• Basic Research •

Novel mutations in *PDE6A* and *CDHR1* cause retinitis pigmentosa in Pakistani families

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Abstract

● **AIM:** To investigate the genetic basis of autosomal recessive retinitis pigmentosa (arRP) in two consanguineous/endogamous Pakistani families.

● **METHODS:** Whole exome sequencing (WES) was performed on genomic DNA samples of patients with arRP to identify disease causing mutations. Sanger sequencing was performed to confirm familial segregation of identified mutations, and potential pathogenicity was determined by predictions of the mutations' functions.

● **RESULTS:** A novel homozygous frameshift mutation [NM_000440.2:c.1054delG, p. (Gln352Argfs*4); Chr5:g.149286886del (GRCh37)] in the *PDE6A* gene in an endogamous family and a novel homozygous splice site mutation [NM_033100.3:c.1168-1G>A, Chr10:g.85968484G>A (GRCh37)] in the *CDHR1* gene in a consanguineous family were identified. The *PDE6A* variant p. (Gln352Argfs*4) was predicted to be deleterious or pathogenic, whilst the *CDHR1* variant c.1168-1G>A was predicted to result in potential alteration of splicing.

● **CONCLUSION:** This study expands the spectrum of genetic variants for arRP in Pakistani families.

● **KEYWORDS:** autosomal recessive retinitis pigmentosa; PDE6A; CDHR1; variants; Pakistani families

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INTRODUCTION

Retinitis pigmentosa (RP) refers to a group of inherited retinal disorders, characterized by progressive degeneration of photoreceptors and retinal pigment deposits resulting in vision loss. Initial symptoms include night blindness, decreased visual acuity and progressive loss of peripheral vision. The majority of RP cases (about 70%-80%) are non-syndromic, where the eye is exclusively affected[1]. The most common forms of syndromic RP, where RP occurs in conjunction with extraocular and/or systemic features, include Usher syndrome and Bardet-Biedl syndrome[2]. The age of onset may vary from childhood to adolescence or early adulthood. The estimated frequency of RP is about 1 in 4000 individuals[3], however, autosomal recessive forms of RP (arRP) have been more frequently reported in Israeli, Saudi, south Indian and Pakistani populations with high rates of consanguinity and endogamy[4-6]. Approximately 130 genes have been associated with RP, including about 90 genes for non-syndromic RP and about 40 genes for syndromic forms[7] (RetNet; https://sph.uth.edu/retnet/). In addition, about 59 genes are known to underlie other forms of inherited retinal dystrophies (RetNet; https://sph.uth.edu/retnet/). Genes commonly reported to cause arRP include *ABCA4* (MIM #601691), *CRB1* (MIM #604210), *CDHR1* (MIM #609502), *PDE6A* (MIM #180071), *PDE6B* (MIM #180072), *RHO* (MIM #180380), *RP1* (MIM #603937), *SPATA7* (MIM #609868), *EYS* (MIM# 612424), *RP1L1* (MIM # 608581), *TULP1* (MIM #602280), and *USH2A* (MIM #608400); among them *CRB1*, *PDE6A*, *PDE6B*, *RP1*, and *TULP1* are the most commonly mutated genes in consanguineous/endogamous families of Pakistani origin[6,8-9].

Despite high genetic heterogeneity, variants in known disease genes are identified in only about 60% of reported cases of RP[10]. Identification of novel genes or genetic variants underlying RP in this cohort of genetically unsolved cases is important in facilitating a better understanding of disease progression and for the development of novel therapies, and, to this end, significant progress has been achieved in recent years through adaptation of high-throughput DNA sequencing technologies[11-12].

In this study, we investigated an endogamous family and a consanguineous family with arRP from Khyber Pakhtunkhwa region of Pakistan. Our clinical and genomic investigations including high-throughput DNA sequencing technologies identified two novel candidate disease variants, a frameshift sequence alteration in *PDE6A*, as well as splice site sequence alteration in *CDHR1* that segregated with the disease phenotype in the affected families.

SUBJECTS AND METHODS

Ethical Approval The study was approved by the Ethical Committee of Kohat University of Science and Technology (KUST; Pakistan), and the study was carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained for participation in the study from families' members and parents of the minor children.

Subjects Family 1 extending over two generations with four affected and six unaffected members, was recruited from the Khyber Pakhtunkhwa region of Pakistan. Further, Family 2 extending over four generations and comprising of eight living affected and 34 unaffected members, was also recruited from same region of Pakistan (Figures 1A and 2A). In Family 1 parents of affected individuals were from same tribe (endogamous family) and in Family 2 parents of affected individuals were cousins (consanguineous family). Blood samples were collected from recruited affected and unaffected individuals, and all affected individuals were clinically evaluated by local ophthalmologists for obtaining medical and family histories and clinical assessment. The clinical assessment procedures used were funduscopic examination and visual acuity measurements performed using Snellen charts, as well as colour fundus photography in selected affected individuals, for diagnosis of RP.

Genetic Analysis Genomic DNA from blood samples was extracted using the ReliaPrep™ kit (Blood gDNA Miniprep System, Promega) according to the manufacturer's protocol. To identify the causative disease variant, whole exome sequencing was performed on a single affected individual in each family (subject II:4 in Family 1 and II:10 in Family 2 in Figures 1 and 2 respectively) to develop a profile of rare sequence variants present at low frequency in publicly available population databases. Coding regions were captured by HiSeq2000 using paired-end (2×100) protocol at a mean coverage depth of 30× at the Otogenetics Corporation (Norcross, GA, USA). The Agilent SureSelect Human All ExonV4 (51 Mb) enrichment kit was used for exome enrichment. The sequence reads were aligned to the human genome reference sequence [hg19] and read alignment, variant calling, and annotation were performed by DNAnexus (DNAnexus Inc., Mountain View, CA; https://dnanexus.com).

