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High Diagnostic Yield of Whole Exome Sequencing in Participants with Retinal Dystrophies in a Clinical Ophthalmology Setting

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Abstract

Purpose—To assess the diagnostic yield and the practicality of implementing whole exome sequencing within a clinical ophthalmology setting.

Design—Evaluation of a diagnostic protocol.

Setting: Patient participants were enrolled during clinical appointments in a university based Ophthalmic Genetics clinic.

Patient Population: Twenty-six patients with a variety of presumed hereditary retinal dystrophies. Intervention: Participants were offered whole exome sequencing in addition to clinically available sequencing gene panels between July 2012 and January 2013 to determine the molecular etiology of their retinal dystrophy.

Main Outcome Measures: Diagnostic yield and acceptability of whole exome sequencing in patients with retinal disorders.

Results—Twenty-six of 29 (~90%) eligible patients who were approached opted to undergo molecular testing. Each participant chose whole exome sequencing in addition to, or in lieu of, clinically available sequencing gene panels. Time to obtain informed consent was manageable in

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the clinical context. Whole exome sequencing successfully identified known pathogenic mutations or suspected deleterious variants in 57.7% of participants. Additionally, one participant had 2 autosomal dominant medically actionable incidental findings (unrelated to retinopathy) that were reported to enable the participant to take preventive action and reduce risk for future disease.

Conclusions—In this study, we identified the molecular etiology for more than half of all participants. Additionally, we found that participants were widely accepting of whole exome sequencing and the possibility of being informed about medically actionable incidental findings.

INTRODUCTION

The pace of progress in ophthalmic genetics has been exponential over the last decade. It is critical for ophthalmologists to understand emerging diagnostic technologies which may have clinical implications for their patients in the very near future. Whole exome sequencing (exome sequencing) and massively parallel sequencing gene panels are attractive new testing approaches for diagnosing genetic disorders that exhibit genetic heterogeneity and overlapping phenotypes. Few Mendelian disorders exhibit the degree of genetic heterogeneity demonstrated by retinitis pigmentosa (RP), one of the most common retinal dystrophies.[1-2] Over 100 genes have been associated with this condition, yet only half of all patients with RP have an identifiable mutation.[3-4] Moreover, other retinal dystrophies, including cone-rod dystrophy, cone dystrophy and Stargardt disease also exhibit genetic heterogeneity.[3,5] Further complicating the clinical assessment of these disorders is the fact that retinal disorders also demonstrate significant phenotypic heterogeneity. For instance, mutations in the *ABCA4 gene* have been associated with several hereditary retinal dystrophies (Stargardt disease, cone-rod dystrophy, cone dystrophy and RP).[1]

Prior to the advent of massively parallel sequencing, genetic testing for heterogeneous disorders was pursued one gene at a time or through limited and expensive gene panels via Sanger sequencing. The benefit of using a broader testing methodology in such circumstances is the potential to eliminate the guesswork inherent in choosing only a subset of genes to test. Another advantage is that many participants seen in Ophthalmic Genetics clinics report no family history of retinal dystrophy, complicating determinations of inheritance patterns that might otherwise guide diagnostic strategies. Since retinal dystrophies are thought to be almost exclusively hereditary in nature, one can assume that there are yet-unidentified genes associated with RP and other retinal dystrophies.[2] Exome sequencing allows the clinician the flexibility of ordering a single test for all suspected heterogeneous disorders and allows the laboratory the flexibility to analyze newly reported genes without continuously updating testing platforms.

A potential complication of exome sequencing testing versus targeted massively parallel sequencing gene panels is the prospect of patients receiving incidental findings unrelated to the retinal diagnosis. That is, when essentially all genes in an individual's genome are sequenced, information will be potentially available regarding other genetic disorders unrelated to the indication for testing. The American College of Medical Genetics (ACMG) recommends that laboratories return selected medically actionable incidental findings as part of any genome-scale clinical test. Thus, a small but predictable subset of patients will have

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such additional findings.[6] It is uncertain how patients might react to this possibility in the clinical setting, therefore, additional time is required to discuss the likelihood and examples of incidental findings as part of the informed consent process

The goals of this study were to investigate the use of exome sequencing to identify the molecular etiology of retinal dystrophies in a clinical ophthalmology setting and to determine the feasibility of using this novel and complex form of genetic testing, with regard to the potential discovery of incidental findings. Previous studies have shown the effectiveness of exome sequencing and targeted gene panels in determining the molecular etiology of retinal dystrophies.[7-10] Here we demonstrate its high diagnostic yield, feasibility and acceptability of exome sequencing for retinal dystrophy patients enrolled in a clinical setting.

