Early Experiences with Genetic Counseling for Incidental Findings from Whole Exome Sequencing

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BACKGROUND: Whole exome sequencing (WES) is an attractive testing methodology for genetic disorders with extreme genetic heterogeneity and overlapping phenotypes, such as retinopathy disorders. However, using a broad testing approach will generate additional severe, medically actionable results termed incidental findings in some patients. NSGC recently released its position statement supporting the return of incidental findings. During a pilot study of the effectiveness of WES to identify disease causing mutations in a cohort of patients with various retinopathies, we identified a higher number of variants associated with incidental findings than anticipated. Determining the significance of many of these variants has been challenging, and some apparently disease causing variants may illustrate a lower penetrance of previously well described conditions.

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.

WES was performed using Agilent's SureSelect XT Target Enrichment System for Illumina paired-end sequencing on the HiSeq 2000 instrument. Potential disease causing variants were confirmed by Sanger sequencing on a duplicate sample in the UNC Clinical Molecular Genetics Laboratory.

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, which we termed “Bin 1” findings.

Patients are asked to return for a follow-up appointment to learn any diagnostic results and/or Bin 1 findings. They were consented to learn of any Bin 1 findings, but were not given the option of learning about non-actionable incidental results in this study. Only clearly pathogenic Bin 1 findings were returned to patients.

PARTICIPANTS: 29 patients were approached and 26 enrolled between July to December 2012; 10 new and 16 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) (13)
- Leber congenital amaurosis (1)
- Cone-rod dystrophy (CRD) (1)
- CRD vs. RP (1)
- Stargardt disease (SD) (3)
- CRD vs. SD (1)
- Cone dystrophy (2)
- Usher syndrome (1)
- FEVR (1)
- Retinal disorder NOS (2)

TABLE - Summary of Bin1 Variants of Unknown Significance & Pathogenic Mutations

Proband	Clinical Diagnosis	Bin 1 Variants	Disease Associated with Variants	Evidence for Pathogenicity	Evidence Against Pathogenicity	Variant Interpretation
A	Retinitis pigmentosa	*TGFBR1* c.1433A>G (p.Asn478Ser)	Loeys-Dietz syndrome	Variant reported in child with LDS	Variant reported in unaffected relative; allele frequency*	VUS
B	Cone-rod dystrophy	*1)BRCA2* c.2857G>T (p.Glu953Ter) *2) MSH6* c.1170_1170delT (p.Phe391fs)	1) Hereditary Breast & Ovarian Cancer 2) Lynch syndrome	1) Nonsense mutation 2) Frameshift mutation	1) None 2) None	1) Pathogenic 2) Pathogenic
C	Stargardt disease	*1) SCN5A* c.6016C>G (p.Pro2006Ala) *2) FBN1* c.3845A>G (p.Asn1282Ser)	1) Long QT syndrome 2) Marfan syndrome	1) Reported as mutation 2) Conservation	1) May only be risk modifier 2) Reported in UMD-FBN1 as benign SNP	1) VUS 2) VUS
D	Retinitis pigmentosa	*RB1* c.1966C>T (p.Arg656Trp)	Retinoblastoma	Reported as a mutation in 2 articles	Neither paper looked for co-segregation or analyzed controls	VUS
E	Cone dystrophy	*1) SERPINC1* c.89T>A (p.Val30Glu) *2) KCNE1* c.253G>A (p.Asp85Asn)	1) Antithrombin III 2) Long QT syndrome	1, 2) Reported as a mutation	1) Conservation; classified as polymorphism in public database 2) Allele frequency*	1) VUS 2) VUS
F	Stargardt disease	*1) APC* c. (p.) *2) KCNH2* c. (p.)	1) FAP 2) Long QT syndrome	1,2) Reported as a mutation	1,2) Higher allele frequency in African Americans	1) VUS 2) VUS
G	Retinitis pigmentosa	*TGFBR1* c.1433A>G (p.Asn478Ser)	Loeys-Dietz syndrome	Variant reported in child with LDS	Variant reported in unaffected relative; allele frequency*	VUS
H	AD RP + hearing loss	*PTCH1* c.1730C>T (p.Thr577Met)	Gorlin syndrome Holoprosencephaly-7	Reported as a mutation	Higher allele freq. in African Americans	VUS
I	Retinal dystrophy NOS	*SCN5A* c.5689C>T (p.Arg1897Trp)	Long QT syndrome	Reported as a mutation	Unclear relationship with LQT syndrome	VUS
J	Retinitis pigmentosa	*KCNE1* c.253G>A (p.Asp85Asn)	Long QT syndrome	Reported as a mutation	Allele frequency*	VUS