Allele-specific primers were designed using Primer3 web software (*PDE6A* F: 5-AGAGATCCACACTTGCCATCA-3, *PDE6A* R*:* 5-GAGCGCAAACACCCAGATTT-3; *CDHR1* F: 5-GGCACTCACAGTCCATTCAC-3, *CDHR1* R: 5-CAAGTTGAATTTGGATGG-3) to evaluate segregation of variants *via* dideoxy sequencing. Polymerase chain reaction (PCR) was undertaken for all recruited family members using allele-specific primers following standard conditions, with products sequenced by Source BioScience LifeSciences (https://www.sourcebioscience.com/). Pathogenicity of the identified sequence variation in the *CDHR1* was analyzed using CRYP-SKIP (https://cryp-skip.img.cas.cz/), Human Splicing Finder (https://www.genomnis.com/access-hsf), and Transcript Inferred Pathogenicity Score (TraP) (http://trap-score.org/) specialized prediction software. The effect of the *PDE6A* gene variant on protein structure was predicted using Phyre2 software (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index). To compare and correlate the *PDE6A* and *CDHR1* gene variants with phenotype, all reported variants were retrieved from HGMD (http://www.hgmd.cf.ac.uk/ac/search.php), OMIM (https://www.ncbi.nlm.nih.gov/omim/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) databases.

RESULTS

Subjects Pedigree analysis of recruited Pakistani families suggested an autosomal recessive mode of disease inheritance in the affected families (Figures 1 and 2). In Family 1, the four affected individuals II:1, II:2, II:4 and II:8 were aged 20, 19, 16 and 4y at time of first examination, whilst in Family 2, the eight affected individuals II:1, II:7, II:10, II:13, II:15, III:15, III:16, and III:18 were aged 51, 46, 43, 40, 36, 30, 18, and 12y respectively. Night blindness and decreased visual acuity were prominent common clinical features in all affected members of both investigated families (Table 1). On the basis of basic clinical ophthalmic assessment, arRP was the major finding in all affected members. Affected member had the classical RP phenotypes *i.e.,* waxy disc pallor, attenuated vessels and bone spicules in both the fundi. In addition, the affected members of the Family 2 had features of macular atrophy along with RP. There was a wide range of severity of clinical features noted in affected members of both families (Figure 3).

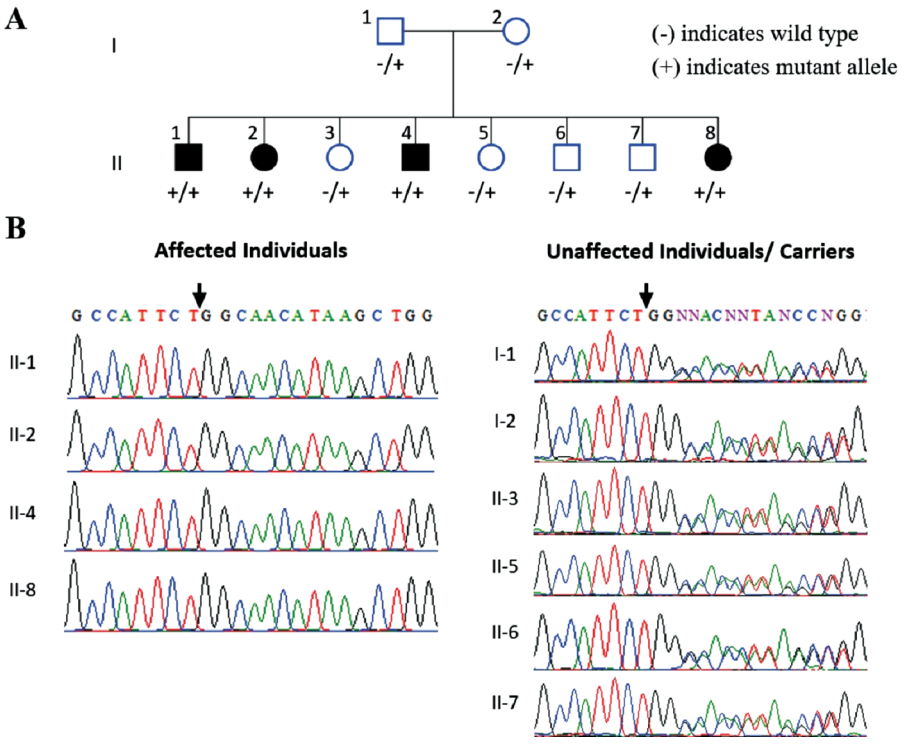

Figure 1 Family 1 pedigree and genetic findings A: Pedigree shows segregation of the *PDE6A* variant identified. B: Sequence chromatograms show the *PDE6A* [NM_000440.2:c.1054delG, p.(Gln352Argfs*4); Chr5:g.149286886del (GRCh37)] variant in affected (homozygous) and phenotypically normal (heterozygous carriers) members of investigated family.

