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EXPLORING THE GENETIC CAUSES OF NON-SYNDROMIC RETINAL DYSTROPHIES IN QATAR

BY

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ABSTRACT

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Title: Exploring the Genetic Causes of Non-syndromic Retinal Dystrophies in Qatar.

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Background. Non-syndromic retinal dystrophies (RDs) are a set of degenerative retinal diseases that vary clinically and genetically. RDs comprise several overlapping disorders such as Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP). RDs are a major cause of vision loss in young adults globally. RDs are genetically heterogeneous and to date, over 250 genes have been associated with the disease pathogenesis.

Aim. This study aims to investigate the genetic basis of non-syndromic RDs in the population of Qatar and to assess the diagnostic yield of the different genetic tests available through a retrospective cohort study.

Methods. A retrospective chart review was conducted to investigate the genetic basis of non-syndromic RDs in Qatar. Data were collected from physical and electronic medical records of patients seen at the Department of Adult and Pediatric Medical Genetics, at Hamad Medical Corporation between "2015" and "2022".

Results. Our study identified 49 eligible patients with a total of 55 variants in 32 RDs-related genes. Qatari patients contributed the most to the study (61.2%). Rod-dominated phenotypes accounted for half (51%) of hereditary retinal diseases in our study cohort. Out of the 49 cases, 38 were solved, where the genetic test identified causative variants explaining the patient's phenotype. Whole exome sequencing and mitochondrial genome testing (WES Plus) was the most utilized test. In our study, the *ABCA4* gene exhibited the highest number of causal variants, with four identified as pathogenic or

likely pathogenic. Among these variants, the c.5882 G>A variant was the most frequently reported.

Conclusions. In conclusion, certain genes have recurrent variations that are most likely the result of regional founder effects. The study also highlighted the patient's preference for WES as first-tier genetic testing in non-syndromic RDs cases. Moreover, family segregation studies play a major role in identifying possible causative variants. The clinical implications of these findings hold promise for improving patient care and management in the field of non-syndromic RDs. More investigation in the geographical region is required before conclusive generalizations. This is the first study of its sort to be conducted in Qatar, and it lays the groundwork for additional research on the epidemiology and genetics of non-syndromic RDs in Qatar.

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TABLE OF CONTENTS

LIST OF TABLES

LIST IOF FIGURES

CHAPTER 1: INTRODUCTION

Retinal dystrophies (RDs) are a set of degenerative retinal diseases that vary clinically and genetically (Nash et al., 2015). Retinal dystrophies (RDs) comprise a number of overlapping disorders such as Leber congenital amaurosis (LCA), Stargardt disease, and retinitis pigmentosa (RP). Common symptoms of RDs include night blindness or color blindness, peripheral vision impairments, and in progressive conditions, this can lead to complete blindness (Khan, 2020b). RDs can be inherited in various patterns (autosomal dominant, autosomal recessive, or X-linked) (Ziccardi et al., 2019). The precise incidence of retinal dystrophies in Qatar is unknown, but the most common form, retinitis pigmentosa, affects approximately one in every 3000 people worldwide (Fahim et al., 1993). Inherited retinal disease is a significant contributor to vision loss in young adults worldwide, with an estimated frequency of up to 1:2,000. Other dystrophies, such as achromatopsia, are rarer, with an incidence of one in every 100,000 people worldwide (Fahim et al., 1993). The majority of RDs cases manifest as non-syndromic, where the retina is only affected. However, in syndromic conditions, additional organs and tissues can be involved (Werdich et al., 2014).

The genetics of retinal dystrophies is complex. Genetic heterogeneity characterizes RD, where pathogenic variation in different genes can cause the same condition, and the same gene can be linked to multiple diseases. To date, over 250 genes have been identified (Nash et al., 2015).

RDs are categorized based on which photoreceptor cells are affected and the extent of the atrophy in the retina (Sung & Chuang, 2010). The two primary cellular units in the retina are the rods and cones photoreceptors, which are responsible for converting light energy into neuronal action potentials and allowing the brain to interpret images (Sung & Chuang, 2010). RDs are typically grouped into three types

based on their impact on photoreceptors: generalized degenerations affecting both rods and cones, diseases primarily affecting rods, and diseases primarily affecting cones (Nash et al., 2015).

In this study, we examine the genetic underpinnings of non-syndromic RDs by analyzing genetic variants and identifying genes that are linked to the disease in the population of Qatar. Additionally, we assess the effectiveness of various genetic tests in diagnosing RDs.

Study hypothesis

Studying the genetic basis of non-syndromic RDs in the population of Qatar will provide a better understanding of the molecular spectrum of RDs in the population of Qatar. As a result, this will assist in providing proper genetic counseling services to patients and families with or suspected to have RDs. The assessment of genetic test diagnostic yield will help to identify the best genetic testing options for RDs in Qatar.

Study aim

This work aims to explore the genetic basis of RDs in Qatar and assess the diagnostic yield of different genetic tests.

Study objectives

a- To understand the molecular spectrum of RDs in Qatar.

b- To explore the genetic basis of RDs in Qatar.

c- To assess the diagnostic yield of different genetic tests available in Qatar, including familial targeted testing, gene panel testing, whole exome sequencing (WES), and WES Plus (WES and mitochondrial genome sequencing).

CHAPTER 2: LITERATURE REVIEW

Retinal dystrophies (RDs) are a diverse category of genetic conditions that causes gradual and severe vision loss by affecting the retina's structure and/or function (Nash et al., 2015). Common manifestations of RDs include night blindness or color blindness, peripheral vision impairments, and in progressive conditions, this can lead to complete blindness (Nash et al., 2015). The majority of RDs cases manifest as non-syndromic, where only the retina is affected. However, in syndromic forms of RDs, additional organs and tissues are affected such as the cardiovascular system, ears, kidneys, and central nervous system (Werdich et al., 2014). Inherited retinal disease is a significant contributor to vision loss in young adults worldwide, with an estimated frequency of up to 1:2,000 (Khan, 2020b).

2.1. Genetics of Retinal Dystrophies

RDs comprise several overlapping disorders such as leber congenital amaurosis (LCA), Stargardt disease, and retinitis pigmentosa (RP) (Chiang et al., 2015). RDs can be inherited in various patterns including autosomal dominant, autosomal recessive, mitochondrial, or X-linked, and may also be sporadic (Ziccardi et al., 2019). RDs are genetically heterogeneous, as they may result from pathogenic variants in different genes. To date, over 300 genes encoding proteins that are expressed in the retina at different levels have been linked to RDs (Ziccardi et al., 2019). Most of the genes encode proteins that have a direct role as photoreceptors, and many of them are expressed in the retinal pigment epithelium, the supportive tissue required for efficient photoreceptors function (Hamel, 2014). Another major group of RD genes codes for proteins involved in cell metabolism, which are not specific to the retina, and these are commonly associated with the syndromic forms of retinopathies (Hamel, 2014). Moreover, studies have also revealed mitochondrial genetic instability as a contributor

to retinal disease (Lefevere et al., 2017). The fact that mitochondria regulate cellular activities including energy production, metabolic regulation, and apoptosis makes them essential for cellular survival and function. Previous investigations showed a link between variants in the mitochondrial genome and monogenic disease, including Leber hereditary optic neuropathy, mitochondrial myopathies, and Kearns-Sayre syndrome (Birtel et al., 2022). Mild to severe symptoms and restricted genotype-phenotype correlations are present in mitochondrial disorders, which can affect one or more organs (Birtel et al., 2022). However, since mitochondrial impairments are more likely to affect multiple tissues, the optic nerves, extraocular muscles, retina, and even retro chiasmal visual pathways, mitochondrial variants are seen mostly in syndromic forms of RDs (Lefevere et al., 2017).

2.2. The different types of Retinal Dystrophies

RDs are classified based on the photoreceptor cells involved and the extent of the atrophy in the retina (Sung & Chuang, 2010). As shown in Figure 1, rod and cone photoreceptors (Figure 1) are the key cellular units in the retina that convert the light energy to a neuronal action potential and allow a picture to be interpreted in the brain (Sung & Chuang, 2010). There are three main types of non-syndromic RDs: generalized retinal degenerations affecting both rod and cone photoreceptors; rod-dominated diseases; and cone-dominated diseases (Nash et al., 2015).

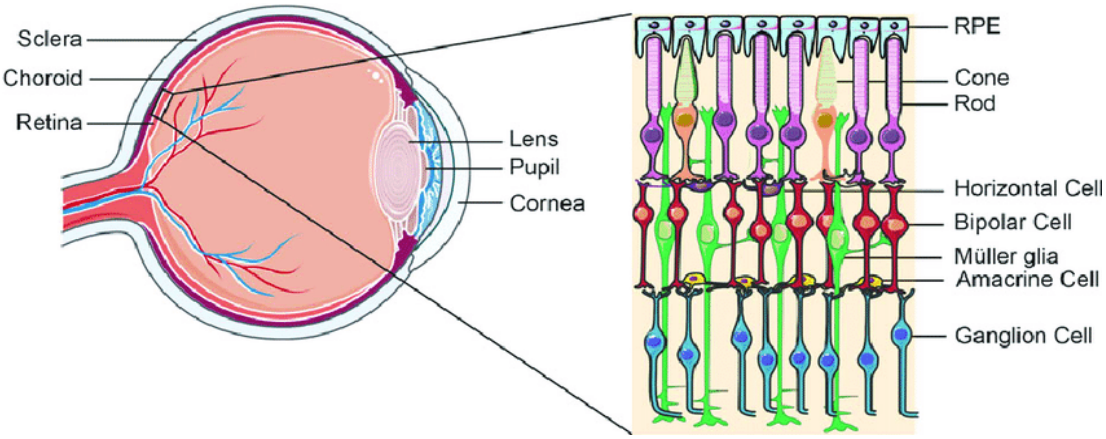

Figure 1. Illustration of the magnified area which represents different cell types in the retina (Fu et al., 2018).

2.2.1. Rod and rod-cone dystrophies

In rod and rod-cone dystrophies, the primary photoreceptors damaged are the rods, or they are the first to be affected (Nash et al., 2015). The damage to the rods leads to reduced peripheral vision, poor night vision, and difficulties with dark adaptation, which can significantly impact daily activities such as driving and navigation (Nash et al., 2015). This category of diseases is further subdivided into stationary forms such as congenital stationary night blindness (CSNB) and progressive forms such as RP (Hamel, 2014).

2.2.1.1. Stationary Rod and rod-cone dystrophies

CSNB refers to a genetically variable non-progressive type of night blindness, also known as nyctalopia (Torben Bech-Hansen et al., 1998). Individuals frequently report symptoms early in life that manifests in the form of night or dim light vision disruption or delayed dark adaption. However, photophobia has also been recorded in a subset of patients. Some types are linked to other ocular symptoms, such as low visual acuity, nystagmus, and strabismus (Boycott et al., 2001). When tested with an electroretinogram (ERG), photoreceptor function may indicate the lack of rod pathway

function or incomplete rod and cone malfunction (Nash et al., 2015). Pathogenic variants in at least 11 different genes have been found in patients with CSNB (Nash et al., 2015). Although autosomal recessive and autosomal dominant inheritance patterns have been described, X-linked inheritance is the most common for CSNB (Zeitz et al., 2015). The incomplete X-linked type of CSNB (CSNB2), is the form in which patients' ERGs show some measurable rod response. CSNB2 results from pathogenic variants in the *CACNA1F* gene. This gene codes for the α1F subunit of calcium channels that are found in the synaptic connection between the bipolar cell and the rod photoreceptor. The calcium channel functions in regulating the glutamate release into the synaptic cleft (Torben Bech-Hansen et al., 1998). It has been demonstrated that loss of function variants in this gene affect calcium ions flow at this synapse, resulting in a dysfunctional channel and hence loss of photoreceptor function (Mansergh et al., 2005). On the other hand, the complete X-linked type of CSNB (CSNB1) results from pathogenic variants in the *NYX* gene (Bech-Hansen et al., 2000). Even though the precise mechanism is unknown, the encoded protein nyctalopin is a small leucine-rich proteoglycan believed to influence bipolar, amacrine, and ganglion cell signaling when altered (Bech-Hansen et al., 2000). Truncated proteins are often nonfunctional and would be predicted to result in the observed phenotype. With the use of ERG, CSNB1 is distinguishable from the CSNB2 phenotype since in CSNB1, both cone and rod photoreceptor functions are compromised (Bech-Hansen et al., 2000).

2.2.1.2. Progressive Rod and rod-cone dystrophies

RP is a progressive type of retinal dystrophy characterized by continuous degeneration of rod and cone photoreceptors, resulting in night blindness and, eventually, loss of vision (Ali et al., 2017). RP is the most prevalent form of RDs with a combined frequency of 1 in 3000 individuals (Fahim et al., 1993). The pace of the

disease progression is determined by the age at which the symptoms appear. Juvenile RP patients experience symptoms during their first years of life, but late-onset RP patients experience symptoms much later (Nash et al., 2015). Clinical manifestations include progressive loss of the capacity to see in low light, leading to nyctalopia or night blindness, followed by the loss of peripheral vision that gradually invades the center of the visual field, culminating in tunnel vision and ultimately, vision loss (Nash et al., 2015). Generally, RP is classified into three stages: early, intermediate, and late (Verbakel et al., 2018a). The manifestations get more prevalent from the early to late stages of the disease (Verbakel et al., 2018a). In the early stages, the primary symptom is nyctalopia. Most individuals do not notice nyctalopia until the mid or end-stage since their quality of life is not adversely affected (Zhang, 2016). In the intermediate stage, nyctalopia becomes more evident, and type III color blindness (yellow, blue) develops alongside photophobia (Ali et al., 2017). During this phase, pigment release from the retinal pigment epithelium (RPE) is also visible (Fahim et al., 1993). This pigment accumulates in the mid-peripheral area as bone spicules, whereas the rest of the regions appear normal. Additionally, the optic disc develops a waxy pallor (Fahim et al., 1993). Patients cannot move independently at the end-stage because only tunnel vision remains functioning and visual acuity is lost (Chang et al., 2011). Fundus examination demonstrates pigment accumulation throughout the retina (Fahim et al., 1993).

RP is a highly heterogeneous disease with more than 90 genes linked to its pathogenesis (Ziccardi et al., 2019). RP is also a complex genetic disorder, in terms of having different patterns of inheritance, a significant number of variants in multiple genes, and the different biological roles in which these genes are engaged. The severity of the condition is usually linked to its mode of inheritance, where autosomal dominant

RP (ADRP) is the mildest form, while X-linked RP is the most severe (Verbakel et al., 2018).

The RP-causing genes are classified according to their roles. As a result of its complex inheritance, the genotype-phenotype correlations are difficult to establish in RP cases (Ali et al., 2017). Several biological functions in which RP genes are involved include phototransduction, visual cycle, ciliary structure, outer segment structure, interphotoreceptor matrix, and retinal metabolism (Dias et al., 2018). The first recognized genetic cause of RP was pathogenic variation in the rhodopsin (*RHO*) gene. Rhodopsin, a 348-amino acid protein that accounts for more than 90 percent of the protein composition of rod outer segment (ROS) discs and enables low-light vision. (Dryja et al., 1990), and its function is to start the phototransduction cascade. Pathogenic variants in this gene are seen in 26 percent of ADRP (Nash et al., 2015). Around 150 variants in the rhodopsin gene have been linked to RP (Luo et al., 2021). These variants are distributed over the whole length of the gene (Luo et al., 2021). The amino acid positions 135, 190, and 347 are among the most variable in the world's population of RP (Wu et al., 2014). Variants in the gene's cytoplasmic domain cause more severe symptoms than variants in the intradiscal domain (Luo et al., 2021; Kremmer, 1997). The most severe type of RP is caused by the *RHO* variant pro347leu (Li et al., 1996). Pathogenic rhodopsin genetic variants are classified into seven types based on their cellular and biochemical features. All of these variants result in the same outcome: the apoptosis of rod cells, leading to RP. Furthermore, several variants have not been thoroughly examined and remain unidentified (Dias et al., 2018).

