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

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• 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.
• METHODS: 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 eligible patients (~90%) 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 the clinical context. Whole exome sequencing successfully identified known pathogenic mutations or suspected deleterious variants in 57.7% of participants. Additionally, 1 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. (Am J Ophthalmol 2015;160(2):354–363. © 2015 by Elsevier Inc. All rights reserved.)

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THE PACE OF PROGRESS IN OPHTHALMIC GENETICS has been exponential over the last decade. It is critical for ophthalmologists to understand emerging diagnostic technologies that 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 1 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 vs targeted massively parallel sequencing gene panels is the prospect of patients receiving incidental findings unrelated

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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 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.

SUBJECTS AND 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 (Agilent Technologies, Santa Clara, California) for Illumina (Illumina, Inc., San Diego, California) 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.

We used 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 nonsyndromic 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, 1 year later. A complete list of these genes is available in Supplemental Table 1 (Supplemental Material available at AJO.com). Variants within this set of genes were then prioritized into computational classes by an informatics algorithm to select: (1) variants previously reported as mutations in the Human Gene Mutation Database[17]; (2) predicted truncating variants that demonstrated <1% minor allele frequency; (3) 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 Table 2 (Supplemental Material available at AJO.com). 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.