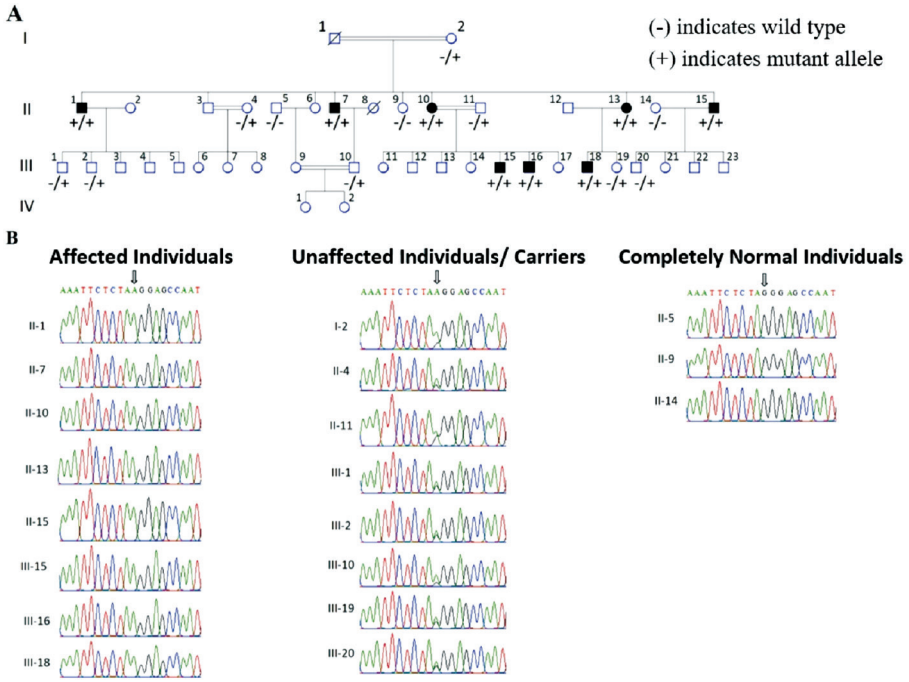

Figure 2 Family 2 pedigree and genetic findings A: Pedigree shows segregation of *CDHR1* variant identified. B: Sequence chromatograms show the *CDHR1* [NM_033100.3:c.1168-1G>A, p.(?); Chr10:g.85968484G>A (GRCh37)] gene variant in the affected and phenotypically normal members of investigated family.

Genetic Findings Initial analysis of exome data excluded previously described variants in genes known to cause ocular disease. Variants were then assessed and filtered for rare, non-synonymous exonic or splice variants, with a population frequency of <0.01 in control databases (including the Genome Aggregation Database; gnomAD, the Exome Aggregation Consortium; ExAC, and the 1000 Genomes Project). A single candidate novel homozygous frameshift variant [NM_000440.2:c.1054delG; Chr5:g.149286886del (GRCh37)] was identified in exon 7 of *PDE6A* (Figure 1)

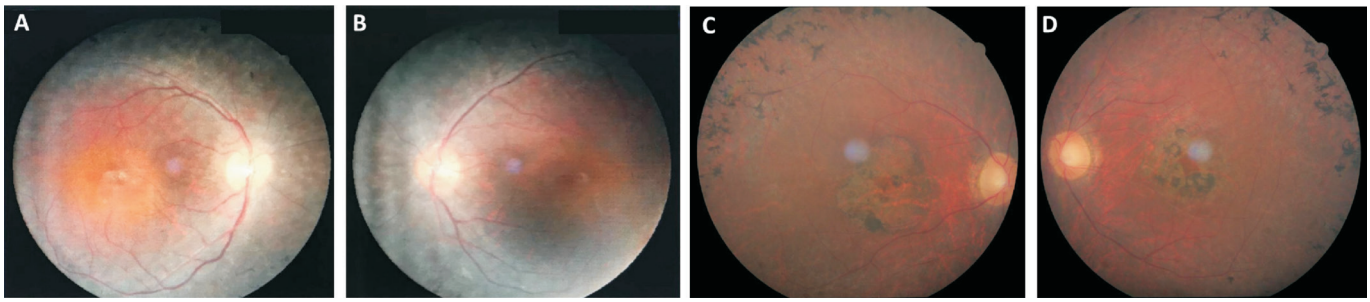

Figure 3 Colour fundus photographs of a single affected individual in each family A, B: Right and left eyes of II:4 in Family 1; C, D: Right and left eyes of II:7 in Family 2. In both affected individuals, typical features of RP are noted, including optic disc pallor, attenuated retinal vessels and mid-peripheral bone spicule pigmentation. Additionally, both affected individuals display features of macular involvement.

Table 1 The age and visual acuity of the affected individuals of both families

Family No.	Patient	Age	Visual acuity	
			Left eye	Right eye
1	II:1	20	6/24	6/36
1	II:2	19	6/24	6/24
1	II:4	16	6/18	6/18
1	II:8	4	6/6	6/6
2	II:7	46	6/60	LP
2	II:10	43	CF	CF
2	III:15	30	6/60	6/60

LP: Light perception; CF: Counting fingers.

in Family 1. This single base pair deletion is predicted to result in a frameshift followed by a premature stop codon (p.Gln352Argfs*4). In Family 2, a novel splice site substitution [NM_033100.3:c.1168-1G>A, Chr10:g.85968484G>A (GRCh37)] was identified at the splice acceptor site in intron 11 of the *CDHR1* gene (Figure 2). In silico analysis of c.1168-1G>A using Human Splicing Finder, CRYP-SKIP and TraP predicted it to result in activation of a new cryptic splice acceptor site within the exon 12 sequence which may lead to alternative splicing and partial skipping of exon 12 or skipping of entire exon 12 sequence during splicing. Both the *PDE6A* p.(Gln352Argfs*4) as well as the *CDHR1* c.1168-1G>A variants are absent in gnomAD. The sequence variants in families 1 and 2 segregate as expected for an autosomal recessive condition in each family (Figures 1 and 2).

