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Review

The Molecular Basis of Retinal Dystrophies in Pakistan

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Abstract: The customary consanguineous nuptials in Pakistan underlie the frequent occurrence of autosomal recessive inherited disorders, including retinal dystrophy (RD). In many studies, homozygosity mapping has been shown to be successful in mapping susceptibility loci for autosomal recessive inherited disease. RDs are the most frequent cause of inherited blindness worldwide. To date there is no comprehensive genetic overview of different RDs in Pakistan. In this review, genetic data of syndromic and non-syndromic RD families from Pakistan has been collected. Out of the 132 genes known to be involved in non-syndromic RD, 35 different genes have been reported to be mutated in families of Pakistani origin. In the Pakistani RD families 90% of the mutations causing non-syndromic RD and all mutations causing syndromic forms of the disease have not

been reported in other populations. Based on the current inventory of all Pakistani RD-associated gene defects, a cost-efficient allele-specific analysis of 11 RD-associated variants is proposed, which may capture up to 35% of the genetic causes of retinal dystrophy in Pakistan.

Keywords: inherited retinal dystrophies; homozygosity mapping; genetic testing

1. Introduction

Inherited retinal dystrophies (RD) belong to a group of clinically and genetically heterogeneous disorders [1]. The clinical sub-classification of this group of diseases is based on the nature of the disease (stationary or progressive), the inheritance pattern, and the dysfunctional part of the retina [2]. The disease is either congenital, occurring early in life, such as Leber congenital amaurosis (LCA; MIM# 204000), and congenital stationary night blindness (CSNB; MIM# 310500), or might have a later onset, such as in retinitis pigmentosa (RP; MIM# 268000), cone-rod dystrophy (CRD; MIM# 604116), and cone dystrophy (CD; MIM# 602093) [3]. In addition to disorders confined to the eye, there are syndromic forms of the disease in which retinal dystrophy is either among the primary clinical symptoms or might manifest at an advanced stage. The most common syndromic form of RD is Usher syndrome (USH; MIM# 276900), in which RP is associated with variable degrees of hearing loss and vestibular dysfunction [4]. Other types of syndromic RD include Bardet-Biedl syndrome (BBS; MIM# 209900), Senior-Loken syndrome (SLSN; MIM# 266900), Joubert syndrome (JBTS; MIM# 213300), and Meckel syndrome (MKS; MIM# 249000). All these syndromes exhibit severe clinical features in addition to retinal degeneration [5,6].

The estimated worldwide prevalence of RD is 1 in 3000 individuals [7]. RP is the most frequent phenotype among the RDs, affecting 1 in 4000 individuals [8,9]. In Pakistan the frequency of RD is not very well defined, but a hospital-based study estimated autosomal recessive RP to be the most prevalent [10]. In several developing countries, as opposed to Western countries, consanguinity has always been a major contributing factor in the high prevalence of autosomal recessive disorders [11]. In Pakistan more than 60% of marriages are consanguineous and among them about 80% are between first cousins [12]. Such consanguineous families are ideal for homozygosity based genetic mapping studies aimed at the identification of the underlying genetic defect [13,14].

As a result of several technological advances, 201 genes implicated in different forms of RD have been identified to date [15]. Among these genes, 132 are linked to non-syndromic forms of the disease with some genetic overlap between different classes [1,3,16]. In the developed countries, genetic testing using medium-to-high throughput genotyping methods are now being routinely used for proper disease diagnosis [17]. This has resulted in the establishment of many genotype-phenotype correlations [17–19]. In the last two decades, several studies have described the genetic causes of different retinal dystrophies in consanguineous Pakistani families. However, to date, there has been no comprehensive ophthalmogenetic overview of all forms of RD that have been identified in Pakistan. Therefore, this literature review provides an overview of all published genetic data of syndromic and non-syndromic RD that have been described for Pakistani families.

2. Experimental

A comprehensive literature review was performed for mutations and loci, which have been described previously for Pakistani individuals with syndromic and non-syndromic retinal diseases. The Retinal Network (RetNet) [15], National Centre for Biotechnology Information (NCBI) [20], Online Mendelian Inheritance in Man (OMIM) [21], The Human Gene Mutation Database (HGMD) [22], and published literature were used to search for the causative genes. In order to predict the pathogenicity of the reported missense mutations, *in silico* analysis including, polymorphism phenotyping (PolyPhen-2) [23], and sorting tolerant from intolerant (SIFT) [24] were performed. The frequency of these variants in the healthy population was checked via the exome variant server (EVS) [25].