PRPF31 is another gene related to RP. The first association between the *PRPF31* gene and RP was discovered in 2001 (Kiser et al., 2019). Many genetic variants have been discovered in *PRPF31* gene, accounting for 5%-10% of ADRP

cases (Rose & Bhattacharya, 2016). This gene has six distinct protein-coding transcripts. *PRPF31* is the most prevalent splicing factor gene for ADRP (Kiser et al., 2019). Splicing factors are required to develop the retina and maintain visual function (Azizzadeh Pormehr et al., 2020). These factors have been shown to regulate splicing in various types of cells. However, disease-causing variants in splicing factors genes are exclusively seen in the retina. The high expression of the *PRPF31* gene in the retina indicates the retina's dependency on alternative splicing (Kiser et al., 2019).

GUCA1B gene encodes for a protein named guanylate cyclase-activating protein 2 (GCAP) (Sato et al., 2005). Pathogenic variants in the *GUCA1B* gene cause ADRP. GCAP proteins are involved in modulating photoreceptor cell light sensitivity and photoresponses. One genetic variant of *GUCA1B* (variant G157R) has been found in RP patients. This variant causes the protein to be retained within the photoreceptors' inner segment (IS), resulting in retinal degeneration and photoreceptor cell apoptosis (Wada et al., 2001).

Pathogenic variants in the *PDE6A* and *PDE6B* genes are the most common causes of autosomal recessive RP (ARRP). These genes encode for the phosphodiesterase 6A and 6B subunits that maintain cytoplasmic cyclic guanosine monophosphate (cGMP) levels, which is required for rod cell phototransduction (S. H. Huang et al., 1995). Pathogenic variants in genes that code for proteins involved in retinal metabolism frequently exhibit an autosomal recessive pattern of inheritance. Another gene is *RPE65,* which encodes the retinal pigment epithelium-specific 65 kDa protein. Pathogenic variants in *RPE65* have been found to segregate in an autosomal dominant manner in RP (Bowne et al., 2011). This protein functions as an isomerase to convert vitamin A into 11 cis retinol and to oxidize 11 cis retinol to 11 cis retinal, which is required to synthesize light-sensitive pigments in photoreceptors. Variants in this

gene are most commonly related to LCA, a severe autosomal recessive type of RD, showing the variability of inheritance among variants within the same gene (Morimura et al., 1998).

2.2.2 Cone and cone-rod dystrophies

Cone and cone-rod dystrophies manifest as more severe conditions compared to rod-cone dystrophies since the perception of color and high acuity are impaired (Nash et al., 2015). Other common clinical manifestation includes photophobia and nystagmus. At later stages, full blindness develops due to degeneration of the rod photoreceptors. Both stationary and progressive types of cone and cone-rod dystrophies may arise, as in rod-dominated dystrophies (Thiadens et al., 2012).

2.2.2.1. Stationary cone dystrophies

Achromatopsia is a rare congenital retinal disease characterized by reduced or missing cone photoreceptor function (Hassall et al., 2017). Patients exhibit poor visual acuity, photophobia, and nystagmus (Hassall et al., 2017). Stationary cone dystrophies are classified into two subtypes: complete or incomplete achromatopsia, which causes an absolute loss of all color vision or the perception of only one color (Nash et al., 2015). Patients often manifest total cone function loss, shown by the absence of cone-isolating ERG recordings. While incomplete phenotypic is relatively uncommon (Kohl et al., 2005). Tritanopia, often known as poor blue vision, is an autosomal dominant condition resulting from variants in the *OPN1SW* gene, which encodes the short-wave sensitive opsin that detects blue light (Weitz et al., 1992). *CNGA3, CNGB3, PDE6H,* and *PDE6C* are linked to autosomal recessive complete achromatopsia. Variants in the *CNGB3* gene alone account for up to 50% of total achromatopsia cases (Kohl et al., 2005). *CNGA3* and *CNGB3* genes encode the α and β and subunits of cGMP-gated

channels found in cone photoreceptors and are implicated in crucial phototransduction processes (Kohl et al., 1998).

2.2.2.2 Progressive cone (COD) and cone-rod dystrophies (CORD)

COD and CORD are characterized by the primary degeneration of cone photoreceptors, typically with subsequent rod involvement (Gill et al., 2019). A progressive cone (COD) or CORD generally appears in childhood ages. Individuals who are affected usually have either cone involvement or cone and then followed by rod degeneration (CORD). These two dystrophies differ in that rod involvement increases the severity. Most patients reach legal blindness by the age of 40 (Thiadens et al., 2012). The macula appearance on fundus examination differs, with some patients presenting with retinal pigment deposits or an atrophic appearance. To date, COD and CORD disease-causing variants have been reported in approximately 30 genes (Thiadens et al., 2012). Such variants might also be linked to other types of RDs, likely because of their extensive activities in photoreceptors and the retina. For instance, *ABCA4* variants have been linked to RP and stargardt disease (Martínez-Mir et al., 1998). *ABCA4* gene is the most commonly detected gene in COD and CORD of autosomal inheritance. Studies indicated that it occurs in 9% and 26% of cases, respectively (Thiadens et al., 2012).

2.2.3. Generalized non-syndromic RDs

Generalized RDs are those that include the simultaneous degeneration of both rod and cone photoreceptor functioning. The vast majority of patients manifest a gradual, often severe, visual decline (Nash et al., 2015).

2.2.3.1. Lebar congenital amaurosis (LCA)

Lieber's congenital amaurosis (LCA) is one of the early onset and severe forms of RDs, with a wide range of symptoms. Dr. Theodore leer documented significant

vision impairment in infants with nystagmus and weak pupillary light reflex in 1869, which were eventually identified as typical manifestations of the latter-named LCA (C. H. Huang et al., 2021). LCA accounts for 5% of all RDs and has a frequency of 1/81,000 to 1/30,000. LCA is also responsible for 20% of childhood blindness. Franchetti's oculo-digital mark, in which patients continually poke and rub their eyes, is a distinctive observation in LCA patients. The appearance of the retina differs in early stages, although retinal pigmentary alterations can be seen as the disease progresses (Nash et al., 2015). Notably, there have been indications of genotype-phenotype retinal appearance patterns, such as a transparent RPE appearance with white spots seen with *RPE65* gene variants and gradual macular degeneration reported in individuals with *AIPL1* and *CRB1* variants (Perrault et al., 2012). So far, approximately 20 genes have been linked to LCA, with almost all having an autosomal recessive pattern of inheritance (C. H. Huang et al., 2021).

2.2.3.2. Choroideremia

Choroideremia is the only X-linked subtype of generalized RDs resulting from variants in the *CHM* gene (Bokhoven et al., 1994). Due to the disease's X-linked inheritance, men are mainly affected, while women are usually asymptomatic carriers or, in rare cases, affected. Patients exhibit night blindness and gradual degradation of the photoreceptors, the choroid, and RPE in their second decade of life (Nash et al., 2015). Affected males are distinguished by the presence of chorioretinal scalloped atrophy in the mid-peripheral fundus. Heterogeneity is prevalent in choroideremia patients, with some uncommon manifestations being misdiagnosed as RP in the clinical assessment (McTaggart et al., 2002). *CHM* gene encodes REP-1, a subunit of the intracellular trafficking rab protein 1 that is important for the movement of proteins and components within cells (Seabra et al., 1993). Multiple non-synonymous, as well as

insertions and deletions variants, have been linked to the condition (McTaggart et al., 2002).

2.3. Genetic & Molecular diagnosis of RDs

Genetic testing is the investigation of a person's DNA to discover genetic variations that may cause diseases (McPherson, 2006). As the knowledge of the human genome and the number of genetic variations linked to RDs (more than 250 causal genes) increases, new genetic testing tools like next-generation sequencing are helping clinicians detect RDs more accurately (Lam et al., 2021a). Implementing advanced molecular techniques has raised the possibility of detecting causative variants in patients with RDs (F. Wang et al., 2014). Determining the causative variants can significantly improve medical care by providing a prognosis, minimizing the need for further electrophysiologic evaluation, and suggesting relevant treatment modifications (Lam et al., 2021a). Genetic testing also enables the proper identification of inheritance patterns, further improving the genetic counseling services for affected patients and families (Lam et al., 2021a).

Given the significant number of genes involved, varying expression, frequent clinical and genetic overlap, and incomplete penetrance, obtaining a molecular diagnosis in RDs is complicated (Nash et al., 2015). Conventional methods, for instance, sanger sequencing, have limited use in identifying genetic variants in conditions with substantial genetic variability due to time consumption and high cost, which makes it unfeasible for analyzing a considerable number of genes (Nash et al., 2015). Different technologies, like the APEX genotype microarray chip, may be used to evaluate several variants in several genes simultaneously and have helped in finding genetic defects (Henderson et al., 2007). This method has been applied in various RDs, including non-syndromic and syndromic RDs (Yoshida et al., 2006). However, the

APEX technique has restricted resolving power because the genotyping array only identifies a limited number of genetic variations from a fixed number of genes (Henderson et al., 2007).

With the introduction of next-generation sequencing (NGS), the quantity of genetic data that can be evaluated in a single sequencing test has increased dramatically. NGS is a relatively new technique that enables rapid and low-cost sequencing of patients' selected exonic regions, complete exomes, and even whole genomes (Lacey et al., 2014). Given the capacity to sequence segments in parallel, hundreds or thousands of sequencing fragments or reads can be used to cover a single region. This method delivers precise large-scale sequencing, which may have diagnostics applications (Audo et al., 2012). Over the past couple of years, efforts have been concentrated on using the NGS technique to identify variants in RDs (Audo et al., 2012). Several organizations have decided to build custom-built gene panels that sequence a specified list of certain disease genes (Audo et al., 2012). For routine genetic diagnosis, targeted gene sequencing offers a rapid, accurate, and relatively cost-effective genotype screening. Even though there are fewer genes evaluated than in whole exome sequencing (WES) and additional novel genes could not be discovered, the variants detection rate significantly rises with a proper approach and sufficient depth of coverage (Gonzàlez-Duarte et al., 2019). Targeted gene sequencing using a customized panel of 332 RD-related genes yielded a diagnostic rate of over 85% in RP patients (Neveling et al., 2012). These remarkable success rates strongly justify custom-targeted gene sequencing in routine genetic diagnosis (Gonzàlez-Duarte et al., 2019). Panel testing has enabled variant identification in around 50% of RP patients (Neveling et al., 2012). In contrast, most patients previously could not obtain a genetic diagnosis due to the high cost and inefficiency of earlier detection approaches

(Neveling et al., 2012). This method lowers the number of identified variants (Daiger et al., 2010). However, since new disease genes in RDs are being identified at an accelerating rate, this technique is constrained in its ability to include more disease genes as they are discovered. Moreover, this technique is restricted in its capacity to discover variants in regulatory regions. They also may be less effective in detecting copy numbers and structural variants (Eisenberger et al., 2013).

A broader next-generation sequence technique, such as WES or whole genome sequencing (WGS), can offer a more comprehensive means of investigating disease gene areas than a specified panel (Guo et al., 2015). WES has evolved into a fundamental methodological tool that also aids in the discovery of new RDs genes and increases the number of cases that have been successfully resolved(Gonzàlez-Duarte et al., 2019). WES involves sequencing all the protein-coding genes in the human genome and to minimize unwanted data, variants in nonrelevant genes can be processed using bioinformatics by focusing on the genes of interest to be investigated. WGS and WES enable the analysis of newly found genes since all genes are sequenced, and bioinformatic filters may be adjusted to evaluate novel disease genes. WGS improves the ability to identify copy numbers and structural variations (Zhao et al., 2013). With several commercial firms investing in various forms of NGS technology, the competition encourages constant product advancements, enhancements, and cost reductions, making this a feasible diagnostic technique (Lam et al., 2021a).

2.4. Retinal dystrophies in the Arab world

The Middle East, which is centered on the Arabian Peninsula, extends from Northern Africa to Western Asia (Davison, 1960). This region's demographics are mostly Arab, with customary intrafamilial marriage (consanguinity), intratribal marriage (endogamy), and a preference for many children. These sociocultural factors

enhance the probability of homozygosity for genetic variants and thus the appearance of many recessive conditions including ocular conditions (Khan, 2013). Multiple studies also revealed genetic causes of RDs in consanguineous families globally, particularly in regions with a high consanguinity rate such as North and sub-Saharan Africa, the Middle East, West, Central, and South Asia (Shen et al., 2021). The State of Qatar is a Middle Eastern country located on the Arabian Peninsula's northeastern coast (Al-Dewik et al., 2018). Qatar's economic progress has resulted in significant advancements in all areas of the country. Qatar's healthcare industry is characterized by its various international accreditations, delivering the highest levels of care both generally and to the practice of genetics and genomics in medical and research contexts (Al-Dewik et al., 2018). The precise incidence of retinal dystrophies in Qatar is unknown. In a study conducted at the Al Noor Institute for the Blinds in Qatar, 90 children participated in research to identify the causes and degree of vision loss (Al Mansouri & Al Laftah, 2003). Those who had consanguineous parents (67.7%) showed a significant frequency of visual impairment among their family members and were predominantly affected by hereditary or congenital ocular disease. This clearly shows that in Qatar, consanguinity may be a substantial risk factor for inherited or congenital visual impairment (Al Mansouri & Al Laftah, 2003). A comprehensive analysis of RDs genetic test results in Arab nations was conducted. Thirty-one papers including 407 individuals from 11 countries were examined (Jaffal et al., 2021). Results showed that next-generation sequencing was the most widely utilized technology (68%) (Jaffal et al., 2021). The most prevalent pattern of inheritance was autosomal recessive (97%). In Saudi Arabia, genetic variants in *RP1* (20%) and *TULP1* (20%) were the most common. On the other hand, the most common genetic variant in Northern Africa was in *MERTK* (18%) and *RLBP1* genes (18%) (Jaffal et al., 2021).

Recent Middle Eastern investigations, many of which used homozygosity mapping, have improved phenotype-genotype correlations for common and uncommon ocular genetic disorders (Khan, 2013). Homozygosity mapping has been a powerful approach for disease gene mapping in the Middle East among current molecular genetic techniques (Khan, 2013). Studies from the region have improved the understanding of ocular genetic diseases. In certain cases, genetic testing showed an undiscovered syndrome. Reports of ocular genetic disorders specific to the region have thus far revealed novel visual pathways (Khan, 2013). Arab communities are characterized by a broad diversity of familial, and social practices. Given these factors, it makes it difficult to design, and provide genetic services using a single model. However, considering the incidence and impact of genetic conditions, the availability of genetic services in Arab populations at all demographic levels remains insufficient (Hamamy & Bittles, 2008). Improving this situation necessitates significant educational efforts, such as increasing the general public's genetic literacy, courses to familiarize healthcare workers with counseling needs and skills, and referral guidelines for high-risk families (Hamamy & Bittles, 2008).

From an ethical standpoint, the use of broad-spectrum genetic testing raises concerns regarding privacy and confidentiality (McGuire et al., 2008). The vast amount of genetic information obtained may contain sensitive data that could be misused or lead to stigmatization. There is also a risk of incidental findings, where unrelated genetic findings with potential health implications are discovered during the testing process. The ethical guidelines and protocols surrounding the management and communication of these findings must be carefully addressed to ensure the well-being and autonomy of the patients (McGuire et al., 2008). These guidelines should emphasize the importance of informed consent, privacy protection, and the responsible

use of genetic information. Healthcare providers and genetic counselors play a crucial role in assisting patients in understanding the implications of the test results, including incidental findings, and supporting them in making informed decisions about further medical interventions or genetic counseling (McGuire et al., 2008).