Variants reported to date in *PDE6A* for arRP in Pakistani families are summarized in Table 2[8,13-16]. A schematic representation of pathogenic variants identified to date in *PDE6A* and *CDHR1* are shown in Figure 4A-4D).

DISCUSSION

Non-syndromic RP cases are frequently reported in South Asian populations, particularly from Pakistan and Southern India, with autosomal recessive modes of inheritance observed in up to 95% of RP cases[6]. The high rates of consanguineous marriages (20% to 50%), as well as endogamous marriages increasing intracommunity genetic homogeneity, both contribute to the high burden of arRP in these populations[6,17-20]. RP is characterized by significant clinical and genetic heterogeneity, leading to diagnostic challenges. Over half of genes currently known to cause non-syndromic RP were identified in South Asian populations[6]. Therefore, the large multigenerational consanguineous/endogamous families of South Asian populations are powerful resources for genetic studies of arRP, facilitating the identification of novel variants or disease genes that contribute to the disease[6].

In current study, genetic analyses identified novel candidate disease variants in *PDE6A* [c.1054delG, p.(Gln352Argfs*4)] and *CDHR1* [c.1168-1G>A, p.(?)] that segregated appropriately with autosomal recessive disease phenotype in Pakistani families. Previously, variants in *PDE6A* (p.Arg102Ser, p.Arg257*, c.1408-2A>G, p.Arg544Trp, p.Tyr700Leufs*21 and c.2028-1G>A) have been described in some Pakistani families with arRP[8,15]. To best of our knowledge, only one mutation (c.1463delG, p.Gly488Alafs*18) in the *CDHR1* gene for retinal dystrophies have been reported in families of Pakistani origin[21-22]. In contrast, disease causing mutations (p.Pro574Ala, c.1485+2T>C, p.Tyr547*, p.Tyr140*, p.Ala128Gly and p.Asp696Alafs) in the *CDHR1* gene have been detected to cause retinal dystrophies in consanguineous/endogamous Spanish, Chinese, Saudi, Lebanese and Israeli populations[23-27]. Previous studies have found that *AIPL1* (MIM #604393), *CRB1* (MIM #604210), *SEMA4A* (MIM #607292), *RPGRIP1* (MIM # 605446), *TULP1* (MIM #602280) *RP1* (MIM #603937), *PDE6B* (MIM #180072), *CNGB1* (MIM # 600724) and *RHO* (MIM #180380) are most commonly mutated genes in Pakistani families to cause arRP and other retinal disorders[6,28]. These studies have also provided evidence of founder effect of few genes' mutations in Pakistani population. In this context, the *AIPL1* mutation p.Trp278* was reported previously founder mutation in Pakistani families with juvenile arRP[8,29-32]. In addition, *TULP1* mutations p.Lys489Arg and p.Thr380Ala were found to be more commonly mutated in Pakistani families with arRP, thus may be referred as Pakistani founder mutations[32-35]. In contrast, no