3. Results

3.1. Overview of Molecular Genetic Studies in Non-Syndromic RD in Pakistan

Thus far, fifty-six studies have reported on the genetic causes of non-syndromic RD including arCRD, arCSNB, arLCA, and arRP in Pakistani persons, most of which belong to consanguineous families. The genetic data of a total of 466 Pakistani RD patients from 103 families (Tables 1 and 2), have been described in the current review. Among these retinal phenotypes, arRP was found to be the most frequently occurring RD (59%), followed by arLCA (19%), arCRD (10%), and arCSNB (9%) (Tables 1 and 2; Figure 1). Autosomal recessive inheritance seems to predominate in the RD families (96%) and only two autosomal dominant RP (adRP) families have been described (Tables 1 and 2). Of these, one adRP family carries a mutation in *RHO* (MIM# 180380) [26], while in one family a frequent variant (c.2138G>A) in *SEMA4A* (MIM# 607292) has been described to cause adRP, however *in silico* prediction and exome variant server (EVS) frequency do not support the pathogenicity of the latter variant (Table 2) [27]. The compiled data demonstrate that out of the 132 genes known to be involved in non-syndromic RD, mutations in 36 different genes are causing disease in patients of Pakistani origin (Table 1; Figure 2), reflecting the genetic heterogeneity of the disease in this population. The most frequently mutated genes were *AIPL1* (MIM# 604392), *CRB1* (MIM# 604210), *TULP1* (MIM# 602280), *RPGRIP1* (MIM# 605446), *RP1* (MIM# 180100), *SEMA4A*, *LCA5* (MIM# 611408), and *PDE6A* (MIM# 180071) (Figure 2). Most of the reported mutations, and those identified in the current cohort, were novel to this population except for mutations in *ABCA4* (MIM# 601691), *CRB1*, *CERKL* (MIM# 608381), *RPE65* (MIM# 180069), *RPGR* (MIM# 312610), and *SPATA7* (MIM# 609868), which were initially identified in persons of different ethnicity (Table 1). As expected, all the reported disease associated alleles are rare variants and *in silico* analysis predicted these variants to have a deleterious effect on protein function (Table S1).

Table 1. Mutations identified in Pakistani patients with non-syndromic retinal dystrophies.

| Gene | RefSeq Id | Nucleotide variant | Protein variant | Phenotype | # Families | # Patients | References |
|----------------|----------------|--------------------|-----------------|-----------|------------|------------|------------|
| <i>ABCA4</i> | NM_000350.2 | c.6658C>T | p.(Gln2220*) | arRP | 1 | 6 | [28,29] |
| <i>ADAM9</i> | NM_003816.2 | c.766C>T | p.(Arg256*) | arCRD | 1 | 4 | [30] |
| <i>AIPL1</i> ‡ | NM_201253.2 | c.116C>A | p.(Thr39Asp) | arLCA | 1 | 6 | [31] |
| <i>AIPL1</i> ‡ | NM_014336.3 | c.834G>A | p.(Trp278*) | EORP | 11 | 25 | [29,31–34] |
| <i>BEST1</i> ‡ | NM_001139443.1 | c.418C>G | p.(Leu140Val) | arRP | 1 | 4 | [35] |

Table 1. Cont.