MATERIALS & METHODS

Patients evaluated for retinal disorders in the University of North Carolina Kittner Eye Center Ophthalmic Genetics Clinic between July 2012 and January 2013 were invited to participate. Participants were enrolled in the research protocol to undergo research genetic testing during their initial or follow-up clinical visits. Return patients were eligible if the molecular etiology of their retinal disorder was unknown. All potential participants were offered clinically available massively parallel sequencing targeted gene panel testing and research exome sequencing through this study. The University of North Carolina at Chapel Hill Institutional Review Board approval was obtained prior to patient enrollment, and this study adhered to the tenets of the Declaration of Helsinki.

All participants were enrolled and consented by a certified genetic counselor and agreed to learn of any diagnostic related findings as well as any medically actionable incidental findings. Known pathogenic mutations and variants of unknown clinical significance that could potentially explain their retinal disease were returned to participants. However, only clearly pathogenic medically actionable incidental findings were returned. Thus, variants of unknown significance within genes associated with medically actionable findings were not returned to participants given their uncertainty and low *a priori* risk of being pathogenic in presumably unaffected individuals. The list of conditions in the category of medically actionable incidental findings was based on a schema previously described by our group and further refined by a committee of medical and molecular geneticists, genetic counselors, a neurologist, a cardiologist and an ethicist as part of the NCGENES Study currently being conducted at University of North Carolina at Chapel Hill.[11,12] This list included all conditions recently recommended by the American College of Medical Genetics for return of incidental findings.[6]

Exome sequencing was performed using Agilent's SureSelect XT Target Enrichment System for Illumina paired-end sequencing on the HiSeq 2000 instrument. The average depth of coverage for all participants across the entire region targeted for enrichment was 58.19.

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We utilized a custom pipeline developed for the NCGENES project to process raw sequence data from FASTQ files to generate variant calls.[13] Variants were stored in a database and extensively annotated.[14]

To facilitate evaluation of variants possibly related to the participants' retinal disorder, we filtered the exome data using a comprehensive list of 186 genes previously associated with syndromic and non-syndromic retinopathies, which was curated using Online Mendelian Inheritance of Man (OMIM)[15], GeneTests.org[16], relevant medical literature and genes currently being evaluated in clinical laboratories. Participants' exome data were reanalyzed using an updated gene list, including 214 genes, one year later. A complete list of these genes is available in supplemental material (Supplemental Table 1). Variants within this set of genes were then prioritized into computational classes by an informatics algorithm to select: A) variants previously reported as mutations in the Human Gene Mutation Database[17]; B) predicted truncating variants that demonstrated <1% minor allele frequency; C) missense variants with <1% minor allele frequency; and several other categories with decreasingly likely pathogenicity. Variants were then analyzed for pathogenicity using a custom user interface.[18] The total number of exome and filtered variants for each participant is available in supplemental material (Supplemental Table 2). Manual analysis of filtered variants entailed a combination of literature searches, publicly available variant databases queries, locus specific database searches, Condel *in silico* modeling and evolutionary conservation.[19]

The veracity of potential disease causing variants identified by exome sequencing and passing manual curation were confirmed by Sanger sequencing on a duplicate sample in the CLIA-certified University of North Carolina McLendon Molecular Genetics Laboratory. All participants were asked to return for a follow-up research appointment to discuss results and were provided with a research report summarizing the yield of the exome sequencing analysis. Participants were not given the option of learning about non-medically actionable incidental results.

RESULTS

During the enrollment period, 29 patients were eligible (15 new and 14 return clinic patients). Twenty-six of the 29 (~90%) patients opted to undergo clinically available testing and/or research exome sequencing. Three new patients declined both clinical and research testing. All 26 patients who wished to proceed with genetic testing chose exome sequencing, and 3 of the new patients (Participants 11, 20 and 25) opted to have simultaneous clinically available genetic testing. Participant 11 had negative clinical testing and Participants 20 and 25 had pathogenic variants identified that were also detected via exome sequencing.

The informed consent process for return patients opting for exome sequencing took 30 minutes or less. New patient appointments lasted 75 minutes, and the informed consent process took approximately 30 minutes. All questions about exome sequencing were addressed, and none of the participants shared any concerns regarding the possibility of learning medically actionable incidental findings.

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Participants represented a wide variety of ages and clinical indications, outlined in Table 1. Five of 26 (~19%) participants were under 18 years of age, and the adults ranged in age from 22 to 69 years. The majority of participants had a firm clinical diagnosis; however, 3 of 26 (11.5%) participants had an uncertain clinical diagnosis at the time of enrollment. Fourteen of 26 participants (~54%) did not have a known family history of retinal dystrophy.