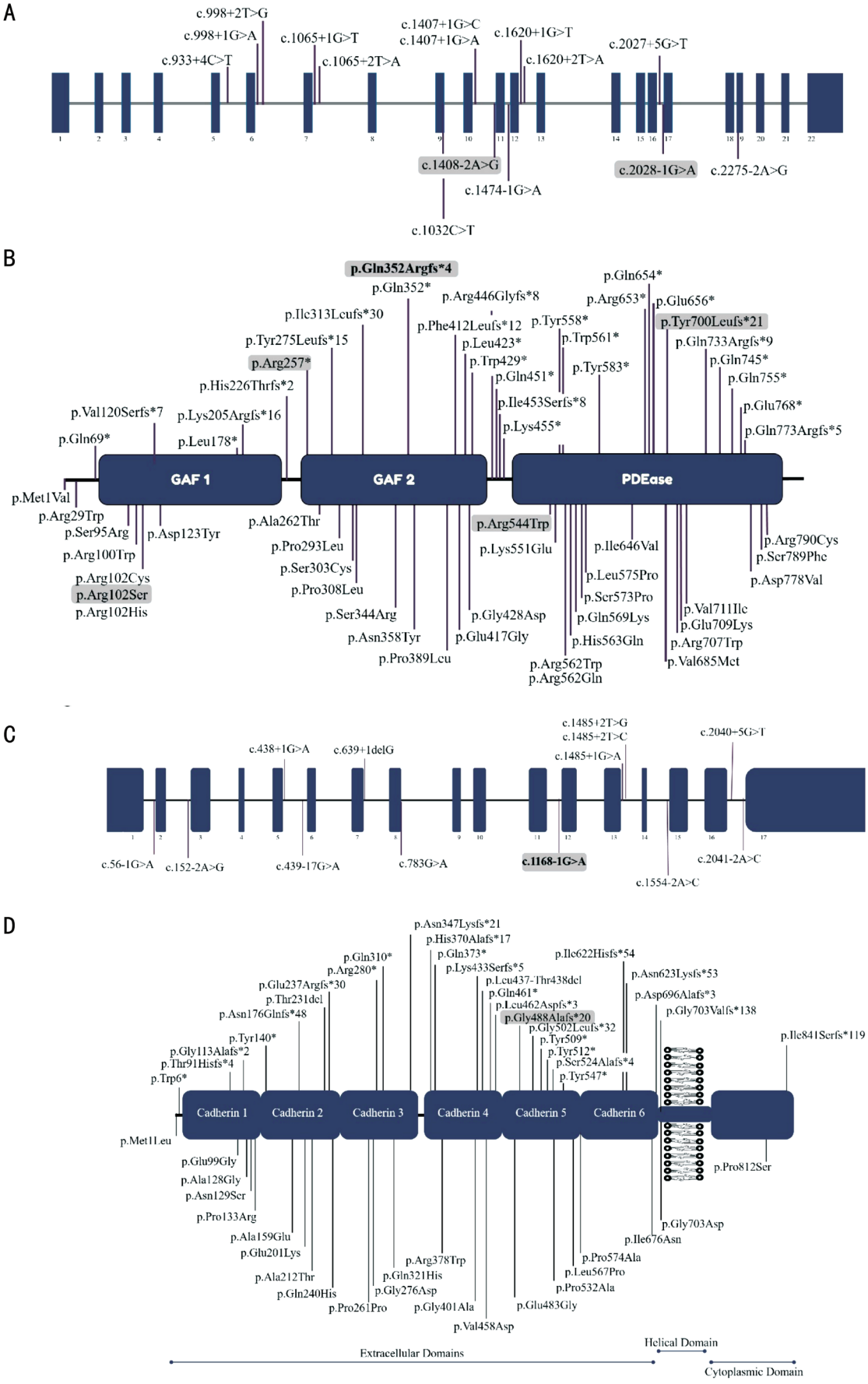

Figure 4 *PDE6A* and *CDHR1* variants associated with arRP A, C: Schematic representation of exons of the *PDE6A* and *CDHR1* gene highlighting the positions of all disease causing mutations identified to date; B, D: Domains of *PDE6A* and *CDHR1* predicted protein [UniProtKB - P16499; UniProtKB - Q96JP9] respectively, highlighting the positions of all disease associated variants identified to date. The variants identified in Pakistani origin patients previously and both the variants identified in this study are in bold and grey color.

Table 2 Summary of all reported *PDE6A* variants associated with arRP in Pakistani families

RefSeqID	Nucleotide variant	Protein variant	Phenotype	Families	Patients	Reference
NM_000440.2	c.304C>A	p.Arg102Ser	arRP	2	8	[13,14]
NM_000440.2	c.769C>T	p.Arg257*	arRP	2	6	[14,15]
NM_000440.2	c.1408-2A>G	p.?	arRP		15	[15,16]
NM_000440.2	c.1630C>T	p.Arg544Trp	arRP	1	3	[8]
NM_000440.2	c.2028-1G>A	p.?	arRP	1	5	[16]
NM_000440.2	c.2098dupT	p.Tyr700Leufs*21	arRP	1	3	[15]
NM_000440.2	c.1054delG	p.Gln352Argfs*4	arRP	1	4	Current Study

founder effect has been reported so far of *PDE6A* mutations identified previously and in current study in Pakistani origin families with arRP[8,15]. However, elucidation of founder effect of reported *PDE6A* mutations in our population may have an impact on efficient diagnosis and counselling of affected families.

The translated PDE6A protein contains 2 GAF domains (GAF-1; 73-222 amino acids and GAF-2; 254-431 amino acids) and a cyclic nucleotide phosphodiesterase (PDEase) domain (483-816 amino acids; UniProtKB - P16499). Normally, the *PDE6A* gene expresses the alpha subunit of phosphodiesterase 6 complex (PDE6), the other members of the PDE6 complex are a beta subunit (*PDE6B*) and two inhibitory gamma subunits (*PDE6G*). The PDE6 complex is directly involved in visual phototransduction pathway, and is localized on the disc membrane of the rod photoreceptor cells[36-37]. During phototransduction in rod photoreceptors, absorption of a photon of light leads to isomerization of rhodopsin. This isomerized rhodopsin activates transducin (a heterotrimeric G-protein) in the disc membrane, which activates the PDE complex, leading to hydrolysis of cyclic guanine monophosphate (cGMP) molecules into GMP molecules and resulting in a fall in the level of cGMP inside the rod photoreceptors. As a result, the cGMP-gated cation channel in the plasma membrane closes and membrane is hyperpolarized[37]. Loss of function in the *PDE6A* protein leads to impairment of the photo-transduction pathway and an increase in cGMP levels. This increase in cGMP levels results in the death of rod and cone photoreceptors cells, leading to the clinical manifestations of RP[38-40].

The identified variant p.Gln352Argfs*4 in the GAF-2 domain like other nonsense variants, is predicted to cause loss of function of *PDE6A via* nonsense-mediated decay[41]. Disease causing variants in *PDE6A* orthologues have also been reported to cause RP[42-43]. Loss of function variants in *PDE6A* are known to cause arRP. Such as a study conducted by Nair and colleagues reported a novel 2-bp frameshift variant p.Ile452Serfs*7 in *PDE6A* in Emirati population[44]. The identified variant (p.Gln352Argfs*4) in current study and the variant p.Arg257* reported previously by Riazuddin *et al*[15] lie in GAF-2 domain that results in loss of function of *PDE6A* in phototransduction pathway and cause arRP in affected individuals. Previously, Jespersgaard and colleagues found a nonsense variant at same amino acid position (p.Gln352*) in *PDE6A* that also caused arRP[45].