| Gene | RefSeq Id | Nucleotide variant | Protein variant | Phenotype | # Families | # Patients | References |
|-----------------|----------------|--------------------|---------------------|-----------------|------------|------------|------------|
| <i>CERKL</i> | NM_001030311.2 | c.316C>A | p.(Arg106Ser) | arRP | 1 | 3 | [36] |
| <i>CERKL</i> | NM_001030311.2 | c.847C>T | p.(Arg283*) | arRP | 1 | 6 | [29,37,38] |
| <i>CLRN1</i> † | NM_001195794.1 | c.92C>T | p.(Pro31Leu) | arRP | 1 | 6 | [39] |
| <i>CLRN1</i> † | NM_001195794.1 | c.461T>G | p.(Leu154Trp) | arRP | 1 | 6 | [39] |
| <i>CNGA1</i> | NM_00142564.1 | c.626_627del | p.(Ile209Serfs*26) | arRP | 1 | 7 | [40] |
| <i>CNGA1</i> | NM_00142564.1 | c.1298G>A | P.(Gly433Asp) | arRP | 1 | 3 | [41] |
| <i>CNGA3</i> | NM_001298.2 | c.822G>T | p.(Arg274Ser) | arCRD (ACHM) | 1 | 4 | [42] |
| <i>CNGA3</i> | NM_001298.2 | c.827A>G | p.(Asn276Ser) | arCRD (ACHM) | 1 | 6 | [43] |
| <i>CNGB1</i> | NM_001297.4 | c.412-1G>A | p.(?) | arRP | 1 | 10 | [44] |
| <i>CNGB1</i> | NM_001297.4 | c.2284C>T | p.(Arg762Cys) | arRP | 1 | 5 | [44] |
| <i>CNGB1</i> | NM_001297.4 | c.2493-2A>G | p.(?) | arRP | 1 | 10 | [41] |
| <i>CNGB3</i> | NM_019098.4 | c.1825del | p.(Val609Trpfs*9) | arCRD (ACHM) | 1 | 2 | [42] |
| <i>CRB1</i> | NM_201253.2 | c.107C>G | p.(Ser36*) | arLCA | 1 | 10 | [33] |
| <i>CRB1</i> | NM_201253.2 | c.2234C>T | p.(Thr745Met) | arRP | 1 | 2 | [41,45] |
| <i>CRB1</i> | NM_201253.2 | c.2536G>A | p.(Gly846Arg) | arRP | 1 | 6 | [31] |
| <i>CRB1</i> | NM_201253.2 | c.3101T>C | p.(Leu989Thr) | arLCA | 1 | 8 | [31] |
| <i>CRB1</i> | NM_201253.2 | c.3296C>A | p.(Thr1099Lys) | arRP | 1 | 9 | [44] |
| <i>CRB1</i> | NM_201253.2 | c.3343_3352del | p.(Gly1115Ilefs*23) | arRP | 1 | 9 | [46] |
| <i>CRB1</i> | NM_201253.2 | c.3347T>C | p.(Leu1071Pro) | arRP | 1 | 7 | [31] |
| <i>CRB1</i> | NM_201253.2 | c.3962G>C | p.(Cys1321Ser) | arRP | 1 | 5 | [46] |
| <i>EYS</i> | NM_001142800.1 | c.8299G>T | p.(Asp2767Tyr) | arRP | 1 | 7 | [47] |
| <i>GNAT1</i> | NM_144499.2 | c.386A>G | p.(Asp129Gly) | arCSNB | 1 | 1 | [48] |
| <i>GRK1</i> | NM_002929 | c.614C>A | p.(Ser205*) | arCSNB (Oguchi) | 1 | 9 | [49] |
| <i>GRK1</i> | NM_002929 | c.827+623_883del | p.(?) | arCSNB (Oguchi) | 1 | 3 | [50] |
| <i>IMPG2</i> ‡ | NM_016247.3 | c.1680T>A | p.(Tyr560*) | arRP | 1 | 2 | [51] |
| <i>LCA5</i> ‡ | NM_181714.3 | c.643del | p.(Leu215Tyrfs*11) | arLCA | 1 | 4 | [52] |
| <i>LCA5</i> ‡ | NM_181714.3 | c.1151del | p.(Pro384Glnfs*17) | arLCA | 3 | 13 | [33,53] |
| <i>MERTK</i> | NM_00634.2 | c.718G>T | p.(Glu240*) | arRP | 1 | 4 | [54] |
| <i>NMNAT1</i> ‡ | NM_022787.3 | c.25G>A | p.(Val9Met) | arLCA | 1 | 5 | [55] |
| <i>NMNAT1</i> ‡ | NM_022787.3 | c.838T>C | p.*280Glnext*16 | arLCA | 1 | 8 | [56] |
| <i>PDE6A</i> | NM_000440.2 | c.889C>T | p.(Gly297Ser) | arRP | 1 | 4 | [57] |
| <i>PDE6A</i> | NM_000440.2 | c.1264-2A>G | p.(?) | arRP | 1 | 5 | [57] |
| <i>PDE6A</i> | NM_000440.2 | c.1630C>T | p.(Arg544Trp) | arRP | 1 | 3 | [29] |
| <i>PDE6A</i> | NM_000440.2 | c.2218_2219insT | p.(Ala740Valfs*2) | arRP | 1 | 3 | [57] |
| <i>PDE6B</i> | NM_000283.3 | c.1160C>T | p.(Pro387Leu) | arRP | 1 | 6 | [58] |
| <i>PDE6B</i> | NM_000283.3 | c.1655G>A | p.(Arg552Gln) | arRP | 1 | 9 | [58] |
| <i>PDE6B</i> | NM_000283.3 | c.1722+1G>A | p.(?) | arRP | 1 | 4 | [44] |
| <i>PROM1</i> | NM_006017.2 | c.1726C>T | p.(Gln576*) | arRP | 1 | 6 | [59] |
| <i>RDH12</i> | NM_152443.2 | c.506G>A | p.(Arg169Gln) | arLCA/EORD | 2 | 2 | [60] |
| <i>RDH12</i> | NM_152443.2 | c.619A>G | p.(Asn207Asp) | arLCA/EORD | 1 | 1 | [60] |
| <i>RDH5</i> | NM_001199771.1 | c.758T>G | p.(Met253Arg) | arCSNB (FA) | 1 | 6 | [61] |
| <i>RDH5</i> | NM_001199771.1 | c.913_917del | p.(Val305Hisfs*29) | arCSNB (FA) | 1 | 2 | [61] |
| <i>RHO</i> | NM_000539.3 | c.448G>A | p.(Glu150Lys) | arRP | 2 | 6 | [62] |
| <i>RHO</i> | NM_000539.3 | c.1045T>G | p.(*349Gluext*52) | adRP | 1 | 8 | [26] |
| <i>RLBP1</i> | NM_000326.4 | c.346G>C | p.(Gly116Arg) | FA | 1 | 4 | [63] |
| <i>RLBP1</i> | NM_000326.4 | c.466C>T | p.(Arg156*) | FA | 1 | 6 | [63] |
| <i>RP1</i> | NM_006269.1 | c.1458_1461dup | p.(Glu488*) | arRP | 2 | 9 | [64,65] |
| <i>RP1</i> | NM_006269.1 | c.4555del | p.(Arg1519Glufs*2) | arRP | 1 | 5 | [65] |
| <i>RP1</i> | NM_006269.1 | c.5252del | p.(Asn1751Ilefs*4) | arRP | 1 | 4 | [65] |
| <i>RPE65</i> | NM_000329.2 | c.131G>A | p.(Arg44Gln) | EORP | 1 | 3 | [41,66,67] |
| <i>RPE65</i> | NM_000329.2 | c.361del | p.(Ser121Leufs*6) | EORP | 1 | 4 | [41,67] |