2.5. Therapeutic approach

The discovery of therapies and treatments for RDs represents the next stages in retinal dystrophy cases. Currently, there is no cure for RDs due to the difficulty of regenerating the affected retinal cells. However, supportive care can help to enhance one's quality of life. Glasses, cataract surgery, and decreased vision aids are all important options to explore for RDs patients (Strong et al., 2017). In recent years, stem cell replacement therapy has been investigated as a potential treatment for RDs, where it will allow stem cells to generate new retinal cells to replace the destroyed cells in the degenerative retina (Öner, 2018). Recent advances in experimental stem cell applications have resulted in the approval of phase I/II clinical studies (Öner, 2018). The most recent stem cell transplantation research indicates that this approach is a viable strategy for recovering visual function in eyes with degenerative retinal disorders such as retinitis pigmentosa, Stargardt disease (Öner, 2018). The human eye has multiple anatomical and immunological advantageous properties which made the eye on the frontline of translational gene therapy (Francis, 2006). These properties include the ability to image patients and perform minimally invasive surgery, the small size of the retina, which only needs small doses of medication to be administrated, and the ocular immunologic advantage provided by the blood-retinal barrier (Cunha-Vaz et al., 2011). Currently, several clinical trials using various approaches are available across the world, to improve results and include additional genes and variants (Varela et al., 2022). Moreover, with officially FDA-approved gene therapy for biallelic *RPE65*

mutation-associated retinal dystrophy Luxturna, validating a genetic diagnosis by genetic testing may enable patients to learn about the newest treatment choices or qualify them for research participation (“FDA Approves Hereditary Blindness Gene Therapy,” 2018).

CHAPTER 3: MATERIALS & METHODS

3.1. Study design and participants

A retrospective chart review of patients' records seen at the Department of Adult and Pediatric Medical Genetics at Hamad Medical Corporation between 2015-2022 was conducted. Ethical approval for the study was granted by Hamad Medical Corporation (HMC) Medical Research Center (MRC-01-22-729) and by the Institutional Review Board (IRB) of Qatar University (QU-IRB 1803-E/23). This study was carried out in accordance with the Declaration of Helsinki.

The approach we applied to search for relevant study participants was through the use of keywords, including the disease names, the names of the RDs genes, and/or the clinical manifestations of RDs. We reviewed the patients' records, and the inclusion criteria included patients of both genders and of any age with a clinical diagnosis of non-syndromic retinal dystrophy and who underwent at least one genetic test. While the exclusion criteria included patients with syndromic retinal dystrophies and patients who did not do any genetic testing. All eligible patients were given a representative numerical code and their sociodemographic data was collected. This includes patients' gender, age, age at diagnosis, nationality, consanguinity, family history, and the clinical manifestations.

3.2. Genetic testing

The genetic testing approach followed in the Department of Adult and Pediatric Medical Genetics, at Hamad Medical Corporation in cases of retinal dystrophy involves RDs gene panels, WES, WES Plus (which includes WES and mitochondrial genome testing), or familial targeted testing of specific gene variants. RDs patients with an unknown genetic cause are offered either WES, WES Plus, or gene panel testing. Different gene panels are used in cases of RDs including the Congenital Stationary

Night Blindness (CSNB) Panel which tests for 12 genes and utilizes both sequencing and deletion/duplication analysis (*CABP4, CACNA1F, CHM, GNAT1, GRM6, NYX, PDE6B, RDH5, RHO, RPE65, SAG, and TRPM1*) (Xiao et al., 2006). Another available gene panel is the Cone-Rod Dystrophies Panel that uses sequencing analysis and test for 31 genes (*ABCA4, ADAM9, AIPL1, BEST1, C8orf37, CABP4, CACNA1F, CDH3, CDHR1, CEP290, CERKL, CNGA3, CNGB3, CRX, DRAM2, ELOVL4, GUCA1A, GUCY2D, PAX6, PITPNM3, POC1B, PROM1, RAB28, RAX2 (QRX), RDH5, RDS (PRPH2), RIMS1, RPGR, RPGRIP1, SEMA4A, TTLL5)* (Hamel CP et al.,2007). The third panel is the Retinal Dystrophy Xpanded Panel, which also uses sequencing analysis and can utilize a "trio" approach, that combines simultaneous investigation of the affected proband and both parents; increasing the possibility of discovering a definite genetic cause in RDs cases. This panel tests for about 780 genes (Sahel et al. 2014; Kocur et al. 2002). Patients who had a negative or inconclusive panel result are offered WES or WES Plus as a more comprehensive test. While in cases where a known RDs familial pathogenic variant has been identified, familial targeted testing is usually offered to the family members to test for a specific gene variant.

We reviewed all patients' genetic test results taking into consideration the type of genetic testing performed (familial targeted testing, gene panel testing, WES, or WES Plus), the variants identified and their type, variant classification, genes associated, zygosity status, the pattern of inheritance, genotype-phenotype correlation and RDs conditions associated.

Patients were classified initially based on the genetic test results into three groups which are solved, unsolved, and uncertain cases, based on their genetic test results and whether the variants identified explained the patient's clinical manifestations. The **solved** cases group included patients with pathogenic or likely

pathogenic variants found in genes related to RDs, and a zygosity status consistent with the known patterns of inheritance associated with the gene. On the other hand, **unsolved** cases included patients with negative results or with benign, or likely benign variants, cases where the variant's zygosity was not consistent with the established inherence pattern (ex., the patient is heterozygous for the autosomal recessive gene), patients with variants found in genes not related to the patient's clinical manifestation and that did not explain the phenotype. **Uncertain** cases included patients with variants of unknown (uncertain) significance in RDs-related genes.

3.3. Investigating identified VUSs

To further explore uncertain cases where variants of unknown significance were identified, patients and their families were offered familial segregation analysis. This involved testing the proband's family members who consented to participate, for the captured variant to verify whether the variants co-segregates with the disease within the family. The cases that were initially uncertain and required family segregation and WES reanalysis (the process of re-evaluating and re-analyzing previously generated WES data using updated or improved bioinformatics pipelines and databases) to be solved were grouped under the category of "cases reconsidered as solved".

Moreover, we have collected the pathogenicity scores of identified variants using InterVar (https://wintervar.wglab.org) which is based on the American College of Medical Genetics and Genomics- American Association for Molecular Pathology (ACMG-AMP) guidelines 2015 for variants classification. The ACMG-AMP established five variant classification classes pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. The classification is based on multiple data about the genetic variants including population data, computational data, segregation, and allelic data (Richards et al., 2015). We also used MutationTaster as an additional

tool to computationally predict the variants' effect on the encoded protein (https://www.mutationtaster.org). Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) was also used to collect data about the variants' clinical significance and to identify whether a variant was previously reported in association with RDs-related phenotypes (Landrum et al., 2018). In addition, different population databases such as The Genome Aggregation Database (gnomAD) and The Greater Middle East (GME) (https://gnomad.broadinstitute.org) (http://igm.ucsd.edu/gme/index.php) were utilized to collect data about the allele frequency of the identified variants among the different populations. GnomAD is a resource developed by an international consortium of researchers to collect and synchronize exome and genome sequencing data from a wide range of large-scale sequencing projects and make summarized data available to the broader scientific community (*GnomAD,* n.d.). GME Variome aims to create a coding base reference for the Greater Middle East nations (Scott et al., 2016).

3.4. Investigations on the variants' novelty

We searched our variants in the literature using Google Scholar (https://scholar.google.com), Pubmed (https://pubmed.ncbi.nlm.nih.gov), Scopus (https://www.scopus.com/search/form.uri?display=basic#basic), and ScienceDirect (https://www.sciencedirect.com) and through publically available population databases, or public archives. Variants that were not reported in the literature, population databases, or public archives were considered novel.

3.5. Statistical analysis

The collected data from all patients was analyzed using the Statistical Package for the Social Sciences (IBM-SPSS v. 28). Frequency and percentage were computed for categorical variables such as nationality, gender, consanguinity, and family history, while the mean and standard deviation were calculated for continuous variables such

as age and age of diagnosis. The diagnostic yield of the genetic tests was calculated by dividing the number of patients who received a positive diagnosis by the total number of patients who underwent the test. The Fisher test was used to assess the significance of the diagnostic yields, with a two-tailed P-value less than 0.05 considered statistically significant.

CHAPTER 4: RESULTS

4.1 Patients demographics and clinical characteristics

The database of patients seen at the Department of Adult and Pediatric Medical Genetics at Hamad Medical Corporation included 20,355 patients. Firstly, 20,161 individuals were excluded as they were patients with other genetic diseases or healthy clients. In addition, 69 patients with syndromic retinal dystrophy were excluded. Lastly, 56 patients with non-syndromic retinal dystrophy who didn't undergo any genetic testing were excluded (Figure 2). Thus, our study identified 49 eligible patients with RDs and 117 family members from the records of January 2015 to December 2022. Table 1 summarizes the participant's demographic and clinical characteristics.

In our study, 44.9% (n = 22) of the participants were males, while 55.1% (n = 27) were females. The mean age of the participants was 20.3 years, and the mean age at diagnosis was 23.3 years. Of the 49 patients, 90% (n = 44) were Arabs, and most of the patients were from Qatar constituting 61.2% (n = 30). Patients from other Arab countries were as follows: Egypt 8.2% (n= 4), Palestine 8.2% (n= 4), Lebanon 4.1% (n=2), Yemen 4.1% (n= 2), Syria 2% (n= 1), and the United Arab Emirates 2% (n= 1). Non-Arab patients included participants from Pakistan 8.2% (n= 4) and Croatia 2% (n= 1), both contributing 10.2% (n = 5). The consanguinity rate in the study was 79.6% (n = 39). The consanguinity rate among Qatari participants was 83.3% (n=25) and 73.6% (n=14) in non-Qatari participants. Of the participants, 67.3% (n = 33) had a family history of RDs. In our study, most patients had clinical manifestations of Rod and Rod-Cone dystrophies (51%, n = 25), with 21 patients diagnosed with RP, 3 patients with congenital stationary night blindness and 1 patient with Rod-Cone dystrophy. Moreover, 32.7% (n = 16) of participants had a clinical diagnosis of Cone and Cone-

Rod dystrophies, including 5 patients with macular dystrophy, 4 patients with Cone-Rod dystrophy, 3 patients with Stargardt disease, 3 patients with Cone dystrophy and 1 patient with achromatopsia. Four patients had generalized retinal dystrophy with a diagnosis of LCA. In addition, 8.2% (n = 4) of participants had unspecified retinal dystrophy.

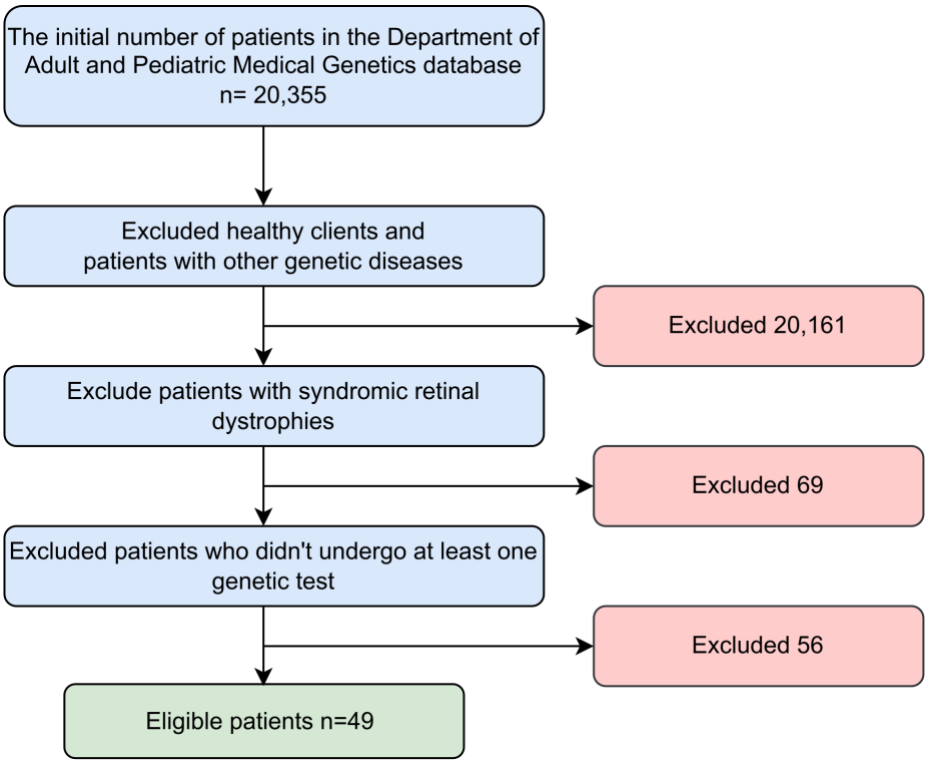

Figure 2. A flowchart demonstrating the approach utilized in identifying eligible study participants.

Table 1. A description of the patient's demographics and clinical features

		Count	Column N %
Age (years)	Mean 20.4	SD	19.03
Age at diagnosis (years)	Mean 23.3	SD	18.9
Gender	Female	27	55.1%
	Male	22	44.9%
Nationality	Qatar	30	61.2%
	United Arab Emirates	1	2.0%
	Yemen	2	4.1%
	Lebanon	2	4.1%
	Palestine	4	8.2%
	Syria	1	2.0%
	Egypt	4	8.2%
	Pakistan	4	8.2%
	Croatia	1	2.0%
Consanguinity	No	6	12.2%
	Yes	39	79.6%
	Not Available	4	8.2%
Family history	Negative	13	26.5%
	Positive	33	67.3 %
	Not available	3	6.1%
Clinical Features	Cone & Cone-Rod dystrophy	16	32.7%
	Macular dystrophy	*5*	
	Cone-Rod dystrophy	*4*	
	Cone dystrophy	*3*	
	Stargardt disease	*3*	
	Achromatopsia	*1*	
	Generalized Retinal dystrophies	4	8.2%
	Leber congenital amaurosis	*4*	
	Rod & Rod-Cone dystrophy	25	51.0%
	Retinitis pigmentosa	*21*	
	Congenital stationary night blindness	*3*	
	Rod Cone dystrophy	*1*	
	Uncategorized Retinal dystrophy	4	8.2%

SD: Standard Deviation

4.2 Genetic findings

In our study, we identified 55 variants in 32 different genes associated with RDs (Figure 3). These variants were detected in 46 of the 49 patients examined. The most common genes included *ABCA4, CRB1, GNAT2, GRM6, GUCY2D, MERTK, PDE6B,*

RDH12, and *RPGRIP1*. The *ABCA4* gene was the most reported in the study, with eight patients harboring variants in the *ABCA4* gene. From the 55 identified variants, 36 were inherited in an autosomal recessive manner, 13 were inherited as both autosomal dominant and autosomal recessive (autosomal dominant/autosomal recessive), 3 as autosomal dominant, 2 as X linked, and one variant with an unknown inheritance pattern. Out of the 55 variants 16 were classified as pathogenic, 15 as likely pathogenic, 23 as variants of uncertain significance, and 1 as a risk allele.