The *CDHR1* protein is an important member of calcium-dependent cadherin superfamily, and is involved in the cell adhesion[46]. *CDHR1* is abundantly expressed in the retina, and variants in this gene have been associated with retinal dystrophies ranging from cone dystrophy to RP[47]. In addition to *CDHR1*, three other members of the cadherin superfamily, *PCDH15*, *CDH3* and *CDH23*, have been implicated in retinal dystrophies[48-50]. *CDHR1* contains six cadherin domains (Cadherin 1; 36-135 amino acids, Cadherin 2; 136-246 amino acids, Cadherin 3; 247-353 amino acids, Cadherin 4; 359-472 amino acids, Cadherin 5; 473-576 amino acids and Cadherin 6; 573-688 amino acids), one transmembrane domain (701-725 amino acid) and one intracellular domain (773-859 amino acids; UniProtKB-Q96JP9). In retinal cells, *CDHR1* protein is abundantly localized to the junction of inner and outer region of rod and cone photoreceptors[51-52] and is thought to be crucial for the normal structure and survival of the rod and cone photoreceptors. Loss of function of *CDHR1* may affect the structure of rod and cone cells that may be the leading cause of retinal dystrophies.

The *CDHR1* splice site variants such as c.1168-1G>C that has been reported in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/955272/) and c.1168-1G>A identified in current study are expected to affect the canonical splice sequence at the intron 11 3' acceptor splice site, and is predicted to result in skipping of exon 12 of *CDHR1* or activation of a cryptic splice site in exon 12 of *CDHR1*. Exon 12 codes for 50 amino acids in the cadherin 4 domain of *CDHR1* and in case of skipping of this exon may lead to the loss of 50 amino acids in *CDHR1* protein. Similarly, a previous study also reported disease causing nonsense variant in same cadherin 4 domain of the *CDHR1* that resulted in a premature truncated protein[53]. Missense variants causing retinal dystrophies have been identified in all 6 cadherin repeat domains of *CDHR1*, supporting the importance of these

domains in maintaining normal *CDHR1* protein function and hence structural integrity of the photoreceptor cells.

In summary, the identification of novel variants in *PDE6A* and *CDHR1* in the present study consolidates the key role of these genes in the pathogenesis of arRP and contributes to the expanding spectrum of disease-causing *PDE6A* and *CDHR1* variants. In addition, our study highlights the fact that disease-causing *PDE6A* and *CDHR1* variants, although rare, can cause arRP in Pakistani endogamous and consanguineous families.

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REFERENCES

1 Ferrari S, di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F. Retinitis pigmentosa: genes and disease mechanisms. *Curr Genomics* 2011;12(4):238-249.

2 Wolfrum U, Nagel-Wolfrum K. The usher syndrome, a human ciliopathy. *Klin Monbl Augenheilkd* 2018;235(3):273-280.

3 Verbakel SK, van Huet RAC, Boon CJF, den Hollander AI, Collin RWJ, Klaver CCW, Hoyng CB, Roepman R, Klevering BJ. Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res* 2018;66:157-186.

4 Aldahmesh MA, Safieh LA, Alkuraya H, Al-Rajhi A, Shamseldin H, Hashem M, Alzahrani F, Khan AO, Alqahtani F, Rahbeeni Z, Alowain M, Khalak H, Al-Hazzaa S, Meyer BF, Alkuraya FS. Molecular characterization of retinitis pigmentosa in Saudi Arabia. *Mol Vis* 2009;15:2464-2469.

5 Sharon D, Banin E. Nonsyndromic retinitis pigmentosa is highly prevalent in the Jerusalem region with a high frequency of founder mutations. *Mol Vis* 2015;21:783-792.

6 Zafar S, Ahmad K, Ali A, Baig R. Retinitis pigmentosa genes implicated in South Asian populations: a systematic review. *J Pak Med Assoc* 2017;67(11):1734-1739.

7 Karali M, Testa F, Brunetti-Pierri R, di Iorio V, Pizzo M, Melillo P, Barillari MR, Torella A, Musacchia F, D'Angelo L, Banfi S, Simonelli F. Clinical and genetic analysis of a European cohort with pericentral retinitis pigmentosa. *Int J Mol Sci* 2019;21(1):E86.

8 Khan MI, Ajmal M, Micheal S, Azam M, Hussain A, Shahzad A, Venselaar H, Bokhari H, de Wijs IJ, Hoefsloot LH, Waheed NK, Collin RW, den Hollander AI, Qamar R, Cremers FP. Homozygosity mapping identifies genetic defects in four consanguineous families with retinal dystrophy from Pakistan. *Clin Genet* 2013;84(3):290-293.

9 Albarry MA, Hashmi JA, Alreheli AQ, Albalawi AM, Khan B, Ramzan K, Basit S. Novel homozygous loss-of-function mutations in *RP1* and *RP1L1* genes in retinitis pigmentosa patients. *Ophthalmic Genet* 2019;40(6):507-513.

10 González-Del Pozo M, Fernández-Suárez E, Martín-Sánchez M, Bravo-Gil N, Méndez-Vidal C, Rodríguez-de la Rúa E, Borrego S, Antiñolo G. Unmasking Retinitis Pigmentosa complex cases by a whole genome sequencing algorithm based on open-access tools: hidden recessive inheritance and potential oligogenic variants. *J Transl Med* 2020;18(1):73.

11 Yang YP, Muzny DM, Reid JG, *et al*. Clinical whole-exome sequencing for the diagnosis of Mendelian disorders. *N Engl J Med* 2013;369(16):1502-1511.

12 Neuhaus C, Eisenberger T, Decker C, *et al*. Next-generation sequencing reveals the mutational landscape of clinically diagnosed Usher syndrome: copy number variations, phenocopies, a predominant target for translational read-through, and *PEX26* mutated in Heimler syndrome. *Mol Genet Genomic Med* 2017;5(5):531-552.