Fifteen of 26 (~58%) participants had clearly deleterious mutations or highly suspicious variants of unknown significance consistent with their phenotype. A complete summary of previous genetic testing and exome sequencing results for each participant is shown in Table 2. Each of these variants was confirmed using Sanger sequencing. Participant 4 had two different medically actionable incidental findings: an apparently novel nonsense variant in *BRCA2,* c.2857G>T (p.Glu593*) which is associated with Hereditary Breast Ovarian Cancer syndrome; and an apparently novel frameshift variant in *MSH6*, c.1170delT (p.Phe391fs) which is associated with Lynch syndrome. Both truncating variants were considered likely pathogenic given their suspected effect on the protein. When questioned further about family history of cancer at results disclosure, he reported that his paternal grandfather had a history of colon cancer at an older age (Figure 1).

DISCUSSION

This study demonstrates the high diagnostic yield, feasibility and acceptability of exome sequencing for retinal dystrophy patients in the clinical ophthalmology setting. In our study, exome sequencing was widely accepted by a heterogeneous clinic population, with ~90% of those approached choosing to participate. Patients who chose not to participate also declined clinically available genetic testing.

The informed consent process for exome sequencing was manageable in a clinic setting. Several participants were not naïve to basic genetic information, which allowed for a brief review and then focused discussion on the differences between traditional genetic testing and exome sequencing along with possible results they might receive. The discussion was lengthier than for traditional clinical testing since it was necessary to explain the nature of the genetic testing as part of a research study. During the consent process, none of the participants expressed concern about the possibility of learning medically actionable incidental findings. However, several participants requested examples of possible results to conceptualize what a medically actionable incidental finding might entail. Several participants expressed that learning this type of information would allow them to be proactive with their health care in the future.

Just over half of the participants were found to have a definitive or possible molecular etiology defined as the cause of their retinal dystrophy, consistent with reports using massively parallel sequencing gene panels in a research setting.[7-10] Previously reported pathogenic variants were identified in several participants, which aided interpretation of exome sequencing results. Novel variants were interpreted as likely pathogenic, if they were expected to result in a truncated protein. Novel missense variants, which are not expected to truncate proteins, and variants within genes that were not a definitive fit for the participant's

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phenotype were more difficult to assess, and were reported back to the participants as variants of unknown significance.

Participants 4 and 7 each had three *ABCA4* gene variants identified with potential clinical significance. Each participant had two known pathogenic mutations and one variant that had been previously reported as a pathogenic mutation and as a variant of unknown significance.[20-24] Since exome sequencing analysis cannot determine the phase of the three variants, these results are consistent with autosomal recessive disease provided at least one pathogenic variant is on each allele. Participant 26 had only one known previously reported pathogenic variant identified in the *ABCA4* gene, c.1927G>A (p.Val643Met).[20,25] Since *ABCA4* mutations are associated with autosomal recessive RP, the identification of one mutation does not fully explain the participant's diagnosis. A current known limitation of exome sequencing is the inability to identify larger deletions and duplications and possibly smaller indels.[26,27] Therefore, it is possible that this participant has a mutation on the other *ABCA4* allele that was not detected by this methodology. Alternatively, he could be a carrier for this form of RP and have an unrelated molecular cause of his RP, or the variant could be erroneously classified as a pathogenic variant. We classified this variant as having uncertain significance due to its minor allele frequency.

Mutations within the *USH2A* gene are typically associated with Usher syndrome or autosomal recessive non-syndromic RP. Participant 19 with RP had one known pathogenic mutation c.2276G>T (p.Cys759Phe)[28] and one possible splice site variant c.12295-3T>A, previously reported as a variant of unknown significance.[29] However, a similar non-canonical splicing variant, *USH2A* c.7595-3C>G, has been described as pathogenic.[28] Participant 12 with late onset RP had two missense variants of unknown significance, c.7130A>G (p.Asn2377Ser) and c.11927C>T (p.Thr3976Met), which have both previously been reported.[28-30]

Participant 25 had an extensive X-linked family history of RP (Figure 1). A known pathogenic mutation in the *RPGR* gene, c.2323_2324delGA (p.Arg775fs) was identified.[31] After enrollment of this patient, eyeGENE (Program through the National Eye Institute) also confirmed this exact mutation by Sanger sequencing.

