Successful Utilization of Enhanced Exome Sequencing to Identify the Genetic Cause of Retinal Disorders in a Case Series

Poster 148

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Jeanie Tirch[1], Michael J. Clark[1], Samuel Strom[2], Ariadna Martinez[2], Sarah Garcia[1], Gemma Chandratillake[1], Jason Harris[1], Anil Patwardhan[1], Stephen Chervitz[1], Ming Li[1], Mark Pratt[1], Gabor Bartha[1], Shujun Luo[1], Richard Chen[1], John West[1], Michael B. Gorin[2]

[1]Personalis, Inc. | 1350 Willow Road, Suite 202 | Menlo Park, CA 94025 • [2]University of California, Los Angeles

Contact:

jeanie.tirch@personalis.com

Abstract

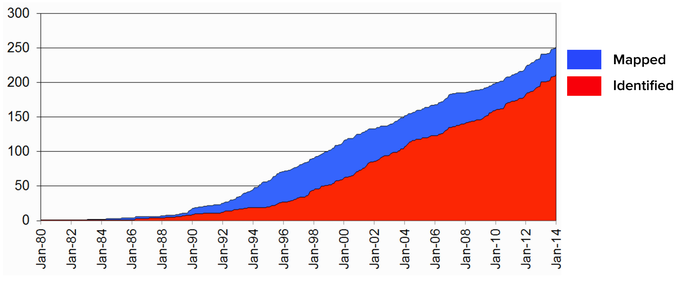
Identifying the genetic etiology for retinal disorders is challenging because of allelic, phenotypic, and locus heterogeneity, as well as environmental toxicities resulting in phenocopies. Patients often endure long diagnostic odysseys involving many single gene and/or gene panel tests. Whole exome sequencing (WES) is a highly appealing alternative to panels, which require frequent revision as new causative genes are discovered; however, incomplete coverage of relevant genes means standard WES is also non-ideal. To address these limitations, we developed the Accuracy and Content Enhanced (ACE) Exome, which improves sensitivity to detect variants by enhancing coverage over genes of biomedical relevance.

We conducted ACE Exome sequencing for members of eleven families with undiagnosed retinal disorders and used a novel automated system to rank variants by integrating family history and phenotypic information with the exome data. For ten of these families, we successfully identified the genetic basis of their retinal disorder.

Several of these diagnoses would have been either missed completely or timely and expensive to pick up via sequential gene testing protocols due to the involvement of genes that are thought to rarely cause disease (*e.g.*, variants in *CRX*, which is associated with only 1% of cases of retinitis pigmentosa (RP), were detected in three of our families). Three diagnoses involve genes not currently present on gene panels available in the US: one family has a novel homozygous variant in *LEPREL1*, a gene that has only been associated with retinal disorders in a single family in the literature; the second involves *NMNAT1*, another recently described gene available only as a single gene test; the third family has a variant in a new gene, previously considered a candidate gene for RP. In addition, one family has an X-linked etiology that would have been missed by standard exome sequencing due to poor gene coverage of *RPGR*.

These successes demonstrate the efficacy of enhanced exome sequencing to diagnose the genetic cause of retinal disorders.

FIGURE 1: The increasing number of identified and mapped genes related to retinal disease



Mapped and Identified Retinal Disease Genes 1980 - 2014

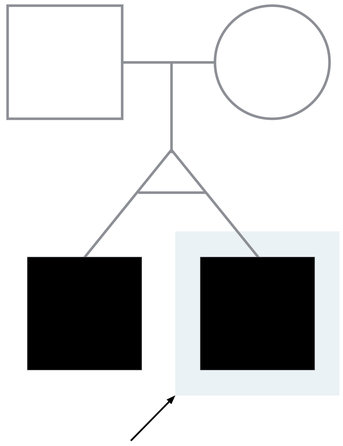
The Challenges to Address

- Panel testing for retinal disorders is challenged by the increasing number of genes identified in association with disease each year (see **FIGURE 1**)
- Exome testing is challenged by regions of absent or low coverage

Making the Case for an Enhanced Exome

Filling in the Gaps

Highlighted Case



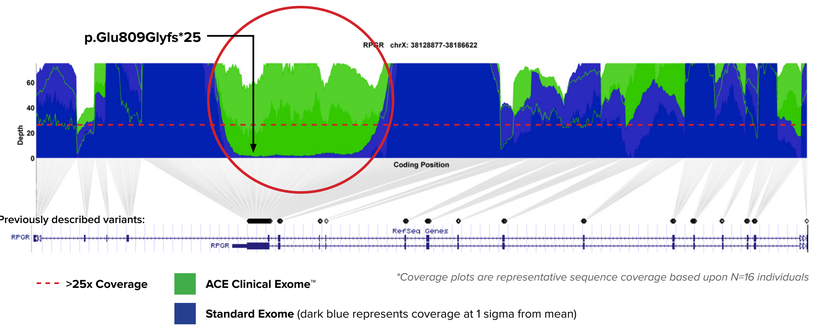
- Identical Twin Brothers
  - Infantile onset retinitis pigmentosa
  - High myopia
- No family history
- Negative for 13-gene autosomal recessive retinitis pigmentosa panel (2012)
- Frameshift variant identified in *RPGR* within a poorly covered region of the gene (using a standard exome), called ORF15 (p.Glu809Glyfs*25 (c.2426_2427del))