13 Maria M, Ajmal M, Azam M, *et al*. Homozygosity mapping and targeted Sanger sequencing reveal genetic defects underlying inherited retinal disease in families from Pakistan. *PLoS One* 2015;10(3):e0119806.

14 Ullah I, Kabir F, Gottsch CB, Naeem MA, Guru AA, Ayyagari R, Khan SN, Riazuddin S, Akram J, Riazuddin SA. Mutations in phosphodiesterase 6 identified in familial cases of retinitis pigmentosa. *Hum Genome Var* 2016;3:16036.

15 Riazuddin SA, Zulfiqar F, Zhang QJ, Yao WL, Li SL, Jiao XD, Shahzadi A, Amer M, Iqbal M, Hussnain T, Sieving PA, Riazuddin S, Hejtmancik JF. Mutations in the gene encoding the alpha-subunit of rod phosphodiesterase in consanguineous Pakistani families. *Mol Vis* 2006;12:1283-1291.

16 Khan SY, Ali S, Naeem MA, Khan SN, Husnain T, Butt NH, Qazi ZA, Akram J, Riazuddin S, Ayyagari R, Hejtmancik JF, Riazuddin SA. Splice-site mutations identified in PDE6A responsible for retinitis pigmentosa in consanguineous Pakistani families. *Mol Vis* 2015;21:871-882.

17 Yunis K, Rafei RE, Mumtaz G. International perspectives: consanguinity: perinatal outcomes and prevention-a view from the middle east. *NeoReviews* 2008;9(2):e59-e65.

18 Krishnaiah S, Subba Rao B, Lakshmi Narasamma K, Amit G. A survey of severe visual impairment in children attending schools for the blind in a coastal district of Andhra Pradesh in South India. *Eye* (*Lond*) 2012;26(8):1065-1070.

19 Hamamy H. Consanguineous marriages. *J Community Genet* 2012;3(3):185-192.

20 Bittles AH. *Consanguinity in context*. Vol. 63. 2012: Cambridge University Press.

21 Ba-Abbad R, Sergouniotis PI, Plagnol V, Robson AG, Michaelides M, Holder GE, Webster AR. Clinical characteristics of early retinal disease due to CDHR1 mutation. *Mol Vis* 2013;19:2250-2259.

22 Li L, Chen YB, Jiao XD, *et al*. Homozygosity mapping and genetic analysis of autosomal recessive retinal dystrophies in 144 consanguineous Pakistani families. *Invest Ophthalmol Vis Sci* 2017;58(4):2218-2238.

23 Abu-Safieh L, Alrashed M, Anazi S, *et al*. Autozygome-guided exome sequencing in retinal dystrophy patients reveals pathogenetic mutations and novel candidate disease genes. *Genome Res* 2013;23(2):236-247.

24 Beryozkin A, Shevah E, Kimchi A, Mizrahi-Meissonnier L, Sharon D. Whole exome sequencing reveals mutations in known retinal disease genes in 33 out of 68 Israeli families with inherited retinopathies. *Sci Rep* 2015;5(1):13187.

25 Nikopoulos K, Avila-Fernandez A, Corton M, Lopez-Molina MI, Perez-Carro R, Bontadelli L, di Gioia SA, Zurita O, Garcia-Sandoval B, Rivolta C, Ayuso C. Identification of two novel mutations in CDHR1 in consanguineous Spanish families with autosomal recessive retinal dystrophy. *Sci Rep* 2015;5:13902.

26 Fu JW, Ma L, Cheng JL, Yang LS, Wei CL, Fu SY, Lv HB, Chen R, Fu JJ. A novel, homozygous nonsense variant of the CDHR1 gene in a Chinese family causes autosomal recessive retinal dystrophy by NGS-based genetic diagnosis. *J Cell Mol Med* 2018;22(11):5662-5669.

27 Nair P, Sabbagh S, Mansour H, *et al*. Contribution of next generation sequencing in pediatric practice in Lebanon. A Study on 213 cases. *Mol Genet Genomic Med* 2018;6(6):1041-1052.

28 Khan MI, Azam M, Ajmal M, Collin RW, den Hollander AI, Cremers FP, Qamar R. The molecular basis of retinal dystrophies in Pakistan. *Genes* (*Basel*) 2014;5(1):176-195.

29 Sohocki MM, Bowne SJ, Sullivan LS, Blackshaw S, Cepko CL, Payne AM, Bhattacharya SS, Khaliq S, Qasim Mehdi S, Birch DG, Harrison WR, Elder FFB, Heckenlively JR, Daiger SP. Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis. *Nat Genet* 2000;24(1):79-83.

30 Damji KF, Sohocki MM, Khan R, Gupta SK, Rahim M, Loyer M, Hussein N, Karim N, Ladak SS, Jamal A, Bulman D, Koenekoop RK. Leber's congenital amaurosis with anterior keratoconus in Pakistani families is caused by the Trp278X mutation in the AIPL1 gene on 17p. *Can J Ophthalmol* 2001;36(5):252-259.

31 Khaliq S, Abid A, Hameed A, Anwar K, Mohyuddin A, Azmat Z, Shami SA, Ismail M, Mehdi SQ. Mutation screening of Pakistani families with congenital eye disorders. *Exp Eye Res* 2003;76(3):343-348.

32 McKibbin M, Ali M, Mohamed MD, Booth AP, Bishop F, Pal B, Springell K, Raashid Y, Jafri H, Inglehearn CF. Genotype-phenotype correlation for leber congenital amaurosis in Northern Pakistan. *Arch Ophthalmol* 2010;128(1):107-113.