Table 1. Cont.

| Gene | RefSeq Id | Nucleotide variant | Protein variant | Phenotype | # Families | # Patients | References |
|------------------|----------------|--------------------|--------------------|--------------|------------|------------|------------|
| <i>RPE65</i> | NM_000329.2 | c.751G>T | p.(Val251Phe) | arLCA | 1 | 6 | [33] |
| <i>RPGR</i> | NM_001034853.1 | c.2426_2427del | p.(Glu809Glyfs*25) | xLRP | 1 | 8 | [41,68] |
| <i>RPGRIP1</i> | NM_020366.3 | c.587+1G>C | p.(?) | arLCA | 1 | 1 | [33] |
| <i>RPGRIP1</i> | NM_020366.3 | c.1180C>T | p.(Gln394*) | arLCA | 1 | 1 | [33] |
| <i>RPGRIP1</i> | NM_020366.3 | c.2480G>T | p.(Arg827Leu) | arCRD, arLCA | 2 | 9 | [33,69] |
| <i>RPGRIP1</i> | NM_020366.3 | c.3620T>G | p.(Leu1207*) | arLCA | 1 | 1 | [33] |
| <i>SAG</i> | NM_000541.4 | c.916G>T | p.(Glu306*) | arCSNB | 1 | 1 | [70] |
| <i>SEMA4A</i> † | NM_022367.3 | c.1033G>C | p.(Asp345His) | arCRD, arRP | 4 | 4 | [27] |
| <i>SEMA4A</i> † | NM_022367.3 | c.1049T>G | p.(Phe350Cys) | arCRD, arRP | 4 | 4 | [27] |
| <i>SLC24A1</i> † | NM_004727.2 | c.1613_1614del | p.(Phe538Cysfs*23) | arCSNB | 1 | 5 | [71] |
| <i>SPATA7</i> | NM_018418.4 | c.253C>T | p.(Arg85*) | arLCA/arRD | 2 | 3 | [72] |
| <i>SPATA7</i> | NM_018418.4 | c.960dup | p.(Pro321Thrfs*6) | arLCA/arRD | 1 | 6 | [72,73] |
| <i>TTC8</i> † | NM_144596.2 | c.115-2A>G | p.(?) | arRP | 1 | 4 | [74] |
| <i>TULP1</i> | NM_003322.3 | c.1138A>G | p.(Thr380Ala) | arRP | 3 | 34 | [33,75,76] |
| <i>TULP1</i> | NM_003322.3 | c.1445G>A | p.(Arg482Gln) | arRP | 1 | 8 | [75] |
| <i>TULP1</i> | NM_003322.3 | c.1466A>G | p.(Lys489Arg) | arRP | 4 | 19 | [41,76,77] |
| <i>ZNF513</i> | NM_144631.5 | c.1015T>C | p.(Cys339Arg) | arRP | 1 | 4 | [78,79] |

ACHM, achromatopsia; ad, autosomal dominant; ar, autosomal recessive; CSNB, congenital stationary night blindness; CRD, cone rod dystrophy; EORD, early onset retinal dystrophy; EORP, early onset RP; FA, fundus albipunctatus; LCA, Leber congenital amaurosis; RD, retinal dystrophy; RefSeq Id, reference sequence identifier; RP, retinitis pigmentosa; xLRP, X-linked RP; † novel gene identification; † novel phenotype association.

Out of the 47 non-synonymous variants identified in Pakistani non-syndromic RD families (Table 1) three variants (*SEMA4A*, c.2138G>A; *RPI*, c.1118C>T; *RPGRIP1*, c.1639G>T), are reported as single nucleotide polymorphisms (SNP) with high frequencies in the EVS (Table 2) [27,64,69]. In addition, SIFT also predicts these changes to be tolerated while except for the *RPGRIP1* variant, the other two are considered to be benign by PolyPhen-2 (Table 2). Therefore, these variants could be segregating with the disease in the family by chance and the causative mutation may reside in another gene.