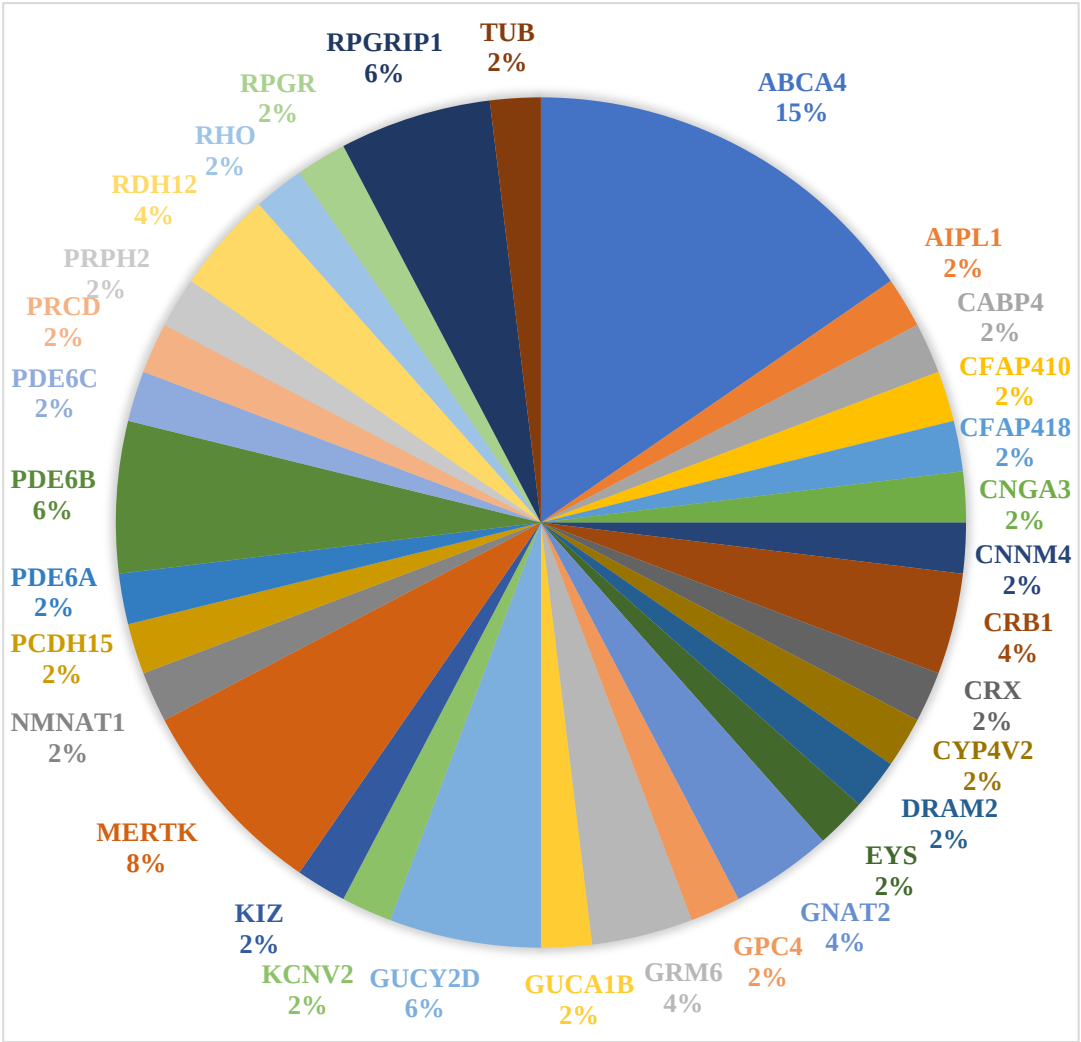

Figure 3. A pie chart demonstrating the percentage of identified genes (with genetic variants) among our patient cohort (n=49)

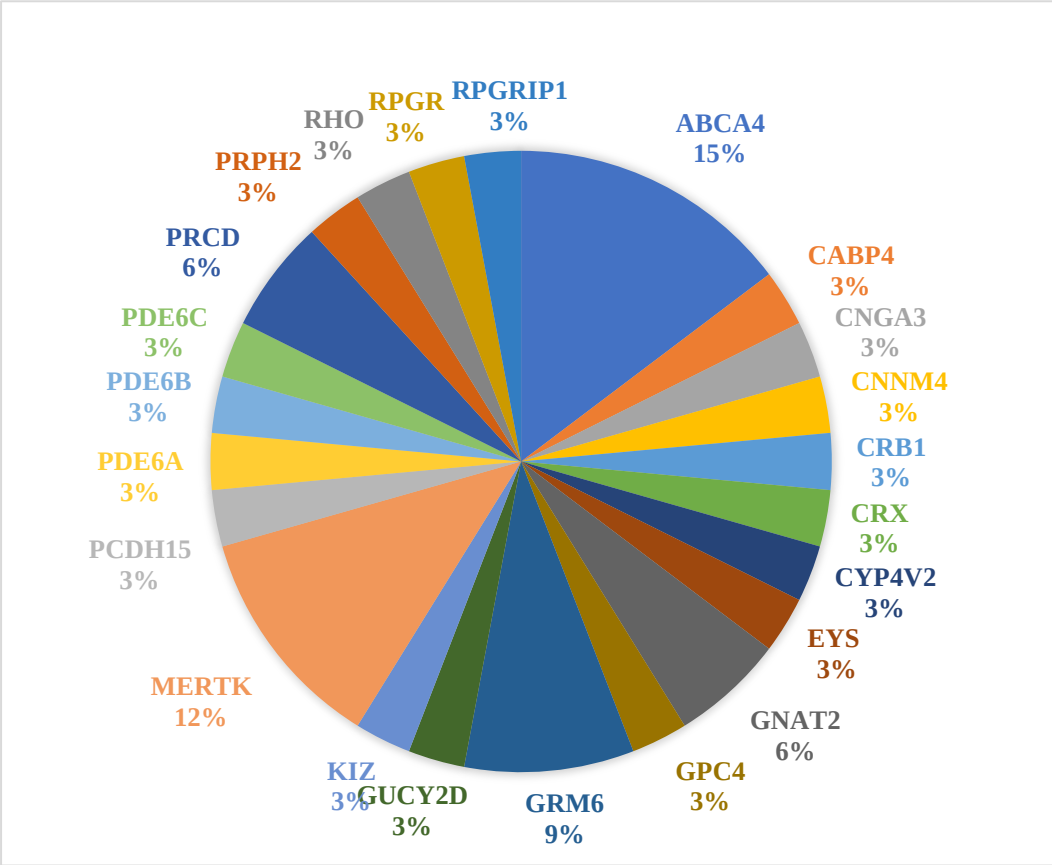

Figure 4. A pie chart demonstrates the percentage of captured genes with identified variants among Qatari patients in our cohort (n=30)

4.2.1 Solved cases

In the current study, 28 out of 49 cases were initially classified as "solved," where the genetic test identified a pathogenic/likely pathogenic causative variant in RDs-related genes and explains the patient phenotype. A total of 21 genes and 31 variants were identified in the solved group (Table 2). Out of these 31 variants, 16 were classified as pathogenic and 15 as likely pathogenic. The most reported gene in the solved cases was the *ABCA4*, which was reported in 5 patients with 4 different variants. To a lesser extent, the genes *MERTK*, *PDE6B*, *GRM6*, *RDH12*, *RPGRIP1*, and *GNAT2*

were all reported in at least 2 patients from our cohort. All patients were found to have causative variants in a single gene except for two patients RDs-1 and RDs-9 who were found to have causative variants in two genes. Patient RDs-1 was found to be homozygous for the pathogenic variant c.2214delT in the *MERTK* gene and the pathogenic variant c.1040_1041delTT in the *GUCY2D* gene, while RDs-9 was compound heterozygous for the pathogenic variant c.5582 G>C and likely pathogenic variant c.1609 C>T in *ABCA4* gene and heterozygous for the likely pathogenic variant c.128 G>A in the *CRX* gene. Twenty-one variants were inherited in an autosomal recessive pattern, 7 variants were inherited as autosomal dominant/autosomal recessive, two variants as autosomal dominant, and one variant as X-linked. Of the 28 solved cases, 20 were homozygous for causative variants, 6 were compound heterozygous, 1 was heterozygous, and 1 was hemizygous. Familial segregation studies were conducted in 5 out of the 6 cases who carry compound heterozygous variants and showed that in 4 cases each parent was found to be a carrier for one of the variants, except for one patient RDs-35 from Lebanon. This patient was found to be compound heterozygous for the pathogenic variant c.3278dupC and the pathogenic variant c.2935 C>T in *RPGRIP1* and parental testing showed that one variant (c.2935 C>T) was inherited from the mother, while the other variant was not detected in both parents and most likely the variant occurs spontaneously during the development and is not inherited from either parent (de novo). Family segregation studies also showed that in patient RDs-9 with 3 identified variants, the pathogenic variant c.5582 G>C in the *ABCA4* gene was inherited from the father, while the likely pathogenic variant c.1609 C>T in the *ABCA4* gene was inherited from the mother. However, the likely pathogenic variant c.128 G>A in the *CRX* gene was not detected in both parents indicating that the variant is most likely de novo. The most reported clinical manifestation was retinitis

pigmentosa (n=13), while the least reported phenotype was Achromatopsia as it was reported in 1 patient. In addition, 18 cases were initially classified as uncertain cases based on the genetic test results that showed variants of uncertain significance. Following the family segregation analyses, 9 cases were reconsidered as solved as the variants were segregating with the disease in family members. One case was reconsidered as solved following WES reanalysis as the variant identified was reclassified from a variant of uncertain significance to a likely pathogenic variant.

4.2.2 Unsolved cases

Unsolved cases included 3 participants, 2 of whom had a negative genetic test result (1 had WES Plus conducted, and 1 had gene panel testing). One patient, RDs-26 from Palestine, underwent gene panel testing and was found to be heterozygous for 2 autosomal recessive variants, c.8177_8187del in *ALMS1* and c.190+2 T>C in *MKS1*, where both genes are related to syndromic retinal dystrophy, and such findings did not explain the patient's phenotype.

4.2.3 Uncertain cases

Eighteen participants were initially included in the uncertain cases group, with 24 identified variants in 18 genes (Table 3). The most reported genes included *ABCA4* (3 patients) and *MERTK* (2 patients). Among the 18 patients, 2 participants had identified variants in 2 different genes. Patient RDs-34 was heterozygous for the risk allele c.5603 A>T in *ABCA4* and homozygous for a variant of uncertain significance (VUS) c.246 T>G in *DRAM2*. Patient RDs-17 was homozygous for a VUS c.103 G>A in *PDE6A*, and hemizygous for the variant c.156 C>G in *GPC4* (Table 3). Out of the 24 variants, 23 were classified as VUS, while one variant was classified as a risk allele. In this group, 16 variants were inherited in an autosomal recessive manner, 5 variants as autosomal dominant/autosomal recessive, 1 as autosomal dominant, 1 as X-linked,

and 1 variant was of unknown mode of inheritance. Family segregation analyses were conducted, where other family members of the probands were tested for the same identified variant which may be helpful in assessing the clinical significance of a patient's uncertain test result. Following family segregation studies, 9 cases were reconsidered as solved as the genetic variant was segregating with the disease among family members. One participant, RDs-13 from Qatar had a reclassified variant result by WES reanalysis. This patient was initially found to be homozygous for 2 different VUSs in the same gene, c.2020 A>G and c.2435 A>C in the *MERTK* gene. Following WES reanalysis, the c.2020 A>G variant in *MERTK* was reclassified from VUS to likely pathogenic, while the c.2435 A>C variant was reclassified to likely benign. It's worth mentioning that the same variant c.2020 A>G in *MERTK* was identified in another patient from Qatar, and the case was reconsidered as solved through family segregation studies. Both patients RDS-13 and RDs-11 had a similar clinical presentation of rod and cone dystrophy. Out of the 18 cases, 10 were reconsidered as solved, while 8 remained uncertain (Table 3).

4.2.4 Shared vs. Novel variants

Overall, 49 of the identified variants have been reported before, while six were novel (Table 4). Out of the 55 variants, 13 were reported in other patients from the literature, population databases and public archives, with some sharing similar ethnic backgrounds to our study participants, such as the variant c.2214delT in the *MERTK* gene, which was identified in two Qatari patients and was also reported in patients from Saudi Arabia and the United Arab Emirates with similar clinical manifestations (Khan, 2020b) (Patel et al., 2018). In addition, patient RDs-4 from Qatar shared the variant c.81_82insA in the *CABP4* gene with patients from Saudi Arabia (Khan et al., 2013). Another shared variant was c.821 T>C in the *RDH12* gene; this variant was seen in 2

of our patients from Palestine and was also reported in patients from a similar ethnic background (Sharon et al., 2020) (Table 4). On the other hand, multiple variants were reported in patients from different ethnic backgrounds, such as the variant c.2137+1 G>A in *EYS* reported in a Qatari patient from our study and reported in a patient from Denmark (Jespersgaard et al., 2019). The variant c.5882 G>A in the *ABCA4* gene was reported in 3 Qatari patients from our study and was shared by patients from multiple ethnicities, including those from China, Spain, the United Arab Emirates, and Italy (Maltese et al., 2022; X. F. Huang et al., 2015; Jespersgaard et al., 2019). The variant with the highest frequency was c.5603 A>T in the *ABCA4* gene with a heterozygous/homozygous frequency of 0.04042 in GnomAD, 0.039314516 frequency in GME and being associated with complex retinal dystrophy phenotype on ClinVar (Table 4). We identified 5 patients with 6 novel variants in 4 genes that were not reported before (Table 5). The two most reported genes were *RPGRIP1* (c.3278dupC and c.105dupA*)* and *CRB1* (c.3613 G>T and c.4211 G>C*)* where both include 2 novel variants (Table 5). Two variants were classified as pathogenic, 2 as likely pathogenic, and 2 as variants of uncertain significance. All novel variants were inherited in an autosomal recessive pattern except the variant c.2213_2215del in *GUCY2D* which was inherited in an autosomal dominant/autosomal recessive pattern. Out of the 5 patients, 3 were homozygous and 2 were found to be compound heterozygous. RDs-36 was found to be compound heterozygous for 2 novel variants in the *CRB1* (c.3613 G>T and c.4211 G>C) while RDs-35 was compound heterozygous for 1 novel variant c.3278dupC and 1 shared variant c.2935 C>T in the *RPGRIP1* gene (Table 5). In order to predict their effect, prediction databases were used to evaluate the possible effects of these variants on the protein structure and function. All 6 novel variants were found to be disease-causing (Table 5).

Table 2. Identified causative variants in the solved group

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG Subclassification	VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	Reports from other populations/ethnicities	Phenotype	References
GUCY2D	rs763890649	c.1040_1041delTT	p.Phe347TrpfsX5	Deletion	Frameshift	Homozygous	AD/AR	WES	Pathogenic	–	–	–	disease causing	RDs-1	Qatar	2 years	3 months	Retinitis pigmentosa	–	–	–
MERTK	rs886039422	c.2214delT	p.Cys738TrpfsX32	Deletion	Frameshift	Homozygous	AR	WES Familial targeted testing	Pathogenic	–	–	–	disease causing	RDs-1 RDs-21	Qatar Qatar	2 years 34 years	3 months 28 years	Retinitis pigmentosa Retinitis pigmentosa	Saudi Arabia, United Arab Emirate	Retinitis pigmentosa & Rod cone dystrophy	(Patel et al., 2016) (Khan, 2020)
GRM6	rs752205220	c.1478 G>A	p.Trp493Ter	single nucleotide variant	Nonsense	Homozygous	AR	WES WES Plus	Likely Pathogenic	Pathogenic	PVS1	–	–	RDs-2 RDs-6	Qatar Qatar	3 years 6 years	3 years 6 years	Congenital stationary night blindness leber congenital amaurosis	– –	– –	– –
CABP4	rs786205852	c.81_82insA	p.Pro28ThrfsX4.	single nucleotide variant	Frameshift	Homozygous	AR	WES Plus	Pathogenic	–	–	–	disease causing	RDs-4	Qatar	4 years	6 months	Uncategorized Retinal dystrophy	Saudi Arabia	segregated with congenital retinal dysfunction in 11 affected individuals (aged 2–26 years) from four consanguineous families	(Khan et al., 2013)
ABCA4	–	c.5584 G>C	p.Gly1862Arg	single nucleotide variant	Missense	Homozygous	AD/AR	WES Plus	Pathogenic	Variant of uncertain significance	PM1	Hot	disease causing	RDs-8	Qatar	26 years	23 years	Stargardt disease	China	Stargardt disease	(X. F. Huang et al., 2015)
	rs61748556	c.1609 C>T	p.Arg537Cys	single nucleotide variant	Missense	Heterozygous	AD/AR	Gene Panel testing	Likely Pathogenic	Likely Pathogenic	PM1	–	–	RDs-9	Qatar	14 years	11 years	Macular dystrophy	Germany	Stargardt disease	(Schulz et al., 2017)
	rs61750155	c.4793 C>A	p.Ala1598Asp	single nucleotide variant	Missense	Homozygous	AD/AR	WES Plus	Pathogenic	Likely Pathogenic	PM1	–	–	RDs-25	Yemen	20 years	18 years	Stargardt disease	Germany	Stargardt disease	(Maugeri et al., 2000)
	rs1800553	c.5882 G>A	p.Gly1961Glu	single nucleotid	Missense	Heterozygous	AD/AR	WES	Pathogenic	Likely Pathogenic	PS3	–	–	RDs-22	Qatar	56 years	53 years	Retinitis pigmentosa	China, Spain, United Arab Emirates, and Italy	Stargardt disease	(Maltese et al., 2022)(X. F. Huang