Mutations in *RPGR* cause ~80% of X-linked retinitis pigmentosa

Exonic coverage is sub-optimal with Standard Exome as various exons are completely devoid of coverage (see **FIGURE 2**)

FIGURE 2: Depth coverage plot of *RPGR*

Depth Coverage Plot of *RPGR**

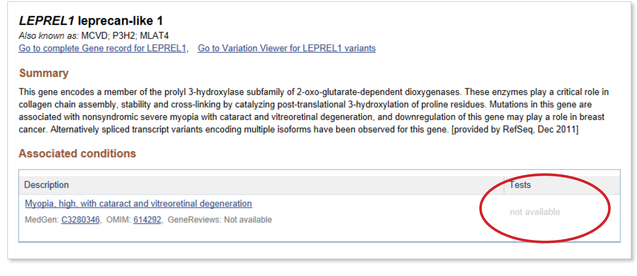


*Coverage plots are representative sequence coverage based upon N=16 individuals

Exome's Potential

- Achieving panel-grade coverage gives value to a negative result
- Many standard exomes address 'average coverage', but the metric of clinical relevance is that of 'gene finishing' -- the percent of bases covered at ≥ 15x such that accurate variant calls can be made

Providing Flexibility



***LEPREL1* leprecan-like 1**

Also known as: MCVD; P3H2; MLAT4

Go to complete Gene record for LEPREL1. Go to Variation Viewer for LEPREL1 variants

Summary

This gene encodes a member of the prolyl 3-hydroxylase subfamily of 2-oxo-glutarate-dependent dioxygenases. These enzymes play a critical role in collagen chain assembly, stability and cross-linking by catalyzing post-translational 3-hydroxylation of proline residues. Mutations in this gene are associated with nonsyndromic severe myopia with cataract and vitreoretinal degeneration, and downregulation of this gene may play a role in breast cancer. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene. [provided by RefSeq, Dec 2011]

Associated conditions

Description	Tests
Myopia, high, with cataract and vitreoretinal degeneration	not available
MedGen: C3280346, OMIM: 614292, GeneReviews: Not available	

- Several genes in our study do not have clinical single-gene or panel tests available.
- In at least two cases, the presumed inheritance and family history were later found to be incorrect.

Exome's Potential

- Dynamic and updated curation efforts identify clinically relevant genes prior to the addition of those genes onto panels
- Sensitivity to detect the cause of a syndrome is reliant on lab-clinician interactions

Results

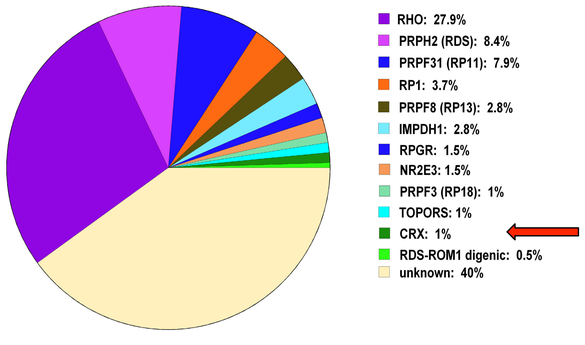
TABLE 1: ACE Exome identified the genetic basis for disease in 10 of 11 sequenced families

Family	Diagnosis	Inheritance Pattern	Ethnicity	Gene
Family 1 (trio)	Cone-Rod Dystrophy	Sporadic	East Asian	*CRX*
Family 2 (trio)	Retinitis Pigmentosa	Autosomal Dominant, possibly X-linked	French	*CRX*
Family 3 (parent-child)	Retinitis Pigmentosa, Cone-Rod Dystrophy	Autosomal Dominant	Mexican	*CRX*
Family 4 (trio)	Achromatopsia	Sporadic	Caucasian	*CNG83*
Family 5 (sib pair)	Atypical Stickler Syndrome	Autosomal Recessive	Lebanese	*LEPREL1*
Family 6 (proband)	Cone-Rod Dystrophy	Autosomal Recessive	Ashkenazi Jewish	*CACNA1F*
Family 7 (trio)	Leber Congenital Amaurosis	Sporadic	Caucasian	*NMNAT1*
Family 8 (three affected individuals)	Retinal Degeneration	Autosomal Dominant	Caucasian	Candidate gene for RP
Family 9 (three affected individuals)	Retinitis Pigmentosa	Autosomal Dominant	Caucasian	*PRPF31*
Family 10 (proband)	Retinitis Pigmentosa; Leber Congenital Amaurosis	Autosomal Recessive or X-linked	Caucasian	*RPGR*
Family 11 (three affected individuals)	Retinitis Pigmentosa	Autosomal Recessive	Greek	Unidentified

ACE Exome identified the genetic basis for disease in 10 of 11 sequenced families.

We identified mutations in genes that are very rarely the cause of retinal disorders (*CRX*, see **FIGURE 3**), in patients that had previously tested negative for variants in the same gene (*CNBG3*), in a gene that has only been linked to retinal disorders in one other family (*LEPREL1*), in a single proband from a family with variable phenotypes (*CACNA1F*) and in a gene only recently described as linked to the disorder in question (*NMNAT1*) [see **TABLE 1**].

FIGURE 3: In the context of retinal disorders as a whole, variants in *CRX* are responsible for only 1% of all cases of retinitis pigmentosa



Key Takeaways

- Exome may be appropriate for conditions that have allelic, phenotypic, and locus heterogeneity if the desired coverage over genes of interest is achieved
- Enhancing exomic coverage increases diagnostic yield and adds value to a negative result

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