Table 2. Common variants reported as mutations in Pakistani patients with non-syndromic retinal dystrophies and their *in silico* pathogenicity prediction.

| Gene | RefSeq Id | Nucleotide variant | Protein variant | Phenotype | # Families | # Patients | Ref. | phyloP | Grantham distance | PolyPhen | SIFT | EVS |
|----------------|-------------|--------------------|-----------------|-----------|------------|------------|------|--------|-------------------|--------------------------|------------------|---|
| <i>RPI</i> | NM_006269.1 | c.1118C>T | p.(Thr373Ile) | arRP | 2 | 11 | [64] | 0.61 | 89 | Benign (0.01) | Tolerated (0.50) | T = 152; C = 12,854 (rs77775126) |
| <i>RPGRIP1</i> | NM_020366.3 | c.1639G>T | p.(Ala547Ser) | arCRD | 3 | 12 | [69] | 0.29 | 99 | Probably damaging (1.00) | Tolerated (0.49) | T = 2,792; G = 9,214 (rs10151259) |
| <i>SEMA4A</i> | NM_022367.3 | c.2138G>A | p.(Arg713Gln) | adRP | 1 | 4 | [27] | 1.25 | 43 | Benign (0.23) | Tolerated (0.43) | A = 451; G = 12,555 (rs41265017) |

Ad, autosomal dominant; ar, autosomal recessive; CRD, cone-rod dystrophy; EVS, exome variant server; PolyPhen, polymorphism phenotyping; RefSeq Id, reference sequence identifier; RP, retinitis pigmentosa; SIFT, sorting tolerant from intolerant.

Figure 1. Distribution of non-syndromic Pakistani RD families according to their phenotypes. Ad, autosomal dominant; ar, autosomal recessive; CRD, cone-rod dystrophy; CSNB, congenital stationary night blindness; LCA, Leber congenital amaurosis; RP, retinitis pigmentosa; xl, X-linked.

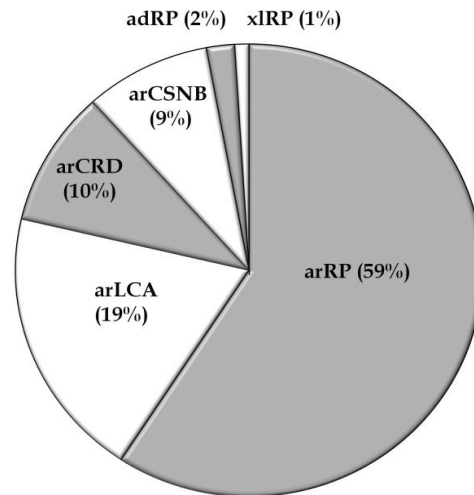
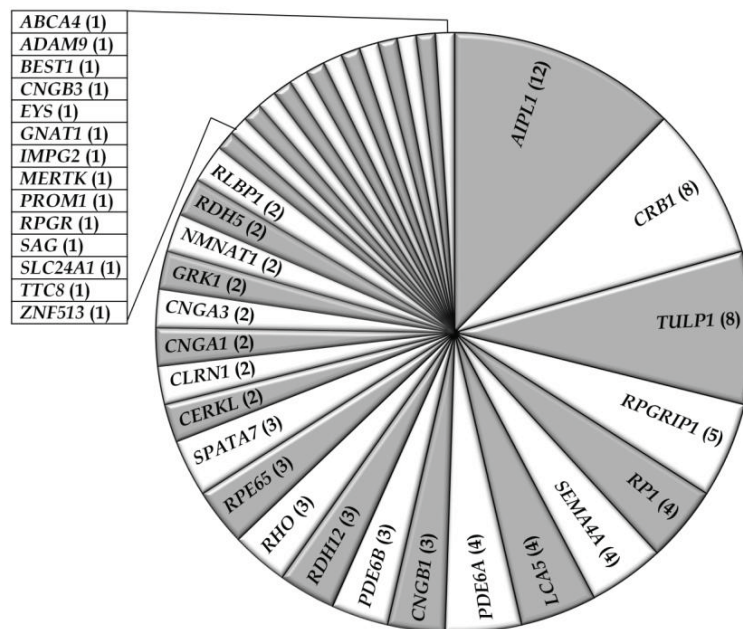


Figure 2. Occurrence of gene defects in non-syndromic RD families in Pakistan. Numbers of families with mutations in respective genes are indicated between parentheses.



3.2. Overview of Molecular Genetic Studies in Syndromic RDs in Pakistan

In addition to the non-syndromic families, data of 52 syndromic RD families with a total of 139 affected individuals were collected from 22 studies. Usher syndrome represented about 36% of the families in this group, whereas BBS (33%), MKS (13%), JBTS (10%), and SLSN (8%), accounted for the other families (Table 3; Figure 3). The most commonly mutated gene associated with syndromic RD in the Pakistani population was cadherin 23 (*CDH23*; MIM# 605516), which has been reported to

be mutated in persons with Usher type 1, followed by *TMEM67* (MIM# 609884), the gene mutated in persons with autosomal recessive MKS (Table 3; Figure 4). As expected for the syndromic mutations, all the reported disease associated alleles are rare variants and *in silico* analysis predicted these variants to have a deleterious effect on protein function (Table S2).

