Investigating Whole Exome Sequencing as a Diagnostic Test for Hereditary Retinal Disorders

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BACKGROUND: Whole exome sequencing (WES) is an attractive testing methodology for retinal disorders given their extreme genetic heterogeneity and overlapping phenotypes. Currently available clinical genetic testing for these conditions is costly and has low yield due to poor characterization of the totality of genes associated with eye development and function. Developing methods to determine the precise etiology of patients' conditions will become even more essential as gene-based therapies become more widely available. We proposed that WES is a more cost effective and efficient methodology in determining the molecular etiology of retinal disorders than currently available Sanger sequencing tests or Next-generation sequencing gene panels.

STUDY DESIGN: Patients diagnosed with a heterogeneous retinal disorder were eligible for study. Patients were enrolled during their initial clinic visit or previously seen patients were invited to return for a research visit if the molecular etiology of their retinal disorder was unknown. Patients approached during their initial clinic visit were offered clinical and/or research testing.

WES was performed using Agilent's SureSelect XT Target Enrichment System for Illumina paired-end sequencing on the HiSeq 2000 instrument. To narrow down the number of possible disease causing variants, we created a comprehensive list of 203 genes that have been associated with retinal disorders, which was curated using OMIM, GeneReviews, relevant medical literature and genes currently being evaluated in clinical laboratories. Potential disease causing variants were confirmed by Sanger sequencing on a duplicate sample in the UNC Clinical Molecular Genetics Laboratory.

Patients are asked to return for a follow-up appointment to learn any diagnostic results of their WES test. They were consented to learn of any medically actionable incidental results, but were not given the option of learning about non-actionable incidental results in this study.

PARTICIPANTS: 31 patients were approached and 28 enrolled between July to December 2012; 10 new and 18 previously seen patients. Three new patients opted to pursue clinical and WES testing simultaneously. Three patients declined all genetic testing. No patient preferred clinical testing over WES. The informed consent process took an average of 30 minutes. Patients were referred for the following clinical diagnoses:

- Retinitis pigmentosa (RP) (11)
- Rod-cone dystrophy vs. RP (2)
- Cone-rod dystrophy (CRD) (1)
- CRD vs. RP (1)
- Stargardt disease (SD) (3)
- CRD vs. SD (1)
- Refsum disease vs. RP (1)
- Cone dystrophy (2)
- Leber congential amaurosis (1)
- Usher syndrome (1)
- Bardet-Biedl syndrome (BBS) (1)
- FEVR (1)
- Retinal disorder NOS (2)

TABLE - Summary of the first 10 whole exome sequences

Proband	Clinical Diagnosis	Onset	Diagnostic Results	Incidental Findings
A	Retinitis pigmentosa	Early teens	***RHO*** **c.1040C>T (p.Pro347Leu)**	none
B	Cone-rod dystrophy	early 20s	***ABCA4*** **c.1622T>C (p.Leu541Pro)** ***ABCA4*** **c.1805G>A (p.Arg602Gln)** ***ABCA4*** **c.3113C>T (p.Ala1038Val)**	***BRCA2*** **c.2857G>T (p.Glu953Ter)** ***MSH6*** **c.1170_1170delT (p.Phe391fs)**
C	Stargardt disease	16yrs	***ABCA4*** **c.6320G>A (p.Arg2107His)** ***ABCA4*** **c.4139C>T (p.Pro1380Leu)** ***ABCA4*** **c.2546T>C (p.Val849Ala)**	none
D	Bardet-Biedl syndrome	birth	***BBS10*** **c.2119_2120delTG (p.Val707fs)** ***BBS10*** **c.1185C>G (p.His395Gln)**	none
E	Cone dystrophy	20s	none	none
F	Cone dystrophy	birth	none	none
G	Retinitis pigmentosa	29yrs	*CACNA1F* c.1556G>A (p.Arg519Gln) *RPGR* c.2606_2620del	none
H	AD RP vs. CRD + hearing loss	5yrs	*CRX* c.724G>A (p.Val242Met)	none
I	Refsum disease vs. RP	33yrs	***CEP290*** **c.5434_5435delAG (p.Glu1812fs)**	none
J	Cone-rod dystrophy vs. Stargardt disease	13yrs	***BBS9*** **c.1255C>G (p.Pro419Ala)** ***BBS9*** **c.2138A>T (p.Glu713Val)**	none

Results listed in bold black are considered pathogenic variants responsible for the patient's disorder or reportable medically actionable incidental findings. Results listed in bold red are variants of unknown significance related to their disorder. Additional variants are noted here for review, but were not reported to patients due to lack of substantial evidence that these variants are disease causing. Variants of unknown significance identified in genes associated with incidental findings are not reported to patients. The list of conditions considered to fall under the category of incidental findings were assigned by a committee of medical and molecular geneticists, genetic counselors, a neurologist, a cardiologist, a psychiatrist and an ethicist convened as part of the NCGENES Study being conducted at UNC. See poster 0299 for additional information about the NCGENES Study.