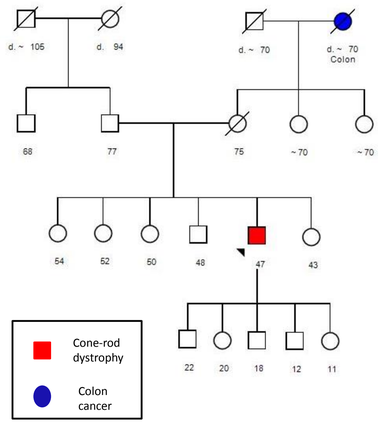
Summary of variants of unknown significance (VUS) and pathogenic variants identified within a predefined subset of genes associated with Bin 1 findings. Please note that known and likely benign variants identified in these patients are not listed. Data on conservation, allele frequency, *in silico* models, locus specific databases and medical literature were used in variant analysis. *This allele frequency refers to the population of patients enrolled in this study and the NCGENES Study, a WES study also being conducted at UNC. This frequency was slightly higher than frequencies reported from other cohorts, such as the 1000 genomes data set, which could suggest artifact data in some instances or simply be a result of our smaller sample size.

RESULTS: WES was a well received option for our patients, 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.

Variants in Bin 1 genes were filtered, and only truncating variants and variants listed as pathogenic mutations in Human Genetic Mutation Database and had <5% minor allele frequency were reviewed. Overall, 15 of 24 (62.5%) patients had variants within Bin 1 genes. After subsequent review, 4 of these 15 (26.6%) patients only had variants that were classified as known or likely benign. Eleven of 14 (78.5%) patients had VUS results. One patient had 2 novel pathogenic mutations associated with hereditary cancer syndromes with only one relative diagnosed with cancer in his family (see pedigree). Pathogenic and VUS results are listed in the table above. Two patients’ analyzes are still pending.

Proband B

47 year old Caucasian male enrolled due to a clinical diagnosis of cone-rod dystrophy. Was provided genetic counseling for the presumed pathogenic mutations in the *BRCA2* and *MSH6* genes. He later informed us of only one relative with cancer. We will offer genetic counseling and testing to relatives. He will be followed in a high risk colon cancer clinic.



DISCUSSION: The incidental findings identified in our study highlight potential challenges for genetic counseling. Several patients had variants that were very difficult to classify. Many variants had previously been reported as mutations in the literature with varying degrees of evidence. Some variants had more convincing functional and/or co-segregation data, while many had simply been identified in a patient with an associated phenotype. We chose to err on the side classifying variants as VUS if they lacked substantial evidence of pathogenity to prevent unnecessary harm; however, we plan to reanalyze these variants for up to three years. This process has shown to be a delicate balance between protecting patients from harm while not withholding important information.

Two variants were presumed pathogenic given their severity. We cannot be confident regarding the risks that novel apparently truncating variants confer in the context of a negative personal and family history. Moreover, the penetrance rates of well-studied genetic disorders (and previously described mutations) are likely inflated due to ascertainment bias.

Genetic counselors need to be mindful of these limitations with interpreting variants in genes associated with incidental findings when counseling patients that have undergone genome-scale sequencing tests.

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