4. Discussion

The Pakistani population is known for its high rate of consanguinity (>60%), but it is still remarkable that 97% of the families with inherited RDs had an autosomal recessive mode of inheritance. It is, therefore, not surprising that Pakistani families have been instrumental in pinpointing a number of the underlying gene defects through homozygosity mapping [80,81]. Genetic studies of Pakistani families with RD have previously facilitated the identification of eleven novel RD genes, *i.e.*, *AIPL1* [34], *BEST1* [35], *CC2D2A* (MIM# 612013) [82], *CDH23* (MIM# 605516) [83], *IMPG2* (MIM# 607056) [51], *LCA5* (MIM# 611408) [53], *NMNAT1* (MIM:608700) [55,56], *ZNF513* (MIM# 613598) [78], *PCDH15* (MIM# 605514) [84], *SEMA4A* [27], and *SLC24A1* (MIM# 603617) [71]. In addition, mutations in *CLRN1* (MIM# 606397) and *TTC8* (MIM# 608132), which had been previously implicated in the syndromic retinal phenotypes USH3 (MIM# 276902), and BBS (MIM# 209900), respectively, were found to cause non-syndromic arRP [39,74]. Mutations in *RPI*, which had previously been shown to be involved in adRP, were found to segregate in a recessive manner in 3 Pakistani families [64]. In addition to the novel genes identified in the affected Pakistani families, five novel RD loci including three non-syndromic, *i.e.*, *CORD8* (MIM# 605549), [85], *RP29* (MIM# 612165), [86], and *RP32* [87], and two syndromic, *i.e.*, *USH1H* (MIM# 612632), [88], and *USH1K* [89], have also been identified in Pakistani families.

Table 3. Mutations identified in Pakistani patients with syndromic retinal dystrophies.

| Gene | RefSeq Id | Nucleotide variant | Protein variant | Phenotype | # Families | # Patients | References |
|-----------------|----------------|--------------------|-------------------|-----------|------------|------------|------------|
| <i>AH11</i> | NM_017651.4 | c.2370dup | p.(Lys791*) | arJBTS | 1 | 2 | [90] |
| <i>ARL6</i> | NM_032146.3 | c.281T>C | p.(Ile94Thr) | arBBS | 1 | 5 | [91] |
| <i>ARL6</i> | NM_032146.3 | c.123+1119del | p.(?) | arBBS | 1 | 1 | [92] |
| <i>ARL13B</i> | NM_182896.2 | c.236G>A | p.(Arg79Gln) | arJBTS | 1 | 3 | [93] |
| <i>BBS1</i> | NM_02464.9.4 | c.47+1G>T | p.(?) | arBBS | 1 | 2 | [94] |
| <i>BBS1</i> | NM_02464.9.4 | c.442G>A | p.(Asp148Asn) | arBBS | 1 | 2 | [94] |
| <i>BBS2</i> | NM_031885.3 | c.1237C>T | p.(Arg413*) | arBBS | 1 | 1 | [95] |
| <i>BBS5</i> | NM_152384.2 | c.2T>A | p.(Met1Lys) | arBBS | 2 | 2 | [95] |
| <i>BBS10</i> | NM_024685.3 | c.271dup | p.(Cys91Leufs*5) | arBBS | 2 | 4 | [96] |
| <i>BBS10</i> | NM_024685.3 | c.1075C>T | p.(Gln359*) | arBBS | 1 | 7 | [91] |
| <i>BBS10</i> | NM_024685.3 | c.1091del | p.(Asn364Thrfs*5) | arBBS | 1 | 1 | [96] |
| <i>BBS10</i> | NM_024685.3 | c.1958_1967del | p.(Ser653Ilefs*4) | arBBS | 1 | 2 | [97] |
| <i>BBS10</i> | NM_024685.3 | c.2121dup | p.(Lys708*) | arBBS | 1 | 1 | [96] |
| <i>BBS12</i> | NM_152618.2 | c.1589T>C | p.(Leu530Pro) | arBBS | 2 | 2 | [95] |
| <i>BBS12</i> | NM_152618.2 | c.2102C>A | p.(Ser701*) | arBBS | 1 | 3 | [98] |
| <i>CC2D2A</i> † | NM_001080522.2 | c.2003+1G>C | p.(?) | arJBTS | 1 | 5 | [82] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.1114C>T | p.(Gln372*) | arUSH1 | 1 | 3 | [83] |
| <i>CDH23</i> | NM_022124.5 | c.2587+1G>A | p.(?) | arUSH1 | 1 | 4 | [99] |

Table 3. Cont.