				e variant		Heterozygous		Gene Panel testing					RDs-9	Qatar	14 years	11 years	Macular dystrophy			et al., 2015) (Jespersgaard et al., 2019)
						Heterozygous		WES Plus					RDs-47	Qatar	69 years	64 years	Retinitis pigmentosa			
Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG Subclassification	VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	Reports from other populations/ethnicities	Phenotype	References
CRX	rs777176389	c.128G>A	p.Arg43His	single nucleotide variant	Missense	Heterozygous	AD	Gene Panel testing	Likely Pathogenic	Variant of uncertain significance	PM1	Hot	disease causing	RDs-9	Qatar	14 years	11 years	Macular dystrophy	–	–	–
	rs370898371	c.1107+3A>G	IVS8+3A>G	single nucleotide variant	Intron Variant	compound Heterozygous	AD/AR	Gene Panel testing	Likely Pathogenic	–	–	–	–	RDs-15	Croatia	41 years	38 years	Retinitis pigmentosa	–	–	–
PDE6B	rs1737315492	c.1859A>G	p.His620Arg	single nucleotide variant	Missense		AD/AR		Likely Pathogenic	Variant of uncertain significance	PM1	Hot	disease causing						–	–	–
	rs751859807	c.1655G>A	p.Arg552Gln	single nucleotide variant	Missense	Homozygous	AR	Gene Panel testing	Likely Pathogenic	Likely Pathogenic	PM1	–	–	RDs-18	Qatar	25 years	24 years	Retinitis pigmentosa	–	–	–
PDE6C	rs1057518244	c.724-1G>T	IVS3-1G>T (in intron3)	single nucleotide variant	splice acceptor	Homozygous	AR	WES Plus	Likely Pathogenic	–	–	–	–	RDs-10	Qatar	15 years	9 years	Retinitis pigmentosa	–	–	–
RDH12	rs1594867597	c.821T>C	p.Leu274Pro	single nucleotide variant	Missense	Homozygous	AR	WES Plus	Pathogenic	Likely Pathogenic	PM1	–	–	RDs-19	Palestine	21 years	17 years	Retinitis pigmentosa	Israel	Retinitis Pigmentosa (5), Leber congenital amurosis(1)	(Sharon et al., 2020)
								Familial targeted testing					RDs-31	Palestine	6 years	2.5 years	Uncategorized Retinal dystrophy			
KCNV2	-	9p24.2(2684449-27668756)x1	-	copy number variation	copy number variation	compound Heterozygous	AR	WES	Pathogenic	–	–	–	–	RDs-20	Egypt	14 years	12 years	Cone dystrophy	–	–	–
	rs1819788466	c.757C>G	p.Pro253Ala	single nucleotide variant	Missense		AR		Likely Pathogenic	Variant of uncertain significance	PM2	Warm	disease causing						–	–	–
KIZ	rs775124094	c.247C>T	p.Arg83Ter	single nucleotide variant	Nonsense	Homozygous	AR	Gene Panel testing	Pathogenic	Variant of uncertain significance	PM2	Tepid	–	RDs-24	Qatar	50 years	49 years	Retinitis pigmentosa	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG Subclassification	VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	Reports from other populations/ethnicities	Phenotype	References
RPGR	rs1186795749	c.3092del	p.Glu1031Glyfs*58	Deletion	Frameshift	Hemizygous	XLR	Gene Panel testing	Pathogenic	–	–	–	disease causing	RDs-29	Qatar	37 years	37 years	Retinitis pigmentosa	Denmark	Retinitis pigmentosa	(Jespersgaard et al., 2019)
AIPL1	rs62637014	c.834G>A	p.(Trp278Ter)	single nucleotide variant	Nonsense	Homozygous	AR	WES Plus	Pathogenic	Variant of uncertain significance	PM2	Warm	disease causing	RDs-30	Syria	14 years	12 years	Macular dystrophy	Romania	LCA	(Maltese et al., 2022)
GNAT2	rs1553226581	c.720+5G>C	IVS6+5G>C	single nucleotide variant	splicing site	Homozygous	AR	WES Plus	Likely Pathogenic	–	–	–	–	RDs-32	Qatar	44 years	41 years	Retinitis pigmentosa	–	–	–
								WES						RDs-44	Qatar	16 years	11 years	Achromatopsia	–	–	–
NMNAT1	rs201994921	c.634G>A	p.Val212Met	single nucleotide variant	Missense	compound Heterozygous	AR	Familial targeted testing	Likely Pathogenic	Likely Pathogenic	PM1	–	–	RDs-33	Pakistan	9 years	6 years	leber congenital amaurosis	–	–	–
	–	chr1:10035650_10035833	–	Deletion	Deletion		AR		Pathogenic	–	–	–	–						–	–	–
RPGRIP1	–	c.3278dupC	p.Gln1094Thrfs*6	Duplication	Frameshift	compound Heterozygous	AR	WES Plus	Pathogenic	–	–	–	disease causing	RDs-35	lebanon	7 years	6 years	Rod & Rod-Cone dystrophy	–	–	–
	rs1371805993	c.2935 C>T	p.Gln979Ter	single nucleotide variant	Missense		AR		Pathogenic	Pathogenic	PVS1	–	–						Israel	Retinitis pigmentosa	(Sharon et al., 2020)
CRB1	rs61751266	c.1107delA	p.Glu370AsnfsX5	Deletion	Frameshift	Homozygous	AR	WES	Pathogenic	Pathogenic	–	–	–	RDs-14	Qatar	5 years	2 years	Uncategorized Retinal dystrophy	Saudi Arabia	Cone-Rod Dystrophy, Leber congenital amaurosis	(Patel et al., 2018)
	–	c.3613 G>T	p.Gly1205Ter	single nucleotide variant	Nonsense	compound Heterozygous	AR	WES Plus	Pathogenic	Variant of uncertain significance	PM1	–	–	RDs-36	Qatar	10 years	6 years	Cone-Rod dystrophy	–	–	–
	–	c.4211 G>C	p.Arg1404Thr	single nucleotide variant	Missense		AR		Likely Pathogenic	–	–	–	–								
CFAP418	–	c.478dupA	p.Met160Asnfs*25	Duplication	Frameshift	Homozygous	AR	WES Plus	Likely Pathogenic	–	–	–	disease causing	RDs-42	lebanon	27 years	27 years	Retinitis pigmentosa	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG Subclassification	VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	Reports from other populations/ethnicities	Phenotype	References
PRPH2	rs1799986489	c.936del	p.Pro313Argfs*11	Deletion	Frameshift	Heterozygous	AD	Familial targeted testing	Likely Pathogenic	–	–	–	disease causing	RDs-46	Qatar	61 years	61 years	Macular dystrophy	–	–	–
CYP4V2	rs199476204	c.1348 C>T	p.Gln450Ter	single nucleotide variant	Nonsense	Homozygous	AR	WES Plus	Likely Pathogenic	Pathogenic	PVS1	–	–	RDs-47	Qatar	69 years	64 years	Retinitis pigmentosa	–	–	–
CNGA3	rs104893613	c.847C>T	p.Arg283Trp	Single nucleotide variant	Missense	Homozygous	AR	WES	Pathogenic	Likely Pathogenic	PM1	–	–	RDs-12	Qatar	7 years	7 years	Retinitis pigmentosa	–	–	–

Identified variants in cases reconsidered as solved after family segregation studies

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG subclassification	ACMG VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age	Age of diagnosis	Phenotype	Mother	Father	Affected sibs	Healthy sibs	Justification	Reports from other populations/ethnicities	Phenotype	References
PDE6B	rs771338607	c.2407A>G	p.Asn803Asp	single nucleotide variant	Missense	Homozygous	AD/AR	WES PlusTrio	Variant of uncertain significance	Likely Pathogenic	PM1	–	–	RDs-7	Pakistan	15 years	14 years	Retinitis pigmentosa	Heterozygous	Heterozygous	No affected siblings	Heterozygous in 1 bro not detected in 2 sis	This finding is most likely associated with Retinitis Pigmentosa in this patient as the variant homozygosity is segregating with the disease in the family	–	–	–
ABCA4	rs745512565	c.4753C>T	p.Arg1585Trp	single nucleotide variant	Missense	Homozygous	AD/AR	WES Trio	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	RDs-40	Egypt	20 years	14 years	Cone-Rod dystrophy	Heterozygous	Heterozygous	No affected siblings	Heterozygous in 1 sis Not detected in 1 bro	This finding is most likely associated with Cone-Rod dystrophy in this patient as the variant homozygosity is segregating with the disease in the family	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG subclassification	ACMG VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age	Age of diagnosis	Phenotype	Mother	Father	Affected sibs	Healthy sibs	Justification	Reports from other populations/ethnicities	Phenotype	References
PRCD	rs757471313	c.74C>T	p.Pro25Leu	single nucleotide variant	Missense	Homozygous	AR	WES PlusTrio	Variant of uncertain significance	Variant of uncertain significance	PM1	Hot	disease causing	RDs-23	Qatar	48 years	46 years	Retinitis pigmentosa	Heterozygous	Heterozygous	Homozygous in 1 affected sis	Heterozygous in 1 sib not detected in 1 sib	This finding is most likely associated with Retinitis Pigmentosa in this patient as the variant homozygosity is segregating with the disease in the family	–	–	–
CNNM4	–	c.509T>C	Leu170Pro	single nucleotide variant	Missense	Homozygous	AR	Familial targeted testing	Variant of uncertain significance	Variant of uncertain significance	PM2	Cool	disease causing	RDs-48	Qatar	5 years	2 years	Cone-Rod dystrophy	Heterozygous	Heterozygous	No affected siblings	Heterozygous in 1 unaffected sis	This finding is most likely associated with Cone-Rod dystrophy in this patient as the variant homozygosity is segregating with the disease in the family	Qatar, United Arab Emirates	Cone-Rod Dystrophy, Jalili	(Khan, 2020; Patel et al., 2018)
MERTK	–	c.2020A>G	p.Met674Val	single nucleotide variant	Missense	Homozygous	AR	WES Trio	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	RDs-11	Qatar	21 years	20 years	Retinitis pigmentosa	Heterozygous	Heterozygous	Homozygous in 1 affected sister	Heterozygous in 3 unaffected siblings not detected in 1 sibling	This finding is most likely associated with Retinitis Pigmentosa in this patient as the variant homozygosity is segregating with the disease in the family	–	–	–
PCDH15	rs568865061	c.2897G>C	p.Arg966Thr	single nucleotide variant	Missense	compound Heterozygous	AR	WES PlusProband	Variant of uncertain significance	likely benign	PM1	–	–	RDs-16	Qatar	38 years	33 years	Retinitis pigmentosa	Not detected	Heterozygous	No affected siblings	Not detected in 2 siblings	This finding is most likely associated with Retinitis Pigmentosa in this patient as the variant compound heterozygosity is segregating with the	–	–	–
	rs750302536	c.131T>C	p.Val44Ala	single nucleotide variant	Missense		AR		Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing						Heterozygous	Not detected	No affected siblings	Not detected in 2 siblings		–	–	–

																						disease in the family			
Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG subclassification	ACMG VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age	Age of diagnosis	Phenotype	Mother	Father	Affected sibs	Healthy sibs	Justification	Reports from other populations/ethnicities	Phenotype	References
GRM6	–	c.281G>C	p.Arg94Pro	single nucleotide variant	Missense	Homozygous	AR	WES PlusTrio	Variant of uncertain significance	Variant of uncertain significance	PM1	Hot	disease causing	RDs-37	Pakistan	12 years	8 years	Cone-Rod dystrophy	Heterozygous	Heterozygous	No affected siblings	Not detected in 2 siblings	This finding is most likely associated with Cone-Rod dystrophy in this patient as the variant homozygosity is segregating with the disease in the family	–	–	–
GUCY2D	–	c.2213_2215del	p.Glu738del	Deletion	Frameshift	Homozygous	AD/AR	WES Trio	Variant of uncertain significance	–	–	–	disease causing	RDs-39	Yemen	3 years	1.5 years	leber congenital amaurosis	Heterozygous	Heterozygous	No affected siblings	Heterozygous in 1 sibling	This finding is most likely associated with leber congenital amaurosis in this patient as the variant homozygosity is segregating with the disease in the family	–	–	–
CFAP410	rs771024688	c.209G>A	p.Arg70Gln	single nucleotide variant	Missense	Homozygous	AR	WES PlusTrio	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	RDs-41	Egypt	13 years	9 years	Cone-Rod dystrophy	Heterozygous	Heterozygous	No affected siblings	Heterozygous in 1 sis Not detected in 1 bro	This finding is most likely associated with Cone-Rod dystrophy in this patient as the variant homozygosity is segregating with the disease in the family	–	–	–

Identified variants in cases reconsidered as solved after WES reanalysis

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG Subclassification	ACMG VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	Justification	Reports from other populations/ethnicities	Phenotype	References
MERTK	–	c.2020 A>G	p.Met674Val	single nucleotide variant	Missense	Homozygous	AR	WES	Likely Pathogenic	Variant of uncertain significance	PM1	Warm	disease causing	RDs-13	Qatar	48 years	40 years	Retinitis pigmentosa	The variant was initially classified as a VUS and then reclassified after reWES	–	–	–
	rs141361084	c.2435 A>C	p.Tyr812Ser	single nucleotide variant	Missense	Homozygous	AR		Likely Benign	Variant of uncertain significance	PM1	Warm	disease causing						The variant was initially classified as a VUS then reclassified after reWES	–	–	–

AD: Autosomal dominant, AR: Autosomal recessive, AD/AR: Autosomal dominant & Autosomal recessive, XL: X-linked, PVS1: Very strong evidence of pathogenicity, PS3: Strong evidence of pathogenicity, PM1-PM6: Moderate strength evidence of pathogenicity