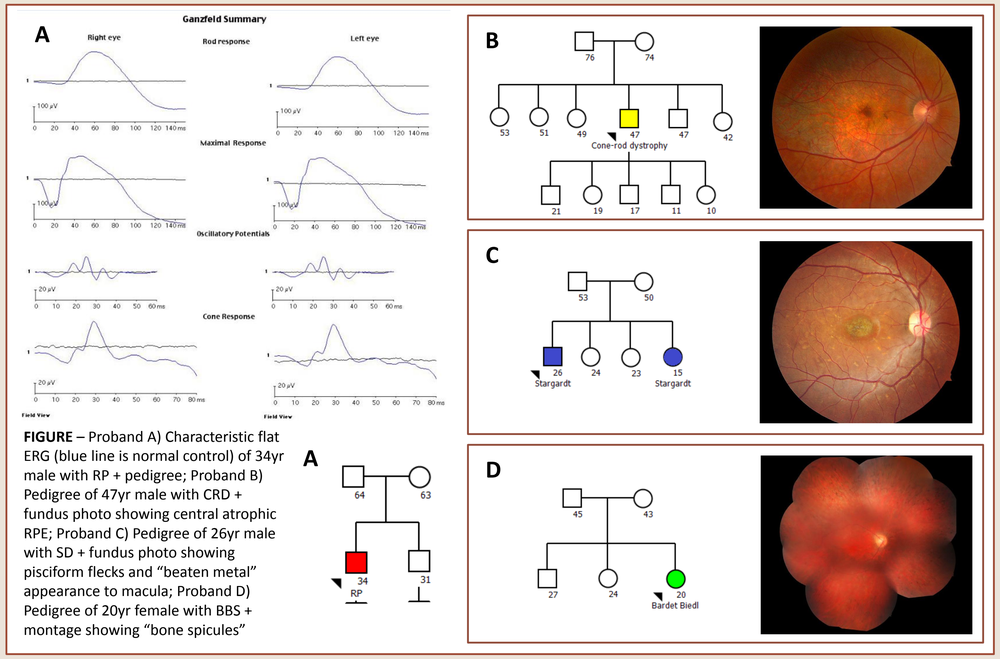

FIGURE – Proband A) Characteristic flat ERG (blue line is normal control) of 34yr male with RP + pedigree; Proband B) Pedigree of 47yr male with CRD + fundus photo showing central atrophic RPE; Proband C) Pedigree of 26yr male with SD + fundus photo showing pisciform flecks and "beaten metal" appearance to macula; Proband D) Pedigree of 20yr female with BBS + montage showing "bone spicules"

RESULTS: WES was a well received option for our patients. All patients who pursued genetic testing chose WES research testing with or without clinical testing, and none of our patients expressed concern about the possibility of learning incidental findings. The consent process for genetic testing was lengthier but manageable for clinic visits; however, the consent process would be longer if non-actionable incidental results were made available to patients.

Our average coverage for the exome was 60 fold. Definitive pathogenic mutation(s) were identified in 4 of the first 10 patients giving a detection rate of 40%. Variants of unknown significance will be followed up with family studies when possible.

SUMMARY AND FUTURE DIRECTIONS: WES proves to be a reasonable and potentially cost effective method of testing for a myriad of syndromic and non-syndromic retinal disorders in the clinical setting. A single test for hereditary retinal conditions is advantageous due to their considerable overlapping features. Several of our patients had multiple differential diagnoses, such as SD and CRD. The potential cost effectiveness of WES over many clinically available NGS gene panels and traditional Sanger sequencing was highlighted by Proband A, who has an apparently *de novo RHO* mutation, which is an autosomal dominant form of RP. Based on the lack of family history, genetic testing would have been focused on autosomal recessive and X-linked forms of this condition before considering an autosomal dominant form as a last resort. This one case alone illustrates the potential for cost savings when not dependent on complete and correct family pedigrees to predict inheritance patterns.

The detection rate using WES is still limited by our knowledge of which genes are associated with retinal disorders. For patients with no clear molecular etiology using our current gene list, we plan to reanalyze the exome data using an updated list of retinal genes at a minimum of annually for at least the next four years. In addition, we plan to query the data for rare or novel potential pathogenic variants to look for candidate genes that may play a role in retinal disorders.

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