| Gene | RefSeq Id | Nucleotide variant | Protein variant | Phenotype | # Families | # Patients | References |
|-----------------|----------------|--------------------------|-------------------------|-----------|------------|------------|------------|
| <i>CDH23</i> | NI | NI | p.(Arg1305*) | arUSH1 | 1 | 4 | [99] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.3106_3106+11delinsTGGT | p.(Gly1036delinsTrpCys) | arUSH1 | 1 | 5 | [83] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.6050-9G>A | p.(?) | arUSH1 | 4 | 13 | [83] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.6050-1G>C | p.(?) | arUSH1 | 1 | 6 | [83] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.6054_6074del | p.(Val2019_Val2025del) | arUSH1 | 1 | 3 | [83] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.6845del | p.(Asn2282Thrfs*91) | arUSH1 | 1 | 3 | [83] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.7198C>T | p.(Pro2400Ser) | arUSH1 | 1 | 4 | [83] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.8150A>G | p.(Asp2717Gly) | arUSH1 | 1 | 3 | [83] |
| <i>CDH23</i> ‡ | NM_022124.5 | c.8208_8209del | p.(Val2737Alafs*2) | arUSH1 | 2 | 11 | [83] |
| <i>CEP290</i> | NM_025114.3 | c.5668G>T | p.(Gly1890*) | arJBTS | 1 | 1 | [100,101] |
| <i>IQCB1</i> | NM_001023570.2 | c.488-1G>A | p.(?) | arSLSN | 1 | 1 | [41,102] |
| <i>IQCB1</i> | NM_001023570.2 | c.1465C>T | p.(Arg489*) | arSLSN | 1 | 1 | [102] |
| <i>IQCB1</i> | NM_001023570.2 | c.1796T>G | p.(*599Serext*2) | arSLSN | 1 | 1 | [102] |
| <i>NPHP4</i> | NM_015102.3 | c.3272dup | p.(Ser1092Valfs*11) | arSLSN | 1 | 1 | [102] |
| <i>PCDH15</i> ‡ | NM_001142763.1 | c.7C>T | p.(Arg3*) | arUSH1 | 1 | 5 | [84] |
| <i>PCDH15</i> ‡ | NM_001142763.1 | c.1927C>T | p.(Arg643*) | arUSH1 | 1 | 3 | [103] |
| <i>PCDH15</i> ‡ | NM_001142763.1 | c.3389-2A>G | p.(?) | arUSH1 | 1 | 3 | [84] |
| <i>TCTN2</i> | NM_024809.3 | c.1873C>T | p.(Gln625*) | arJBTS | 1 | 4 | [104] |
| <i>TMEM67</i> | NM_153704.5 | c.647del | p.(Val217Leufs*5) | arMKS | 1 | 2 | [105] |
| <i>TMEM67</i> | NM_153704.5 | c.715-2A>G | p.(?) | arMKS | 1 | 1 | [105] |
| <i>TMEM67</i> | NM_153704.5 | c.1127A>C | p.(Gln376Pro) | arMKS | 2 | 2 | [105] |
| <i>TMEM67</i> | NM_153704.5 | c.1575+1G>A | p.(?) | arMKS | 3 | 5 | [105] |
| <i>TTC8</i> | NM_144596.2 | c.1049+2_1049+4del | p.(?) | arBBS | 1 | 3 | [106] |
| <i>USH1G</i> | NM_173477.2 | c.163_164+13del | p.(Gly56*) | arUSH1 | 1 | 4 | [107] |

Ar, autosomal recessive; BBS, Bardet-Biedl syndrome; JBTS, Joubert syndrome; MKS, Meckel syndrome; NI, not indicated; RefSeq Id, reference sequence identifier; SLSN, Senior-Loken syndrome; USH1, Usher syndrome type 1; ‡ novel gene identification; † novel phenotype association.

Figure 3. Prevalences of syndromic RD phenotypes. BBS, Bardet-Biedl syndrome; JBTS, Joubert syndrome; MKS, Meckel syndrome; SLS, Senior-Loken syndrome; USH, Usher syndrome.

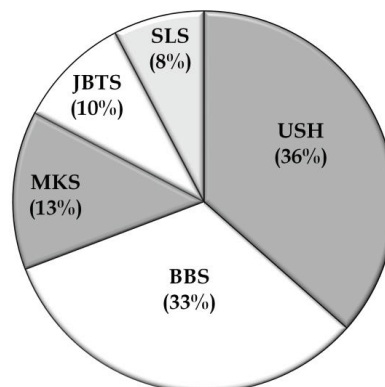
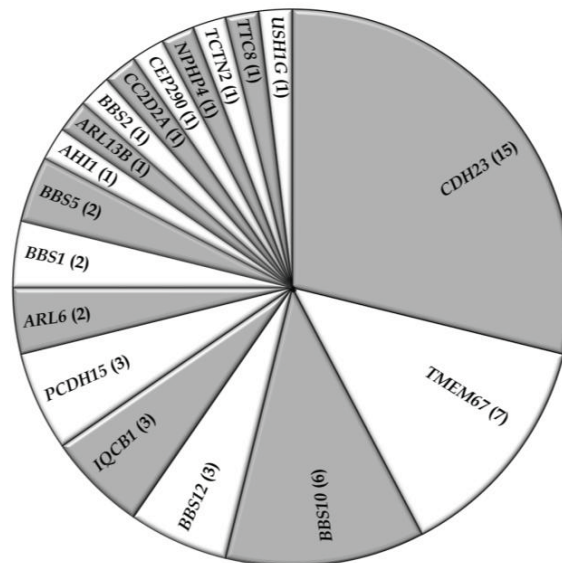


Figure 4. Occurrence of gene defects in syndromic RD families in Pakistan. Numbers of families with mutations in respective genes are indicated between parentheses.