Table 3. Identified variants in the uncertain cases

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG Subclassification	ACMG VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age	Age of diagnosis	Phenotype	reports from other populations/ ethnicities	Phenotype	References
RHO	_	c.697-3C>A	IVS3-3C>A	single nucleotide variant	splicing site	Homozygous	AD/AR	WES Plus	Variant of uncertain significance	_	_	_	_	RDs-3	Qatar	46 years	43 years	Retinitis pigmentosa	_	_	_
PDE6A	rs374847529	c.103 G>A	p.Asp35Asn	single nucleotide variant	Missense	Homozygous	AR	WES	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	RDs-17	Qatar	3 years	2 years	Retinitis pigmentosa	–	–	–
GPC4	rs1412463359	c.156 C>G	p.lle52Met	single nucleotide variant	Missense	Hemizygous	XL		Variant of uncertain significance	Variant of uncertain significance	PM2	Cool	disease causing						–	–	–
ABCA4	rs1801466	c.5603 A>T	p.Asn1868Ile	single nucleotide variant	Missense	Heterozygous	AD/AR	WES	Risk Allele	Variant of uncertain significance	PM1	Tepid	polymorphism	RDs-34	Pakistan	56 years	53 years	Stargardt disease	Germany	Stargardt disease	(Schulz et al., 2017)
	rs752850266	c.6218 G>C	p.Gly2073Ala	single nucleotide variant	Missense	Homozygous	AR	WES Plus	Variant of uncertain significance	Variant of uncertain significance	PM1	Hot	disease causing	RDs-45	Qatar	54 years	50 years	Retinitis pigmentosa	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG Subclassification	ACMG VUS Description	Mutationtaster	PATIENT ID	Country of origin	Age	Age of diagnosis	Phenotype	reports from other populations/ ethnicities	Phenotype	References
CRB1	rs1571522690	c.1313 G>A	p.Cys438Tyr	single nucleotide variant	Missense	Homozygous	AR	Gene Panel testing	Variant of uncertain significance	Variant of uncertain significance	PM1	Hot	disease causing	RDs-28	United arab emirates	10 years	6 years	Macular dystrophy	–	–	–
DRAM2	rs772262465	c.246 T>G	p.Ser82Arg	single nucleotide variant	Missense	Homozygous	AR	WES	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	RDs-34	Pakistan	56 years	53 years	Stargardt disease	–	–	–
EYS	rs199740930	c.2137+1 G>A	IVS13+1 G>A	single nucleotide variant	splice site donor	compound Heterozygous	AR	WES Plus	Variant of uncertain significance	–	–	–	–	RDs-38	Qatar	14 years	8 years	Congenital stationary night blindness	–	–	–
	rs1383398602	c.3709 G>C	p.Gly1237Arg	single nucleotide variant	Missense		AR		Variant of uncertain significance	likely benign	PM2	–	–						–	–	–
GUCA1B	rs1554186885	c.593 C>G	p.Ala198Gly	single nucleotide variant	Missense	Heterozygous	AD	WES	Variant of uncertain significance	Variant of uncertain significance	PM2	Cool	disease causing	RDs-43	Palestine	13 years	6 years	Cone dystrophy	–	–	–
TUB	rs575184271	c.1357_1360delAGAG	p.Arg453SerfsX13	Deletion	Frameshift	Homozygous	Unknown		Variant of uncertain significance	–	–	–	disease causing						–	–	–
RPGRIP1	–	c.105dupA	p.Pro36Thrfs*35	Duplication	Frameshift	Homozygous	AR	Gene Panel testing	Variant of uncertain significance	–	–	–	disease causing	RDs-49	Egypt	12 years	3 years	leber congenital amaurosis	–	–	–

Table 4. Shared variants

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethniciites	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicitiies	Phenotype	References
	rs763890649	c.1040_1041delTT	p.Phe347TrpfsX5	Deletion	Frameshift	Homozygous	AD/AR	Pathogenic	–	–	–	disease causing	Likely Pathogenic	Leber congenital amaurosis	Heterozygous - 0.000004164	–	–	RDs-1	Qatar	2 years	3 months	Retinitis pigmentosa	–	–	–
GUCY2D	rs138836357	c.1093C>T	p.Arg365Trp	single nucleotide variant	Missense	Homozygous	AD/AR	VUS	VUS	PM1	Warm	polymorphism	Conflicting interpretations of Pathity	Leber congenital amaurosis	Heterozygous - 0.0007357	Middle Eastren Lationo/ Admixed American African/ African American European (non-finnish) European (finnish) Ashkenazi Jewish South Asian	0.002014099	RDs-30	Syria	14 years	12 years	Macular dystrophy	–	–	–
	rs886039422	c.2214delT	p.Cys738TrpfsX32	Deletion	Frameshift	Homozygous	AR	Pathogenic	–	–	–	disease causing	Pathogenic	Retinitis Pigmentosa (AR)	–	–	–	RDs-1 RDs-21	Qatar Qatar	2 years 34 years	3 months 28 years	Retinitis pigmentosa Retinitis pigmentosa	Saudi Arabia, United Arab Emirate	Retinitis pigmentosa & Rod cone dystrophy	(Patel et al., 2016) (Khan, 2020b)
MERTK	–	c.2020A>G	p.Met674Val	single nucleotide variant	Missense	Homozygous Homozygous	AR	Variant of uncertain significance Likely Pathogenic	Variant of uncertain significance	PM1	Warm	disease causing	Likely Pathogenic	–	-	-	-	RDs-11	Qatar	21 years	20 years	Retinitis pigmentosa			
	rs141361084	c.2435A>C	p.Tyr812Ser	single nucleotide variant	Missense	Homozygous	AR	likely benign	Variant of uncertain significance	PM1	Warm	disease causing	Variant of uncertain significance	Retinitis pigmentosa	Heterozygous - 0.00007884	Middle Eastren Lationo/ Admixed American African/ African American European (non-finnish)	0.003021148	RDs-13	Qatar	48 years	40 years	Retinitis pigmentosa	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethniciities	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicitiies	Phenotype	References
GRM6	rs752205220	c.1478G>A	p.Trp493Ter	single nucleotide variant	Nonsense	Homozygous	AR	Likely Pathogenic	Pathogenic	PVS1	_	disease causing	-	-	Heterozygous - 0.000007983	Lationo/ Admixed American	_	RDs-2 RDs-6	Qatar Qatar	3 years 6 years	3 years 6 years	Congenital stationary night blindness Congenital stationary night blindness	_	_	_
	_	c.281 G>C	p.Arg94Pro	single nucleotide variant	Missense	Homozygous	AR	Variant of uncertain significance	Variant of uncertain significance	PM1	Hot	disease causing	Variant of uncertain significance	_	-	-	-	RDs-37	Pakistan	12 years	8 years	Retinitis pigmentosa	_	_	_
CABP4	rs786205852	c.81_82insA	p.Pro28ThrfsX4.	single nucleotide variant	Frameshift	Homozygous	AR	Pathogenic	_	_	_	disease causing	Pathogenic	_	-	-	-	RDs-4	Qatar	4 years	6 months	Uncategorized Retinal dystrophy	Saudi Arabia	segregated with congenital retinal dysfunction in 11 affected individuals (aged 2–26 years) from four consanguineous families	(Khan et al., 2013)

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethniciities	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicities	Phenotype	References
ABCA4	–	c.5584G>C	p.Gly1862Arg	single nucleotide variant	Missense	Homozygous	AD/AR	Pathogenic	Variant of uncertain significance	PM1	Hot	disease causing	Pathogenic	–	-	-	-	RDs-8	Qatar	26 years	23 years	Stargardt disease	China	Stargardt disease	(X. F. Huang et al., 2015a)
	rs61748556	c.1609C>T	p.Arg537Cys	single nucleotide variant	Missense	Heterozygous	AD/AR	Likely Pathogenic	Likely Pathogenic	PM1	–	–	Pathogenic/Likely pathogenic	Retinal dystrophy	Heterozygous - 0.00002387	African/ African American European (non-finnish) South Asain European (finnish Lationo/ Admixed American European (non-finnish)	–	RDs-9	Qatar	14 years	11 years	Macular dystrophy	Germany	Stargardt disease	(Schulz et al., 2017)
	rs61750155	c.4793C>A	p.Ala1598Asp	single nucleotide variant	Missense	Homozygous	AD/AR	Pathogenic	Likely Pathogenic	PM1	–	–	Pathogenic/Likely pathogenic	Retinal dystrophy	Heterozygous - 0.00002631		0.001007049	RDs-25	Yemen	20 years	18 years	Stargardt disease	Germany	Stargardt disease	(Maugeri et al., 2000a)
																		RDs-9	Qatar	14 years	11 years	Macular dystrophy			
	rs1800553	c.5882G>A	p.Gly1961Glu	single nucleotide variant	Missense	Heterozygous	AD/AR	Pathogenic	Likely Pathogenic	PS3	–	–	Pathogenic	Complex Retinal dystrophy	–	–	–	RDs-47	Qatar	69 years	64 years	Retinitis pigmentosa	China, Spain, United Arab Emirates, and Italy	Stargardt disease	(Maltese et al., 2022) (X. F. Huang et al., 2015a) (Jespersgaard et al., 2019)
																		RDs-22	Qatar	56 years	53 years	Retinitis pigmentosa			

	rs1801466	c.5603A>T	p.Asn1868Ile	single nucleotide variant	Missense	Heterozygous	AD/AR	Risk Allele	Variant of uncertain significance	PM1	Tepid	polymorphism	Conflicting interpretations of pathogenicity	Complex Retinal dystrophy	Heterozygous / Homozygous - 0.04042	Lationo/ Admixed American African/ African American European (non-finnish) Amish Middle Eastren Askenazi Jewish European (finnish) South Asian	0.039314516	RDs-34	Pakistan	56 years	53 years	Stargardt disease	Germany	Stargardt disease	(Schulz et al., 2017)
	rs745512565	c.4753C>T	p.Arg1585Trp	single nucleotide variant	Missense	Homozygous	AD/AR	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	Variant of uncertain significance	–	Heterozygous - 0.000006579	African/ African American	–	RDs-40	Egypt	20 years	14 years	Cone-Rod dystrophy	–	–	–
	rs752850266	c.6218G>C	p.Gly2073Ala	single nucleotide variant	Missense	Homozygous	AR	Variant of uncertain significance	Variant of uncertain significance	PM1	Hot	disease causing	Variant of uncertain significance	–	Heterozygous - 0.00003942	African/ African American European (non-finnish)	–	RDs-45	Qatar	54 years	50 years	Retinitis pigmentosa	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethnicities	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicities	Phenotype	References
CRX	rs771736389	c.128 G>A	p.Arg43His	single nucleotide variant	Missense	Heterozygous	AD	Likely Pathogenic	Variant of uncertain significance	PM1	Hot	disease causing	Pathogenic/Likely pathogenic	Cone-rod dystrophy Leber congenital amaurosis	Heterozygous - 0.000006574	European (non-finnish)	–	RDs-9	Qatar	14 years	11 years	Macular dystrophy	–	–	–
PDE6B	rs370898371	c.1107+3A>G	IVS8+3A>G	single nucleotide variant	Splice site	compound Heterozygous	AD/AR	Likely Pathogenic	–	–	–	–	Conflicting interpretations of pathogenicity	Retinitis pigmentosa	Heterozygous - 0.00002627	European (non-finnish)	–	RDs-15	Croatia	41 years	3 years	Retinitis pigmentosa	–	–	–
	rs1737315492	c.1859A>G	p.His620Arg	single nucleotide variant	Missense		AD/AR	Likely Pathogenic	Variant of uncertain significance	PM1	Hot	disease causing	Variant of uncertain significance	Retinitis pigmentosa	-	-	–						–	–	–

	rs751859807	c.1655G>A	p.Arg552Gln	single nucleotide variant	Missense	Homozygous	AR	Likely Pathogenic	Likely Pathogenic	PM1	–	–	Pathogenic/Likely pathogenic	–	Heterozygous - 0.00001972	East Asian European (non-finnish) African/ African American	–	RDs-18	Qatar	25 years	24 years	Retinitis pigmentosa	–	–	–
	rs771338607	c.2407A>G	p.Asn803Asp	single nucleotide variant	Missense	Homozygous	AD/AR	Variant of uncertain significance	Likely Pathogenic	PM1	–	–	Variant of uncertain significance	Retinitis pigmentosa	Heterozygous - 0.00000398	South Asian	–	RDs-7	Pakistan	15 years	14 years	Retinitis pigmentosa	–	–	–
PDE6C	rs105751824 4	c.724-1G>T	IVS3-1G>T (in intron3)	single nucleotide variant	Splice site	Homozygous	AR	Likely Pathogenic	–	–	–	–	Likely pathogenic	–	-	-	-	RDs-10	Qatar	15 years	9 years	Retinitis pigmentosa	–	–	–
RDH12	rs159486759 7	c.821 T>C	p.Leu274Pro	single nucleotide variant	Missense	Homozygous	AR	Pathogenic	Likely Pathogenic	PM1	–	–	Pathogenic	Retinitis pigmentosa	-	-	-	RDs-19 RDs-31	Palestine Palestine	21 years 6 years	17 years 2.5 years	Retinitis pigmentosa Uncategorized Retinal dystrophy	Israel	Retinitis Pigmentosa (5), Leber congenital amurosis(1)	(Sharon et al., 2020)
KCNV2	–	9p24.2(2684449-2766856)x1	–	copy number variation	copy number variation	compound Heterozygous	AR	Pathogenic	–	–	–	–	–	–	–	–	–	RDs-20	Egypt	14 years	12 years	Cone dystrophy	–	–	–
	rs1819788466	c.757 C>G	p.Pro253Ala	single nucleotide variant	Missense		AR	Likely Pathogenic	Variant of uncertain significance	PM2	Warm	disease causing	Variant of uncertain significance	–	-	-	-						–	–	–
KIZ	rs775124094	c.247 C>T	p.Arg83Ter	single nucleotide variant	Nonsense	Homozygous	AR	Pathogenic	Variant of uncertain significance	PM2	Tepid	–	Pathogenic/Likely pathogenic	Retinal dystrophy	Heterozygous - 0.00003287	African/ African American European (non-finnish)	-	RDs-24	Qatar	50 years	49 years	Retinitis pigmentosa	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethnicities	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicities	Phenotype	References
RPGR	rs1186795749	c.3092del	p.Glu1031Glyfs*58	Deletion	Frameshift	Hemizygous	XLR	Pathogenic	–	–	–	disease causing	Pathogenic	Complex Retinal dystrophy	-	-	-	RDs-29	Qatar	37 years	37 years	Retinitis pigmentosa	Denmark	Retinitis pigmentosa	(Jespersgaard et al., 2019)
AIPL1	rs62637014	c.834 G>A	p.Trp278Ter	single nucleotide variant	Nonsense	Homozygous	AR	Pathogenic	Variant of uncertain significance	PM2	Warm	disease causing	Pathogenic	Leber congenital amaurosis	Heterozygous - 0.0003291	Lationo/ Admixed American African/ African American European (non-finnish) South Asian	0.000503525	RDs-30	Syria	14 years	12 years	Macular dystrophy	Romania	LCA, Leber congenital amaurosis	(Maltese et al., 2022)
GNAT2	rs155322658 1	c.720+5G>C	IVS6+5G>C	single nucleotide variant	Splice site	Homozygous	AR	Likely Pathogenic	–	–	–	–	Likely pathogenic	–	-	-	-	RDs-32	Qatar	44 years	41 years	Retinitis pigmentosa	–	–	–
																		RDs-44	Qatar	16 years	11 years	Achromatopsia	–	–	–
NMNAT1	rs201994921	c.634G>A	p.Val212Met	single nucleotide variant	Missense	compound Heterozygous	AR	Likely Pathogenic	Likely Pathogenic	PM1	–	–	Conflicting interpretations of pathogenicity	Leber congenital amaurosis	Heterozygous - 0.0000 3286	African/ African American European (non-finnish) European (finnish)	–	RDs-33	Pakistan	9 years	6 years	Generalized Retinal dystrophies	–	–	–
	–	chr1: 10035650_ 10035833	–	Deletion	Copy number variation		AR	Pathogenic	–	–	–	–	–	-	-	-	–						–	–	–
RPGRIP1	rs137180599 3	c.2935 C>T	p.Gln979Ter	single nucleotide variant	Missense	Heterozygous	AR	Pathogenic	Pathogenic	PVS1	–	–	Pathogenic	Leber congenital amaurosis Cone-rod dystrophy, Retinitis pigmentosa	–	–	–	RDs-35	Lebanon	7 years	6 years	Rod-Cone dystrophy	Israel	Retinitis pigmentosa	(Sharon et al., 2020)

	rs6175 1266	c.1107del A	p.Glu370 AsnfsX5	Dele tion	Fram eshif t	Homo zygou s	AR	Patho genic	Patho genic	–	–	–	Pathoge nic	Leber congen ital amauro sis	–	–	–	RDs -14	Qat ar	5 ye ars	2 year s	Uncate gorized Retinal dystrop hy	Saudi Arabia	Cone-Rod Dystro phy, Leber congeni tal amauro sis	(Patel et al., 2018)