Approximately one-third of patients with autosomal recessive Stargardt disease have only one identifiable mutation in the *ABCA4* gene.[23,32] Participant 10 previously had testing that identified a single known pathogenic mutation in the *ABCA4* gene, c.161G>A (p.Cys54Tyr).[32] Exome sequencing identified the known *ABCA4* mutation and revealed a variant of unknown significance in the *PROM1* gene, c.1345G>A (p.Val449Met), which has been associated with autosomal dominant Stargardt disease. To our knowledge, this variant has not previously been reported. It is highly evolutionarily conserved and was predicted as deleterious by Condel, supporting its pathogenicity.[19] However, parental samples were collected after results disclosure and revealed that both the *ABCA4* and *PROM1* variants were inherited from the participant's unaffected father making the pathogenicity of the *PROM1* variant unlikely.

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Participant 18 had two apparently novel frameshift mutations in *USH2A*, c.923_924insGCCA (p.His308fs) and c.12152_12153insTT (p.Glu4051fs), which explained his clinical diagnosis of Usher syndrome type 2.

Three participants in this cohort had an unclear clinical diagnosis at the time of enrollment, and exome sequencing helped identify a probable cause in one. Participant 16 was an 11 year old male with retinal features consistent with a rare diagnosis of pigmented paravenous chorioretinal atrophy or an RP-like syndrome. Exome sequencing identified the two missense variants in the *RDH12* gene, c.697G>C (p.Val233Leu) and c.869T>G (p.Val290Gly). The Val233Leu variant was previously reported in an individual with retinopathy who had a frameshift mutation on the other allele.[33] To our knowledge, the Val290Gly has not previously been reported.

Two participants had results suggesting a different inheritance pattern than inferred by their family history. Participant 1 is a 34 year old male with a personal history of RP and no known family history who was found to have a previously reported pathogenic variant in the *RHO* gene, *RHO* c.1040C>T (p.Pro347Leu).[34] Mutations within the *RHO* gene are associated with autosomal dominant RP, which is not consistent with this participant's family history. However, *de novo* dominant mutations have been reported in the *RHO* gene.[33,34]

Participant 24 is a 55 year old male with a personal and family history of Stargardt disease who was found to have an apparently novel heterozygous variant in the *PRPH2* gene, c.457A>G (p.Lys153Glu). The *PRPH2* gene is associated with autosomal dominant Stargardt disease. Two of the participant's siblings were reported to have the same phenotype (Figure 1). Neither the parents nor any other relatives carry a clinical diagnosis of Stargardt disease, and thus, an autosomal recessive form of this condition was expected. Interestingly, his mother reportedly was diagnosed with age related macular degeneration (AMD). The mother also likely has this variant and has been diagnosed with AMD due to the later onset of symptoms in this family. The mother was not available for testing.

One rationale for using exome sequencing versus massively parallel sequencing gene panels is to determine the mutation(s) within a gene that typically caused a syndromic form of a retinal disease. For example, there are reports of individuals with non-syndromic RP having mutations in at least three genes originally described as associated with Bardet-Biedl syndrome (BBS): *BBS1*, *TTC8* and *ARL6*, an autosomal recessive disease characterized by truncal obesity, postaxial polydactyly, cognitive impairment, genitourinary malformations, renal dysfunction and cone-rod dystrophy or RP.[35-37]

Participant 9 is a 22 year old female diagnosed initially with Stargardt disease around 10 years of age. However, when she was evaluated in the Ophthalmic Genetics clinic, her electroretinogram demonstrated normal cone and rod photoreceptor function, and her exam revealed bilateral macular atrophy consistent with a macular dystrophy. She was found to have two missense variants in the *BBS9* gene, which is associated with BBS. Thus, this participant appears to have non-syndromic cone-rod dystrophy possibly caused by *BBS9* mutations. We were unable to locate a previous report linking *BBS9* pathogenic variants

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with non-syndromic cone-rod dystrophy, and thus, would represent an expansion of the phenotype if these variants are indeed pathogenic.

Variants of unknown significance were identified in seven participants. These participants were informed that these variants could be disease causing or simply human variation. Some variants of unknown significance are more likely to be pathogenic than others, and this information was also shared with participants. For instance, the *USH2A* variant of unknown significance in Participant 19 is likely pathogenic, since this individual has a second known pathogenic variant within the same gene that fits the phenotype. Alternatively, participants with one variant of unknown significance identified along with two known pathogenic variants within a gene that fits the phenotype suggests that the variant of unknown significance is less likely to be pathogenic; as in the case of Participants 4 and 7. Participants 9, 12 and 16 had two variants of unknown significance within genes that fit their phenotype which are usually inherited in an autosomal recessive fashion. These variants rose to the level of suspicion to report to participants; however, they could undoubtedly be distractions rather than disease causing variants. Participant 26 had one only variant of unknown significance within a gene that fits the phenotype, which suggests that he has a deletion on the other allele that was undetected, he is simply a carrier for that form of RP and has other disease causing variants, or that this variant is not disease causing, contrary to previous reports in the literature. Each of these possible scenarios were discussed with participants.

