Figure 4.

(A) Sequence chromatograms of *MERTK* in family PKRP173: unaffected individual 07 (top row) heterozygous and affected individual 09 (bottom row) homozygous for c.718 G→T transversion that leads to a premature termination (p.E240X). (B) A schematic representation of *MERTK* domains. Structure domains were predicted with simple modular architecture tools (SMART) algorithms (http://smart.embl-heidelberg.de/). FNB, fibronectin type 3 domain; IG, immunoglobulin domain; TM, transmembrane domain; Tyrkc, Tyrosine kinase catalytic domain.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Clinical characteristics of affected individuals of PKRP173 diagnosed with autosomal recessive retinitis pigmentosa (RP)

ID	Age (years)	Age at first diagnosis of RP (years)	Visual acuity	First symptom	Disease progression	Fundus findings
9	48	9	PL	Night blindness	Progressive	Macular atrophy, pigment deposition, vascular attenuation
10	45	10	PL	Night blindness	Progressive	Macular atrophy, pigment deposition, vascular attenuation
11	43	9	PL	Night blindness	Progressive	Macular atrophy, pigment deposition, vascular attenuation
18	45	10	PL	Night blindness	Progressive	Macular atrophy, pigment deposition, vascular attenuation

PL, perception of light.

Br J Ophthalmol. Author manuscript; available in PMC 2012 July 11.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

Two point LOD scores of chromosome 2q markers for PKRP173

Marker	cM	Mb	0	0.01	0.05	0.09	0.1	0.2	0.3	Z_{max}	θ_{max}
D2S2264	114.4	102.4	– ∞	–0.01	0.54	0.64	0.65	0.58	0.4	0.65	0.10
D2S293	118.1	107.2	– ∞	–0.06	0.47	0.61	0.60	0.53	0.3	0.60	0.09
D2S1896	122.9	112.6	3.20	3.13	2.87	2.61	2.54	1.86	1.18	3.20	0.00
D2S2269	122.9	112.9	3.25	3.18	2.91	2.67	2.62	1.96	1.26	3.25	0.00
D2S160	122.9	112.9	3.18	3.10	2.84	2.59	2.49	1.85	1.16	3.18	0.00
D2S347	131.5	124.2	– ∞	–0.01	0.54	0.64	0.65	0.58	0.4	0.65	0.10

LOD, logarithm of the odds.

LOD scores were calculated at different θ values for each marker with the FASTLINK version of MLINK from the LINKAGE program package Maximum LOD scores for each marker were calculated using ILINK.