Gen e	RSID	Variant (c.DNA)	Variant (protein)	Vari ant type	Vari ant Impa ct	Zygos ity	Patte rn of Inher itanc e	Varia nts classif icatio n - based on the report	ACM G	ACMG sublass ificatio n (highes t)	VUS Desc riptio n	Mutati ontaste r	ClinVar classific ation	ClinVa r phenot ype	Gnom AD Allele Freque ncy	GnomA D Allele frequenc y in different Ethniciti es	GME Allele Frequency	PAT IEN T ID	Cou ntry of orig in	Ag e (ye ars)	Age of diag nosi s (yea rs)	Phenot ype	reports from other population s/ethnicitie s	Phenot ype	Reference s
CRB 1	rs1571 522690	c.1313 G>A	p.Cys438 Tyr	singl e nucl eotid e varia nt	Miss ense	Homo zygou s	AR	Varia nt of uncert ain signifi cance	Varia nt of uncer tain signif icanc e	PM1	Hot	disease causin g	Variant of uncertai n significa nce	Leber congen ital amauro sis	-	-	-	RDs -28	Unit ed arab emi rate s	10 ye ars	6 year s	Macula r dystrop hy	–	–	–
PRP H2	rs1799 98648 9	c.936del	p.Pro313 Argfs*11	Dele tion	Fram eshif t	Hetero zygou s	AD	Likely Patho genic	–	–	–	disease causin g	Conflicti ng interpret ations of pathoge nicity	Compl ex Retinal dystrop hy	-	-	-	RDs -46	Qat ar	61 ye ars	61 year s	Macula r dystrop hy	–	–	–
CYP 4V2	rs1994 76204	c.1348 C>T	p.Gln450 Ter	singl e nucl eotid e varia nt	Nons ense	Homo zygou s	AR	Likely Patho genic	Patho genic	PVS1	–	–	Pathoge nic	Bietti Crystal line Dystro phy	Hetero zygous - 0.0000 06576	Europea n (non-finnish)	-	RDs -47	Qat ar	69 ye ars	64 year s	Retiniti s pigmen tosa	–	–	–
RH O	–	c.697-3C>A	IVS3-3C>A	singl e nucl eotid e varia nt	Splic e site	Homo zygou s	AD/ AR	Varia nt of uncert ain signifi cance	–	–	–	–	Variant of uncertai n significa nce	–	–	–	–	RDs -3	Qat ar	46 ye ars	43 year s	Retiniti s pigmen tosa	–	–	–
PCD H15	rs5688 65061	c.2897 G>C	p.Arg966 Thr	singl e nucl eotid e varia nt	Miss ense	compo und Hetero zygou s	AR	Varia nt of uncert ain signifi cance	likely benig n	PM1	–	–	Variant of uncertai n significa nce	–	Hetero zygous - 0.0000 1972	South Asian	–							–	–	–
	rs7503 02536	c.131 T>C	p.Val44A la	singl e nucl eotid e varia nt	Miss ense		AR	Varia nt of uncert ain signifi cance	Varia nt of uncer tain signif icanc e	PM1	War m	disease causin g	Conflicti ng interpret ations of pathoge nicity	Usher syndro me type 1F, Autoso mal recessi ve nonsyn dromic HL	Hetero zygous - 0.0000 5263	Europea n (non-finnish) Lationo/ Admixe d Americ an South Asian	–	RDs -16	Qat ar	38 ye ars	33 year s	Retiniti s pigmen tosa	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethnicities	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicities	Phenotype	References
PDE6A	rs374847529	c.103 G>A	p.Asp35Asn	single nucleotide variant	Missense	Homozygous	AR	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	Variant of uncertain significance	Retinitis pigmentosa	Heterozygous - 0.0001251	European (non-finnish) East Asian Lationo/ Admixed American African/ African American	0.002517623363544813 7	RDs-17	Qatar	3 years	2 years	Retinitis pigmentosa	–	–	–
GPC4	rs141246335 9	c.156 C>G	p.lle52Met	single nucleotide variant	Missense	Hemizygous	XL	Variant of uncertain significance	Variant of uncertain significance	PM2	Cool	disease causing	-	-	Heterozygous /Hemizygous - 0.00001102	European (non-finnish)	–						–	–	–
PRCD	rs757471313	c.74 C>T	p.Pro25Leu	single nucleotide variant	Missense	Homozygous	AR	Variant of uncertain significance	Variant of uncertain significance	PM1	Hot	disease causing	Variant of uncertain significance	–	Heterozygous - 0.00001633	European (non-finnish) East Asian	–	RDs-23	Qatar	48 years	46 years	Retinitis pigmentosa	–	–	–
DRAM2	rs772262465	c.246 T>G	p.Ser82Arg	single nucleotide variant	Missense	Homozygous	AR	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	polumorphism	Variant of uncertain significance	–	Heterozygous - 0.00001195	South Asian	–	RDs-34	Pakistan	56 years	53 years	Stargardt disease	–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethniciities	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicities	Phenotype	References
EYS	rs199740930	c.2137+1G>A	IVS13+1G>A	single nucleotide variant	Splice site	compound Heterozygous	AR	Variant of uncertain significance	–	–	–	–	Conflicting interpretations of pathogenicity	Retinitis pigmentosa	Heterozygous - 0.000006409	Ashkenazi Jewish Middle Eastren South Asian European (non-finnish) Lationo/ Admixed American African/ African American	–	RDs-38	Qatar	14 years	8 years	Congenital stationary night blindness	Denmark	Retinitis pigmentosa	(Jespersgaard et al., 2019)
	rs1383398602	c.3709G>C	p.Gly1237Arg	single nucleotide variant	Missense		AR	Variant of uncertain significance	likely benign	PM2	–	–	Variant of uncertain significance	–	Heterozygous - 0.0003553	South Asian	–						-	–	
CFAP410	rs771024688	c.209 G>A	p.Arg70Gln	single nucleotide variant	Missense	Homozygous	AR	Variant of uncertain significance	Variant of uncertain significance	PM1	Warm	disease causing	Variant of uncertain significance	Retinal dystrophy	Heterozygous - 0.00001314	South Asian European (non-finnish)	–	RDs-41	Egypt	13 years	9 years	Cone-Rod dystrophy	–	–	–
GUCA1B	rs155418688 5	c.593 C>G	p.Ala198Gly	single nucleotide variant	Missense	Heterozygous	AD	Variant of uncertain significance	Variant of uncertain significance	PM2	Cool	disease causing	Variant of uncertain significance	–	-	-	-	RDs-43	Palestine	13 years	6 years	Cone dystrophy	–	–	–
TUB	rs575184271	c.1357_1360delAGAG	p.Arg453SerfsX13	Deletion	Frameshift	Homozygous	Unknown	Variant of uncertain significance	–	–	–	disease causing	-	-	Heterozygous - 0.000003977	European (finnish)	–						–	–	–

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	VUS Description	Mutationtaster	ClinVar classification	ClinVar phenotype	GnomAD Allele Frequency	GnomAD Allele frequency in different Ethnicities	GME Allele Frequency	PATIENT ID	Country of origin	Age (years)	Age of diagnosis (years)	Phenotype	reports from other populations/ethnicities	Phenotype	References
CNGA3	*rs104893613*	c.847 C>T	p.Arg283Trp	Single nucleotide variant	Missense	Homozygous	AR	Pathogenic	Likely Pathogenic	PM1	–	–	Pathogenic/Likely pathogenic	Achromatopsia	Heterozygous - 0.00001971	European (non-finnish) African/ African American	–	RDs-12	Qatar	7 years	7 years	Cone dystrophy	–	–	–
CNNM4	–	c.509 T>C	p.Leu170Pro	single nucleotide variant	Missense	Homozygous	AR	Variant of uncertain significance	Variant of uncertain significance	PM2	Cool	disease causing	–	–	–	–	–	RDs-48	Qatar	5 years	2 years	Cone-Rod dystrophy	Qatar, United Arab Emirates	Cone-Rod Dystrophy, Jalili	(Khan, 2020b; Patel et al., 2018)

Table 5. Novel variants

Gene	RSID	Variant (c.DNA)	Variant (protein)	Variant type	Variant Impact	Zygosity	Pattern of Inheritance	Test Done	Variants classification - based on the report	ACMG	ACMG sublassification (highest)	PATIENT ID	Country of origin	Age	Age of diagnosis	Phenotype	Mutationtaster
GUCY2D	–	c.2213_2215del	p.Glu738del	Deletion	Frameshift	Homozygous	AD/AR	WES	Variant of uncertain significance	–	–	RDs-39	Yemen	3 years	1.5 years	leber congenital amaurosis	disease causing
RPGRIP1	–	c.3278dupC	p.Gln1094Thrfs*6	Duplication	Frameshift	compound Heterozygous	AR	WES Plus	Pathogenic	–	–	RDs-35	lebanon	7 years	6 years	Rod-Cone dystrophy	disease causing
	–	c.105dupA	p.Pro36Thrfs*35	Duplication	Frameshift	Homozygous	AR	Panel	Variant of uncertain significance	–	–	RDs-49	Egypt	12 years	3 years	leber congenital amaurosis	disease causing
CRB1	–	c.3613 G>T	p.Gly1205Ter	single nucleotide variant	Nonsense	compound Heterozygous	AR	WES Plus	Pathogenic	Pathogenic	PVS1	RDs-36	Qatar	10 years	6 years	Cone-Rod dystrophy	disease causing

	_	c.4211 G>C	p.Arg1404Thr	single nucleotide variant	Missense		AR		Likely Pathogenic	VUS	PM1							disease causing
CFAP418	_	c.478 dupA	p.Met160Asnfs*25	Duplication	Frameshift	Homozygous	AR	WES Plus	Likely Pathogenic	_	_	RDs-42	lebanon	27 years	27 years	Retinitis pigmentosa		disease causing

4.2.5 Test frequency and diagnostic yield

In order to assess different genetic tests' diagnostic yields. Fisher's exact test was used to determine if there was a significant association between the type of genetic test and solved cases (Table 7), as it best represents the diagnostic yield. All participants in our study underwent one genetic test, with the most used test being WES Plus, which was performed on 21 patients. Out of the 49 patients, 14 patients had WES, 9 had gene panel testing, 5 patients had familial targeted testing, and 21 patients underwent WES Plus. Eighteen cases were solved by both WES and WES Plus, 5 cases by gene panel testing, and 5 cases by familial targeted testing. Through the statistical analysis, no significant association was detected.

Table 6. Different genetic tests diagnostic yield

Genetic Test	Utilization frequency/ per patients (n=49)	Solved cases (diagnostic yield)	Uncertain cases	Unsolved cases
Whole Exome sequencing	35 (70%)	18 (51.4%) *p-value: 0.311**	15 (42.85%)	1 (2.85%)
Mitochondrial Genome testing	21** (42%)	0 -*	0	0
Gene Panel testing	9 (18%)	5 (55.5%) *p-value: 0.635**	2 (22.22%)	2 (22.22%)
Familial Targeted Testing	5 (10%)	4 (80%) *p-value: 0.245**	1 (20%)	0
Total	70	28		

* p-values were collected using the Fisher test to assess the genetic test diagnostic yield
** Mitochondrial genome testing is included in WES

CHAPTER 5: DISCUSSION

In our study, we investigated the genetic factors behind non-syndromic RDs seen at the Department of Adult and Pediatric Medical Genetics, at Hamad Medical Corporation between 2015-2022. In total, we identified 49 eligible patients with 55 variants in 32 different RDs-related genes.

In this study, Qatari patients contributed the most with 61.2% (n = 30), and other Arabs 28.4% (n= 14). The consanguinity rate was about 78% in our cohort, similar consanguinity rates (68%) were seen in previous RDs studies on Saudi Arabian patients (Patel et al., 2018). Notably, 36 (64.45%) of the 55 variants were autosomal recessive variants. This finding is in line with the 79.6% consanguinity rate of our patients, which is significantly higher than the 56% national average (el Mouzan et al., 2008). Out of the 49 participants, 67.3% (n=33) had a positive family history of RDs compared to other cases from England where it was only 35%, and this is explained by the high inbreeding and consanguinity rates among the population of Qatar (Shahid et al., 2012). Our participants' most reported clinical diagnosis was Retinitis pigmentosa, which was also seen in other Arab countries (55%) like Saudi Arabia (Abu-Safieh et al., 2013) and other European countries like Denmark (Jespersgaard et al., 2019).

5. 2. Genetic testing options

Among the 49 participants, 21 underwent WES Plus, 14 underwent WES, 9 underwent panel testing, and 5 were tested by familial targeted testing. WES testing was the most frequent test utilized by patients with unknown familial pathogenic variants 70% (n=35) in comparison to gene panel testing and this can be explained by the comprehensiveness of WES and the ability to include other family members in WES testing. This is done by comparing the proband's genetic data to the family members' data to identify shared genetic variants and thereby diagnosing more than one family

member in the same test. In addition to the cost-effectiveness and reducing the time for the diagnostic odyssey in comparison to the step-wise testing approach. It should be noted that patients who underwent gene panel testing in previous years had limited and less comprehensive panels. On the other hand, participants who did gene panel testing in recent years had access to gene panel tests that are more comprehensive, and, in some panels, parental samples were also included in the testing. Mitochondrial genome testing in RDs can be used in order to capture cases where mitochondrial genetic variants might play a role in the patient's phenotype. However, in our study, mitochondrial genome testing did not identify any causative variants. This finding aligns with previous studies reporting that mitochondrial variants are relatively rare compared to other genetic causes of RDs (Carelli et al., 2004). We did not observe any significant association between using a type of genetic test and the diagnostic yield. The gene panel testing showed a diagnostic yield of 55.5%, while the WES diagnostic yield had a similar result of 51.4%. Previous genetic investigations of hereditary retinal disorders using a 179 RDs gene panel produced molecular diagnosis in 55.3% of patients referred (X. F. Huang et al., 2015a). (Patel et al., 2018) reported a higher molecular diagnostic yield for referred patients in Saudi Arabia (up to 82%) when WES was used. Variations in the genes included in different gene panels among studies could be one of the factors that explain the observed variations in the diagnostic yield associations. In our study, the similarity in the diagnostic yield between gene panel testing and WES may be due to the fact that most recent gene panels are comprehensive and cover a significant proportion of the genes identified by WES testing.

5. 3. Genetic test results

Among the 32 identified genes, the *ABCA4* gene was the most reported, and similar findings were also shared by other studies from the United Arab Emirates and

the United Kingdom (Pontikos et al., 2020) (Khan, 2020b) where *ABCA4* being the most reported gene. Other common genes reported among study participants included *CRB1, GNAT2, GRM6, GUCY2D, MERTK, PDE6B, RDH12*, and *RPGRIP1*, each detected in at least two patients from our cohort. From the 55 identified variants, 36 were inherited in an autosomal recessive manner, 13 as autosomal dominant/autosomal recessive, 3 as autosomal dominant, 2 as X linked, and 1 variant with an unknown inheritance pattern. Out of the 55 variants observed, the majority (n=36 variants; 64.45%) were autosomal recessive. This result is consistent with the fact that 79.6% of the patients came from consanguineous families (el Mouzan et al., 2008).

5. 3.1. Genes Identified in Solved Cases

Twenty-eight cases were initially classified as solved where the genetic test identified a pathogenic/likely pathogenic variant in RDs-related genes. The *ABCA4* was the most reported gene in this group where it was identified in 5 patients. This could be explained by the key function of the *ABCA4* protein for the proper function of the retina (Maugeri et al., 2000b). Pathogenic variants in the *ABCA4* gene are the most common cause of autosomal recessive cone-rod dystrophy, accounting for 30 to 60 % of cases including Stargardt disease and cone-rod dystrophy (Maugeri et al., 2000b). Patients who harbored pathogenic variants in the *ABCA4* gene had clinical manifestations that included Stargardt disease, macular dystrophy, and retinitis pigmentosa. Previous studies that were done on RDs patients from Saudi Arabia and the United Arab Emirates reported the *ABCA4* gene as a significant contributor to RDs in their patients (Patel et al., 2018; Khan, 2020b). While in the Chinese population, for example, the *RPGR* gene is the most common cause of RDs (L. Wang et al., 2018). The second most reported gene was the *MERTK* gene, which was detected in 4 Qatari patients with retinitis pigmentosa. The prevalence of *MERTK* gene variants in RDs patients varies depending

on the population studied (Verbakel et al., 2018b). In contrast to our study, the *MERTK* variants are considered rare causes of RDs, accounting for only around 1% (Strick & Vollrath, 2010). Several studies have reported the prevalence of *MERTK* variants in different populations. For example, a study of Japanese patients with retinitis pigmentosa found that *MERTK* variants accounted for 3.6% of cases (Tada et al., 2006). Our findings on the contribution of *MERTK* variants to cases of RDs are supported by a study of patients from North Africa. According to Jaffal et al. (2021), *MERTK* variants were found to be a major contributor to RDs cases, accounting for 18% of cases studied (Jaffal et al., 2021).