All participants with variants of unknown significance were offered family studies, which, to date, one family has accepted. Parental testing in Participant 10 allowed the downgrade a variant of unknown significance in the *PROM1* gene to a likely benign variant. If the *PROM1* variant was pathogenic, we would have expected that this variant would have been de novo, given that both parents were unaffected and pathogenic variants in this gene are associated with autosomal dominant Stargardt disease.

Exome sequencing, with analysis focused on genes with known associations with retinal disorders, can be considered a “virtual” massively parallel sequencing gene panel, significantly decreasing the number of variants to analyze. However, unlike employing an actual gene panel, exome sequencing allows flexibility to reanalyze data as new gene candidates are identified without the need to develop a new physical test dependent upon gene capture. This will facilitate the identification of novel gene candidates and reanalysis of exome data as additional retinal disease genes are described in participants with as of yet negative results and in participants with variant(s) of unknown significance. Thus, for the time being, a whole-exome approach is readily justifiable as a diagnosis is sought in those with likely genetic retinal pathology. However, as the pace of gene discovery plateaus, there may well come a point at which a captured panel of genes is scrutinized, given the superior analytic performance of such panels due to increased depth of coverage and the lack of concerns about secondary findings in that context.

Participant 4 had medically actionable incidental findings in both the *BRCA2* and *MSH6* genes. The lack of personal or significant family history was surprising and illustrates the complexity of returning medically actionable results. During results disclosure, it was emphasized that these were novel variants that have not been directly associated with

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disease, although most known pathogenic mutations within these two genes are rare or private to families. It was also important to note that previous testing of these two genes has largely been in individuals with a personal and/or family history of cancer. Therefore, as larger numbers of unaffected individuals are tested in the general population through exome sequencing, current estimates of penetrance rates of well-known diseases may be found to be inflated. However, in the meantime, it was recommended that the participant's relatives be tested for these presumed pathogenic mutations, in accordance with the National Comprehensive Cancer Network screening guidelines.

This project was designed as a pilot study. Thus, the sample size is small; however, the yield of exome sequencing for this cohort is similar to other published studies carried out in a research setting. Glöckle et al. reported a diagnostic yield of 50-80% (depending on the phenotype) using massively parallel sequencing gene panels within an unselected set of participants with retinal dystrophies.[10] Audo et al. reported a 57% detection rate in patients with a variety of retinal dystrophies using a massively parallel sequencing gene panel of 254 known and candidate genes.[9]

In this study, relatives were not enrolled at the onset, in order to more closely simulate a clinical ophthalmology setting. Not enrolling relatives limits the ability to determine the phase of variants identified in genes associated with autosomal recessive disorders, or determine whether heterozygous variants in genes associated with dominant conditions are *de novo* versus incompletely penetrant. However, more often than not, informative family members do not attend clinic appointments with patients. In the clinical ophthalmology setting, family studies are initiated only when testing does not point to a clear causality. The same approach was used in this study. However, many informative relatives were unavailable. This is consistent with any clinic patient with genetic testing, and is a limitation that is not unique to retinal dystrophy patients nor exome sequencing.

The pace of progress in ophthalmic genetics has been exponential over the last decade; it is critical for ophthalmologists to understand emerging diagnostic technologies, such as exome sequencing, which may have widespread clinical applications in the very near future. With genetic therapies on the horizon, an accurate molecular diagnosis will be a prerequisite for patients seeking enrollment in clinical trials, confirmation of clinical diagnoses and prognostic information for patients and their family members. In this study, exome sequencing was highly acceptable to the cohort and implementation was manageable in a clinical ophthalmology setting. Such broad testing proved useful in participants with a history of features overlapping among different retinal dystrophies and in participants with no clear clinical diagnosis. Using one test for heterogeneous disorders reduces the need to rely exclusively on family history information for characterizing an individual's disease, and exome sequencing may increase diagnostic yield by allowing review of potentially pathogenic variants within genes that are associated with retinal disorders with somewhat different but similar phenotypes. Exome sequencing is emerging as a powerful tool in determining the molecular etiology for participants with a variety of heterogeneous retinal dystrophies, even in the clinical ophthalmology setting.