33 Gu S, Lennon A, Li Y, Lorenz B, Fossarello M, North M, Gal A, Wright A. Tubby-like protein-1 mutations in autosomal recessive retinitis pigmentosa. *Lancet* 1998;351(9109):1103-1104.

34 Iqbal M, Naeem MA, Riazuddin SA, Ali S, Farooq T, Qazi ZA, Khan SN, Husnain T, Riazuddin S, Sieving PA, Hejtmancik JF, Riazuddin S. Association of pathogenic mutations in TULP1 with retinitis pigmentosa in consanguineous Pakistani families. *Arch Ophthalmol* 2011;129(10):1351-1357.

35 Ajmal M, Khan MI, Micheal S, Ahmed W, Shah A, Venselaar H, Bokhari H, Azam A, Waheed NK, Collin RW, den Hollander AI, Qamar R, Cremers FP. Identification of recurrent and novel mutations in TULP1 in Pakistani families with early-onset retinitis pigmentosa. *Mol Vis* 2012;18:1226-1237.

36 Taylor RE, Shows KH, Zhao Y, Pittler SJ. A PDE6A promoter fragment directs transcription predominantly in the photoreceptor. *Biochem Biophys Res Commun* 2001;282(2):543-547.

37 Lamb TD, Heck M, Kraft TW. Implications of dimeric activation of PDE6 for rod phototransduction. *Open Biol* 2018;8(8):180076.

38 Kjellström U, Veiga-Crespo P, Andréasson S, Ekström P. Increased plasma cGMP in a family with autosomal recessive retinitis pigmentosa due to homozygous mutations in the PDE6A gene. *Invest Ophthalmol Vis Sci* 2016;57(14):6048-6057.

39 Wensel TG, Zhang ZX, Anastassov IA, Gilliam JC, He F, Schmid MF, Robichaux MA. Structural and molecular bases of rod photoreceptor morphogenesis and disease. *Prog Retin Eye Res* 2016;55:32-51.

40 Iribarne M, Masai I. Do cGMP Levels Drive the Speed of Photoreceptor Degeneration? *Adv Exp Med Biol* 2018;1074:327-333.

41 Hillman RT, Green RE, Brenner SE. An unappreciated role for RNA surveillance. *Genome Biol* 2004;5(2):R8.

42 Pandya DY, Kelawala DN, Patel NV, Shah TM, Patel AB, Parmar NR, Reddy B, Patil DB, Joshi CG. Identification of genetic variants in *PDC, RHO, PDE6A* and *PDE6B* in dogs with progressive retinal atrophy. *Curr Sci* 2016;111(10):1640.

43 Winkler PA, Occelli LM, Petersen-Jones SM. Large animal models of inherited retinal degenerations: a review. *Cells* 2020;9(4):E882.

44 Nair P, Hamzeh AR, Malik EM, Oberoi D, Al-Ali MT, Bastaki F. Novel PDE6A mutation in an Emirati patient with retinitis pigmentosa. *Oman J Ophthalmol* 2017;10(3):228-231.

45 Jespersgaard C, Fang M, Bertelsen M, Dang X, Jensen H, Chen Y, Bech N, Dai L, Rosenberg T, Zhang J, Møller LB, Tümer Z, Brøndum-Nielsen K, Grønskov K. Molecular genetic analysis using targeted NGS analysis of 677 individuals with retinal dystrophy. *Sci Rep* 2019;9(1):1219.

46 Nollet F, Kools P, van Roy F. Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. *J Mol Biol* 2000;299(3):551-572.

47 Stingl K, Mayer AK, Llavona P, Mulahasanovic L, Rudolph G, Jacobson SG, Zrenner E, Kohl S, Wissinger B, Weisschuh N. CDHR1 mutations in retinal dystrophies. *Sci Rep* 2017;7(1):6992.

48 Ahmed ZM, Riazuddin S, Bernstein SL, Ahmed Z, Khan S, Griffith AJ, Morell RJ, Friedman TB, Riazuddin S, Wilcox ER. Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F. *Am J Hum Genet* 2001;69(1):25-34.

49 Sprecher E, Bergman R, Richard G, Lurie R, Shalev S, Petronius D, Shalata A, Anbinder Y, Leibu R, Perlman I, Cohen N, Szargel R. Hypotrichosis with juvenile macular dystrophy is caused by a mutation in CDH3, encoding P-cadherin. *Nat Genet* 2001;29(2):134-136.

50 Bolz H, von Brederlow B, Ramírez A, Bryda EC, Kutsche K, Nothwang HG, Seeliger M, del C-Salcedó Cabrera M, Vila MC, Molina OP, Gal A, Kubisch C. Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nat Genet* 2001;27(1):108-112.

51 Rattner A, Chen JC, Nathans J. Proteolytic shedding of the extracellular domain of photoreceptor cadherin. Implications for outer segment assembly. *J Biol Chem* 2004;279(40):42202-42210.

52 Rattner A, Smallwood PM, Williams J, Cooke C, Savchenko A, Lyubarsky A, Pugh EN, Nathans J. A photoreceptor-specific cadherin is essential for the structural integrity of the outer segment and for photoreceptor survival. *Neuron* 2001;32(5):775-786.

53 Gan L, Yang C, Shu Y, Liu F, Sun RT, Deng BL, Xu JX, Huang G, Qu C, Gong B, Li J. Identification of a novel homozygous nonsense mutation in the CDHR1 gene in a Chinese family with autosomal recessive retinitis pigmentosa. *Clin Chim Acta* 2020;507:17-22.