5. 3.2. Unsolved cases

Unsolved cases included 2 patients (RDs-5, RDs-27) with negative test results and 1 patient (RDs-26) being heterozygous for 2 variants in 2 autosomal recessive genes, *ALMS1* and *MKS1*. Patient RDs-5 with a clinical diagnosis of LCA was tested by WES Plus and the test came back negative. Previous studies reported that the absence of positive outcomes from WES Plus may be indicative of the limitations of the test's architecture, rather than the full range of plausible genetic candidates being assessed (Lam et al., 2021b). The patient RDs-27, a 60 year old Qatari female was suspected to have adult-onset vitelliform macular dystrophy (AVMD) and the gene panel results came back negative. AVMD was previously thought to be predominantly a genetic disorder caused by pathogenic variants in several genes such as *PRPH2*, *BEST1*, *IMPG1*, and *IMPG2* (Crincoli et al., 2022). However, recent studies that have focused on genetic testing have revealed that the genetic basis for most cases of AVMD remains unknown, and the majority of cases appear to be idiopathic, meaning that the underlying cause is unclear (Crincoli et al., 2022). Patient RDs-26's finding did not explain his/her clinical phenotype as homozygous pathogenic variants in *ALMS1* are

associated with Alstrom syndrome and *MKS1* is associated with Meckel syndrome (KS), Bardet-Biedl syndrome (BBS), and Joubert syndrome. It should be noted that a patient's heterozygosity for a homozygous variant does not establish a genetic diagnosis. However, previous studies have reported that patients with clinical manifestations of non-syndromic RDs may harbor pathogenic variants in syndromic RDs-related genes (X. F. Huang et al., 2015b). These variants could potentially lead to an isolated non-syndromic retinal disease without any other symptoms (X. F. Huang et al., 2015b).

5. 3.3. Uncertain case

Out of the 18 uncertain cases, 10 were reconsidered as solved through family segregation and WES reanalysis. In family segregation, it was found that the identified variants were segregating with the disease in the family. Patient RDs-23 was found to be homozygous for the variant c.74 C>T in the *PRCD* gene. Family segregation analyses showed that both parents and the healthy siblings were heterozygous carriers while the affected sibling was found to be homozygous.

Patient RDs-48 was tested by familial targeted testing for the variant c.509 T>C in the *CNNM4* gene. This variant was previously identified in a homozygous state in 2 relatives of this patient with a diagnosis of RDs, as it was segregating with the disease in this family. Despite that, this variant was classified as a variant of uncertain significance. These findings emphasized on the importance of functional studies to accurately predict the effect of the variants.

This result indicates the importance of conducting familial segregation when family members are available to further resolve the uncertain cases in RDs genetic diagnosis. As the use of clinical genetic testing evolves from diagnostic to predictive, documentation of such genetic variants could be useful and can influence management

and recommendations, and here comes the genetic counselor's role in identifying such cases with those variants of uncertain significance that are behaving like pathogenic/likely pathogenic variants in certain families. Genetic counselors include the variant data and family segregation evidence so families can utilize these results for disease management and conducting reproductive options such as pre-implantation genetic testing (PGT). Previous studies reported the importance of genetic counseling after a genetic test for understanding and discussing the test results in the context of the patient's medical history (Lam et al., 2021b). Genetic counselors assist patients in comprehending and responding to the results, which may include analyzing how the results relate to their family members, considering options for family planning, addressing any unexpected findings, and managing the emotional and psychological effects of the test (Méjécase et al., 2020).

Moreover, out of the 18 cases, 8 cases reminded uncertain with identified variants of uncertain significance. Previous studies suggest that uncertain genetic variants can add complexity to clinical decision-making and result in harm and costs to patients and the healthcare system (Burke et al., 2022). While efforts to improve variant interpretation are ongoing, VUSs remain an ongoing challenge due to the high prevalence of rare and novel variants in the human genome. Mitigating strategies include limiting VUS identification, subclassifying according to the likelihood of harm, family-based evaluation, and enhanced counseling efforts (Burke et al., 2022).

5. 4. Genotype-phenotype correlation

Finding a robust genotype-phenotype correlation can be a complex task, especially when dealing with a small number of patients carrying a specific variant. In this study, we specifically examined variants that were identified in multiple patients to enhance the validity of our findings. By focusing on these recurrent variants, we

aimed to establish a stronger understanding of the relationship between genotype and phenotype in the context of our research.

The most reported gene in the current study *ABCA4*, the clinical manifestation and the age of onset were variable among them. Patient RDs-9, a 14 years old with a clinical diagnosis of Stargardt disease was found to be compound heterozygous for c.1609 C>T and c.5882 G>A in the *ABCA4* gene. In comparison to the patients RDs-22, 56 years old, and RDs-47, 69 years old, with a clinical diagnosis of retinitis pigmentosa, both were found to be heterozygous for the variant c.5882 G>A in the *ABCA4*. This variability in disease severity and age of onset can be explained by previous studies that reported that biallelic pathogenic variants in the *ABCA4* gene are associated with Stargardt disease which typically presents in the first or second decade of life (Lewis et al., 1999). While heterozygous pathogenic variants in *ABCA4* have also been reported in association with age-related macular degeneration 2 (ARMD2) that manifests later in age (Allikmets et al., 1998; den Hollander & de Jong, 2015; Lewis et al., 1999).

Patients RDs-1 and RDs-21 were both homozygous for the variant c.2214delT in the *MERTK* gene and had a clinical diagnosis of retinitis pigmentosa. The same variant was seen in retinitis pigmentosa patients from Saudi Arabia and the United Arab Emirates (Patel et al., 2016) (Khan, 2020b). Participant RDs-8 was found to be homozygous for c.5584 G>C in the *ABCA4* gene and had a phenotype of Stargardt disease, the same clinical diagnosis was reported in patients from China (X. F. Huang et al., 2015a). Such findings are indicating that specific variants are behaving in the same way and causing similar clinical phenotypes. On the other hand, few participants had a clinical diagnosis that was different from what has been reported in the literature. Patient RDs-48 was clinically diagnosed with cone-rod dystrophy and was found to be

homozygous for the variant c.509 T>C in the *CNNM4* gene. A similar phenotype was reported in patients from Saudia Arabia (Patel et al., 2016). However, the same variant was seen in 1 patient from the United Arab Emirates with a clinical diagnosis of Jalili syndrome (Khan, 2020b) indicating that the variant is pleiotropic. The variant c.81_82insA in *CABP4* was seen in a Qatari participant from our study and his clinical diagnosis was not specified by ophthalmologists. This variant was reported in 11 patients from Saudi Arabia, and all had very similar ophthalmic phenotypes, which was considered cone–rod synaptic disorder (Khan et al., 2013).

Some identified variants had a possible founder effect. The *MERTK* gene variant c.2214delT was discovered in two individuals from Qatar and has also been reported in other studies on patients from Saudi Arabia and the United Arab Emirates with similar clinical symptoms (Khan, 2020b; Patel et al., 2018). Two patients from Palestine also had the *RDH12* gene variation c.821 T>C, which has been described in the literature as a founder mutation in Arabs and Bedouins and was observed in individuals from Palestine (Sharon et al., 2020). Founder effect variants are well-known phenomena in different populations. Endogamy and consanguinity are cultural practices that enhance the probability of homozygosity for genetic variants and thus the appearance of many recessive conditions (Khan, 2013). These genes relate to certain clinical manifestations and thus should be viewed as first-tier testing for individuals in regions with these characteristics. The *ABCA4* variant c.5882 G>A founder effect has previously been identified in the Arabian Peninsula (Khan, 2020a). This variant was also identified in 3 Qatari patients from our cohort and was linked to the identifiable spectrum of Stargardt disease. An individual from the same region who exhibits these clinical characteristics could be tested for the relevant founder first.

Moreover, the issue in identifying the best genetic test may be due to that

referrals for the present cohorts came from a variety of ophthalmologists, where each had a distinct approach to identify and classify inherited retinal diseases. Previous studies reported a higher diagnostic yield when a single ophthalmologist with expertise examined and confirmed the diagnoses in each patient, and a clinical diagnosis that was as specific as possible was obtained to increase the diagnostic yield (Khan, 2020b). This finding highlights the importance of genotype–phenotype correlation when identifying the appropriate genetic test.

5. 5. Therapeutic options

The variant spectrum identified in our cohort provides valuable information for molecular diagnostics and potential gene therapy in RDs patients. Notably, none of our study participants were found to have the *RPE65* gene variant. Novel therapies for inherited retinal dystrophies have rapidly emerged since innovative clinical trials for LCA caused by *RPE65* variants resulted in the first FDA-approved in vivo gene therapy (Ku & Pennesi, 2020). Stargardt disease, caused by pathogenic variants in the *ABCA4* gene was the most reported gene in our cohort (Piotter et al., 2021). Stargardt disease is a common cause of childhood blindness, and gene therapy has not yet shown clinical trial success comparable to that of other hereditary retinal diseases (Piotter et al., 2021). However, Stargardt disease looks to be responsive to therapeutic intervention due to its early age of onset and ongoing disease progression throughout the course of an individual's lifetime (Piotter et al., 2021). Previous clinical trials for Stargardt disease gene therapy showed that EIAV-ABCA4 subretinal gene therapy was well tolerated, with only one episode of ocular hypertension. Macular flecks were also significantly reduced in the treated eye. To fully describe the safety and effectiveness of EIAV-ABCA4, more patient testing and follow-up will be necessary (Piotter et al., 2021). This finding is emphasizing the need for exploring the prevalence of the *ABCA4* gene

variants among the population of Qatar as gene therapy might be an available option in the future. Moreover, advances in viral vectors have resulted in more effective Adeno-associated virus (AAV) transduction and the development of new viral vectors for gene augmentation treatment of large gene targets. Rod-Cone Dystrophies (RCD), the most reported phenotype in our cohort, are characterized by the destruction of rod photoreceptors, followed by a loss of cone photoreceptors, ultimately leading to blindness (John et al., 1998). RCD affects more than 1.5 million people globally, and more than 65 genes are involved (SPVN06, 2022). The *NXNL1* gene codes for proteins that are generated by the photoreceptors. For instance, a new AAV-based therapeutic candidate called SPVN06 encodes human retinal proteins in the same vector. One subretinal injection of SPVN06 is anticipated to prevent cone degeneration in RCD patients, regardless of the mutated gene that causes it (SPVN06, 2022). Such findings can present new therapeutic options for RDs patients in Qatar.

Limitations and Future directions

Our study had several limitations including the relatively small sample size as multiple patients did not undergo genetic tests due to personal/financial reasons, limiting our statistical power. Moreover, several study participants did not have a clear RDs clinical diagnosis by ophthalmologists, thus it was difficult to establish a proper genotype-phenotype correlation.

Future directions in this field should focus on expanding the sample size to increase statistical power and conducting more comprehensive genetic testing to identify potential causative variants in patients who did not undergo testing in this study. Furthermore, investigating the reasons behind patients refusing genetic testing could help to improve patient education and counseling. Additionally, efforts should be made to ensure clear and accurate clinical diagnoses to improve the ability to establish

genotype-phenotype correlations. It may also be beneficial to investigate potential environmental factors that could be contributing to RDs.

CHAPTER 6: CONCLUSION

In conclusion, our study identified a total of 55 variants in 32 different RDs-related genes among 49 patients. Notably, we successfully resolved 38 out of the 49 cases, shedding light on the genetic landscape of RDs. Our findings revealed that rod-dominated phenotypes accounted for a significant proportion (51%) of hereditary retinal diseases in our study cohort. Regional founder effects were observed in certain genes, exemplified by the identification of the variant c.821 T>C in the *RDH12* gene among Palestinian patients and the variant c.2214delT in the *MERTK* gene among the Arabian Peninsula's population. These recurrent variations highlight the importance of considering genetic heritage when studying RDs. Furthermore, our study underscored the patients' preference for WES as the first-tier genetic testing approach in RDs cases. Family segregation studies played a crucial role in identifying potential causative variants, contributing to personalized treatment options, and facilitating genetic counseling services for patients and their families. Importantly, our work expands the understanding of the genetic heterogeneity of RDs among the Arabian population. These findings hold promise for advancing individualized approaches to RDs management and providing comprehensive healthcare in the field. However, further investigations are warranted to validate and generalize these results within the region.

As the first study of its kind conducted in Qatar, our research serves as a foundation for future studies on the epidemiology and genetics of RDs in the country. By offering valuable insights into the molecular spectrum underlying RDs, our work contributes to the broader scientific knowledge in the field. Moving forward, the potential clinical implications and application of these findings in a healthcare setting warrant further exploration, adding an important dimension to the comprehensive impact of our research.

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APPENDICES

Appendix A: The approval letter of the medical research center at HMC.

مؤسسة حمد الطبية
Hamad Medical Corporation

APPROVAL LETTER
MEDICAL RESEARCH CENTER
HMC, DOHA-QATAR

Date: 12 December 2022

Dr. Rehab Ali M.S. Abdulrahman
Pediatrics
Hamad General Hospital (HGH)
Hamad Medical Corporation
Doha-Qatar

Protocol No.	MRC-01-22-729
Study Title	Exploring the genetic causes of non-syndromic retinal dystrophies in Qatar
The above titled research study has been approved to be conducted in HMC and is summarized below:	
Study type	Data Review
Data Collection Period	01/01/2015 to 01/11/2022
Team Member List	Dr. Houssein Khodjet Elkhil, Dr. Mashael Al-Shafai, Dr. Rehab Ali M.S. Abdulrahman, Dr. Tawfeg I M Ben Omran, Ms. Karen El-Akouri, Ms. Reem Ibrahim W Bux, Ms. Sumaya Ibrahim Abiib
Review Type	'Exempt' under MOPH guidelines Category 3: Research involving the collection or study of existing: Data, documents, records and the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.
Decision	Approved
Hospitals/ Facilities Approved	HMC Corporate

This study must be conducted in full compliance with all the relevant sections of the Rules and Regulations for Research at HMC, and the Medical Research Center should be notified immediately of any proposed changes to the study protocol that may affect the 'exempt' status of this study. Wherever amendments to the initial protocol are deemed necessary, it is the responsibility of the Principal Investigator to ensure that appropriate reviews and renewed approvals are in place before the study will be allowed to proceed.

Please note that only **research documentation currently uploaded in ABHATH** is to be utilized at any stage in the conduct of this study. The research team must ensure that changes and progress on the study are appropriately recorded in ABHATH, the online research system of the Medical Research Center. The PI must ensure that any link to patient identifiers is destroyed after data collection and, data security is maintained.

We wish you success in this research and await the outcomes in due course.

Yours Sincerely,

Prof. Michael Paul Frenneaux
Chief of Scientific, Academic and Faculty Affairs
Hamad Medical Corporation

Date: 12 December 2022

Appendix B: Ethical approval of Qatar University IRB

Qatar University Institutional Review Board **QU-IRB**

QU-IRB Registration: IRB-QU-2020-006, QU-IRB, Assurance: IRB-A-QU-2019-0009

DATE:	January 19, 2023
TO:	Mashael Alshafai
FROM:	Qatar University Institutional Review Board (QU-IRB)
PROJECT TITLE:	1999990-1Exploring the genetic causes of non-syndromic retinal dystrophies in Qatar
QU-IRB REFERENCE #:	QU-IRB 1803-E/23
SUBMISSION TYPE:	New Project
ACTION:	DETERMINATION OF EXEMPT STATUS
DECISION DATE:	January 19, 2023
REVIEW CATEGORY:	Exemption category # 3