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Supplementary Material

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Biography

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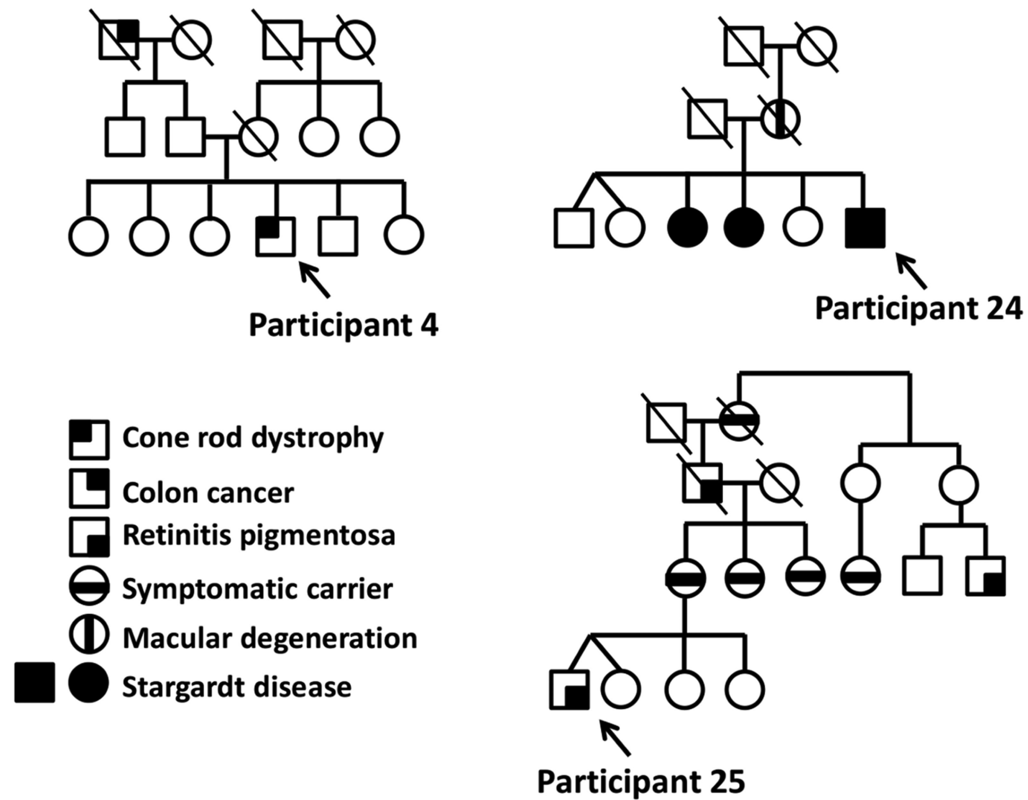

Figure 1. Selected pedigrees of participants with a variety of retinal dystrophies evaluated by whole exome sequencing

(Top Left) Participant 4 with *BRCA2* and *MSH2* mutations with only paternal grandfather with a history of cancer. (Top Right) Participant 24 with *PRPH2* mutation with two sisters with Stargardt disease and mother with diagnosis of age related macular degeneration. (Bottom Right) Participant 25 with *RPGR* mutation with family history consistent with X-linked inheritance.

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Table 1

Summary of participants' clinical diagnoses and presentation, family history and previous record of genetic testing