In the 103 non-syndromic Pakistani RD families described so far, mutations were most frequently found in *AIPL1*, *CRB1*, *TULP1*, *RPGRIP1*, *RP1*, *SEMA4A*, *LCA5*, and *PDE6A* (Table 1; Figure 2). A direct comparison with other RD populations is difficult as comprehensive studies of this kind are rare. In a recent study of Abu-Safieh *et al.* (2012) comprising 150 Saudi Arabian RD families, similar results were observed as *RP1*, *TULP1*, *RPGRIP1*, and *CRB1* were found to be the most frequently mutated genes [108].

A worldwide general literature study revealed arRP-associated mutations distributed in *USH2A* (12%; MIM# 276901), *ABCA4* (8%), *PDE6B* (7%; MIM# 180072), *CNGB1* (6%), and *PDE6A* (5%; MIM# 180071) [109]. In a more recent study of 230 Dutch persons with isolated or arRP [110], the most frequently mutated genes were *EYS* (11%; MIM# 602772), and *CRB1* (11%) followed by *USH2A* (10%), *ABCA4* (9%), and *PDE6B* (7%). As opposed to these studies the absence of *USH2A* variants in individuals of Pakistani origin is probably due to the fact that the most frequent arRP-associated variant, c.2299del;p.(E767fs), is almost invariably found in compound heterozygous states with second mutations that are considered to be mild [111], precluding their detection in a homozygosity mapping approach. Other differences can only be attributed to divergent genetic backgrounds of these populations [112,113].

Although 113/118 variants listed in Tables 1 and 3 have only been identified in Pakistani patients, seven variants (*SEMA4A*, p.(Asp345His) and p.(Phe350Cys); *TULP1*, p.(Thr380Ala); *LCA5*, p.(Pro384Glnfs*17); *RPGRIP1*, p.(Arg827Leu); *TMEM67*, c.1575+1G>A and p.(Gln37Pro)), are more frequent than others, and therefore they seem to be population-specific. The six most frequent variants, p.(Trp278*) in *AIPL1*, p.(Lys489Arg) and p.(Thr380Ala) in *TULP1*, p.(Asp345His) and p.(Phe350Cys) in *SEMA4A*, p.(Pro384Glnfs*17) in *LCA5* (Table 1), explain about 25% of the non-syndromic Pakistani RD families. The p.Trp278* variant has been identified as the most frequent *AIPL1* variant worldwide in many LCA studies [114,115], suggesting that this variant is relatively old. The six frequent variants mentioned above, together with five other variants in *RDH12* (MIM# 608830),

p.(Arg169Gln); *RHO*, p.(Glu150Lys); *RPI*, p.(Glu488*), *RPGRIP1*, p.(Arg827Leu), and *SPATA7*, p.(Arg85*), account for approximately 34% (35/103) of all non-syndromic RD families from Pakistan. A cost-effective initial genetic screening of Pakistani persons with RD therefore could be to analyze these variants using Sanger sequencing. For example, 10 amplicons covers the most frequent variants mentioned above. Alternatively, a larger subset of variants can be captured by arrayed primer extension (APEX) analysis or other allele-specific genotyping methods [116–119].

Three of the 47 missense mutations (*RPI*: c.1118C>T, *RPGRIP1*: c.1639G>T, *SEMA4A*: c.2138G>A) reported to be associated with RD in Pakistani families are found at higher frequencies in EVS. *In silico* analysis also predict them likely to be non-pathogenic, therefore they should be considered as non-causative (Table 2) [27,64,69]. As these variants on their own are not sufficient to explain the phenotype in these six families (two, three and one with *RPI*, *RPGRIP1* and *SEMA4A* mutations, respectively) they must still be considered genetically unresolved.

Of all the non-syndromic and syndromic arRD families ($n = 146$), which are genetically resolved, compound heterozygous mutations were identified in only four non-syndromic RD families ($4/146 = 2.7\%$). These compound heterozygous mutations were identified in *SEMA4A*. This finding on one hand favors the utility of homozygosity based gene identification strategies for Pakistani RD families. While on the other hand it also indicates that in a small but significant proportion of the families ($\sim 2/100$), compound heterozygous mutations might be able to explain the phenotype. These mutations will certainly be overlooked if one only considers homozygosity mapping based approaches to pinpoint causative genetic defects.

5. Conclusions

This review provides a comprehensive overview of genetic causes of non-syndromic and syndromic retinal diseases in Pakistan, the results of which can be used to design a cost-effective screening platform for future genetic testing in Pakistan. For genetically unsolved non-syndromic RD cases, we propose a sequencing-based pre-screening genetic test in which 10 different amplicons capture the most frequent mutations described for Pakistani RD patients. In consanguineous families, homozygosity directed sequence analysis has demonstrated its potential to unravel genetic defect underlying recessive diseases.

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Author Contributions

Conception and design: FPMC, MIK, MAz, RQ, RWJC, and AIH; collected the data: MIK, MAz, and MAj; wrote the manuscript: MIK, MAz, MAj, RWJC, AIH, FPMC, and RQ.

Conflicts of Interest

The authors declare no conflict of interest.

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