Participant	Age at Enrollment	Clinical Diagnosis	Age of Onset	Family History	Previous Testing Results
1	34 yr	retinitis pigmentosa	Early teens	No	not applicable
2	60 yr	cone dystrophy	20's	No	not applicable
3	9 yr	retinal dystrophy NOS + hearing loss	Hearing loss -congenital Retinal dystrophy - ~5	Yes - AD	not applicable
4	46 yr	cone-rod dystrophy	Early 20's	No	not applicable
5	48 yr	cone dystrophy	congenital	Yes - XL	not applicable
6	60 yr	retinitis pigmentosa	29 yr	No	not applicable
7	26 yr	Stargardt disease	16 yr	Yes - AR	not applicable
8	43 yr	retinal dystrophy NOS	41 yr	No	not applicable
9	23 yr	macular dystrophy	10 yr	No	not applicable
10	41 yr	Stargardt disease	6 yr	No	*ABCA4* c.161G>A (p.Cys54Tyr)
11	7 yr	familial exudative vitreoretinopathy	~6 yr	Yes - AD	Negative 4 adFEVR panel + *NDP* gene
12	68 yr	retinitis pigmentosa	~62 yr	No	not applicable
13	52 yr	cone-rod dystrophy	~42 yr	No	not applicable
14	64 yr	retinitis pigmentosa	34 yr	Yes - AR	not applicable
15	22 yr	retinitis pigmentosa	15 yr	No	not applicable
16	12 yr	retinal dystrophy NOS	11 yr	No	Negative 13 recessive retinitis pigmentosa gene panel
17	48 yr	retinitis pigmentosa	~38 yr	No	not applicable
18	23 yr	Usher syndrome type 2	hearing loss - 1 yr retinitis pigmentosa - 13 yr	Yes - AR	not applicable
19	36 yr	retinitis pigmentosa	35 yr	No	not applicable
20	30 yr	retinitis pigmentosa	30 yr	Yes - AD	*RP1* c.2029C>T
21	26 yr	Leber Congenital Amaurosis	Congenital	Yes - AD	not applicable
22	29 yr	retinitis pigmentosa	15 yr	Yes - AD	Negative *RHO, RDS, RP1* (c. 1500-3200), *PRPF31, PRPF8* (exon 42), *PRPF3* (exon11), *NR2E3* (c. 150-210), *TOPORS* (c.1975-2820), *IMPDH1* (exon 10), RP2, *RPGR, SNRNP200*
23	9 yr	retinitis pigmentosa	6 yr	Yes - AD	not applicable
24	54 yr	Stargardt disease	Mid 30's	Yes - AR	not applicable
25	11 yr	retinitis pigmentosa	9 yr	Yes - XL	*RPGR* c.2323_2324delGA (p.Arg775fs)
26	44 yr	retinitis pigmentosa	~42 yr	No	not applicable

Abbreviations: NOS, not otherwise specified; AD, autosomal dominant; XL, X-linked; AR, autosomal recessive

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Table 2

Summary of whole exome sequencing results for each participant and descriptions of previously reported or possibly causative variants identified within genes associated with retinal disease along with the interpretation provided to participants

Participant	WES Diagnostic Results		Previous Reports	Minor Allele Frequency[A]	Conservation	Condel Score[B]	Interpretation
1	NC_000003.11:g.129252554C>T	*RHO* c.1040C>T (p.Pro347Leu)	Pathogenic[34]	8.263e-06	Highly conserved	0.698 deleterious	Known pathogenic
2	Negative						
3	NC_000019.9:g.48343048G>A	*CRX* c.724G>A (p.Val242Met)[C]	Pathogenic[38]	0.001435	Not conserved	0.853 deleterious	Likely benign
4	NC_000001.10:g.94528806A>G	*ABCA4* c.1622T>C (p.Leu541Pro)	Pathogenic[23]	0.0001235	Highly conserved	1.000 deleterious	Known pathogenic
	NC_000001.10:g.94528265C>T	*ABCA4* c.1805G>A (p.Arg602Gln)	Pathogenic[23-24]	1.68e-05	Highly conserved	0.451 deleterious	Known pathogenic
	NC_000001.10:g.94508969G>A	*ABCA4* c.3113C>T (p.Ala1038Val)	Polymorphic[21] Pathogenic[22]	0.001426	Mostly conserved	0.000 neutral	Uncertain variant
	NC_000013.10:g.32911349G>T	*BRCA2* c.2857G>T (p.Glu593*)	None	0	---	---	Likely pathogenic
	NC_000002.11:g.48026292_48026292delT	*MSH6* c.1170delT (p.Phe391fs)	None	0	---	---	Likely pathogenic
5	Negative						
6	Negative						
7	NC_000001.10:g.94466624C>T	*ABCA4* c.6320G>A (p.Arg2107His)	Pathogenic[24]	0.001872	Highly conserved	1.000 deleterious	Known pathogenic
	NC_000001.10:g.94496666G>A	*ABCA4* c.4139C>T (p.Pro1380Leu)	Pathogenic[24,32]	0.0002012	Highly conserved	0.619 deleterious	Known pathogenic
	NC_000001.10:g.94520708A>G	*ABCA4* c.2546T>C (p.Val849Ala)	Pathogenic[24]	0.001335	Not conserved	0.007 neutral	Uncertain variant
8	Negative						
9	NC_000007.13:g.33380565C>G	*BBS9* c.1255C>G (p.Pro419Ala)	None	9.078e-05	Mostly conserved	0.026 neutral	Uncertain variant
	NC_000007.13:g.33545217A>T	*BBS9* c.2138A>T (p.Glu713Val)	None	0	Mostly conserved	0.922 deleterious	Uncertain variant
10	NC_000001.10:g.94577135C>T	*ABCA4* c.161G>A (p.Cys54Tyr)	Pathogenic[20,24,32]	1.65e-05	Mostly conserved	0.026 neutral	Uncertain variant

Participant	WES Diagnostic Results		Previous Reports	Minor Allele Frequency[A]	Conservation	Condel Score[B]	Interpretation
	NC_000004.11:g.16008270C>T	*PROM1* c.1345G>A (p.Val449Met)	None	0.001475	Mostly conserved	0.810 deleterious	Likely benign[D]
11	Negative						
12	NC_000001.10:g.216108128T>C	*USH2A* c.7130A>G (p.Asn2377Ser)	VUS[30] Polymorphism[29]	0.003772	Mostly conserved	0.001 neutral	Uncertain variant
	NC_000001.10:g.215901511G>A	*USH2A* c.11927C>T (p.Thr3976Met)	VUS[28]	0.0005192	Highly conserved	0.966 deleterious	Uncertain variant
13	Negative						
14	Negative						
15	NC_000011.9:g.66293652T>G	*BBS1* c.1169T>G (p.Met390Arg) - Homozygous	Pathogenic[36]	0.001484	Highly conserved	0.627 deleterious	Known pathogenic
16	NC_000014.8:g.68195946G>C	*RDH12* c.697G>C (p.Val233Leu)	Pathogenic[33]	2.491e-05	Highly conserved	0.910 deleterious	Uncertain variant
	NC_000014.8:g.68200483T>G	*RDH12* c.869T>G (p.Val290Gly)	None	8.246e-05	Highly conserved	0.930 deleterious	Uncertain variant
17	Negative						
18	NC_000001.10:g.216498866_216498867insTGGC	*USH2A* c.923_924insGCCa (p.His308fs)	Pathogenic[8]	0.0001074	---	---	Known pathogenic
	NC_000001.10:g.215853632_215853633insAA	*USH2A* c.12152_12153insTT (p.Glu4051fs)	None	0	---	---	Likely pathogenic
19	NC_000001.10:g.216420460C>A	*USH2A* c.2276G>T (p.Cys759Phe)	Pathogenic[28]	0.000784	Highly conserved	1.00 deleterious	Known pathogenic
	NC_000001.10:g.215848961A>T	*USH2A* c.12295-3T>A	None		---	---	Uncertain variant
20	NC_000008.10:g.55538471C>T	*RP1* c.2029C>T (p.R677X)	Pathogenic[39]	0	---	---	Known pathogenic
21	Negative						
22	NC_000007.13:g.128034615C>T	*MERTK* c.1787-2A>C Apparently homozygous[E]	None	0	---	---	Likely pathogenic
23	Negative						
24	NC_000006.11:g.42689616T>C	*PRPH2* c.457A>G (p.Lys153Glu)	None	0	Highly conserved	0.873 deleterious	Uncertain variant

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Participant	WES Diagnostic Results		Previous Reports	Minor Allele Frequency[A]	Conservation	Condel Score[B]	Interpretation
25	NC_000023.10:g.38145928_38145929delCT	*RPGR* c.2323_2324delGA (p.Arg775fs)	Pathogenic[39]	0	---	---	Known pathogenic
26	NC_000001.10:g.94528143C>T	*ABCA4* c.1927G>A (p.Val643Met)	Pathogenic[22,25]	0.001618	Highly conserved	0.383 neutral	Uncertain variant

Exome sequencing results summarized by participant. Variants are described using Human Genome Variation Society (http://www.hgvs.org) nomenclature. Variant interpretation was primarily based on variant type (i.e. predicted truncating versus missense), previous reports in the literature and minor allele frequencies from other large studies. Truncating and canonical splice site variants required less evidence for pathogenicity than missense and intronic variants. Evolutionary conservation and *in silico* model data were considered as supporting evidence, but were not major factors in designating pathogenicity.

[A] Minor allele frequencies were obtained using the Exome Aggregation Consortium (ExAC) Browser40

[B] The Condel19 score is provided as a reference only and was not taken into consideration in variant interpretation.

[C] Not confirmed by Sanger sequencing due to variant being likely benign and not reported back to participant.

[D] Parental studies by Sanger sequencing identified both the *ABCA4* and *PROM1* variants in the unaffected father, suggesting that the *PROM1* variant was likely benign.

[E] An *ad hoc* analysis examining coverage data suggests that this patient has a partial deletion of the *MERTK* gene in lieu of being homozygous for the splice variant. The participant's parents are deceased. Therefore, we are unable to perform parental studies.