Using an Augmented Exome to Improve Diagnostic Yield: Case Studies in Retinal Disorders

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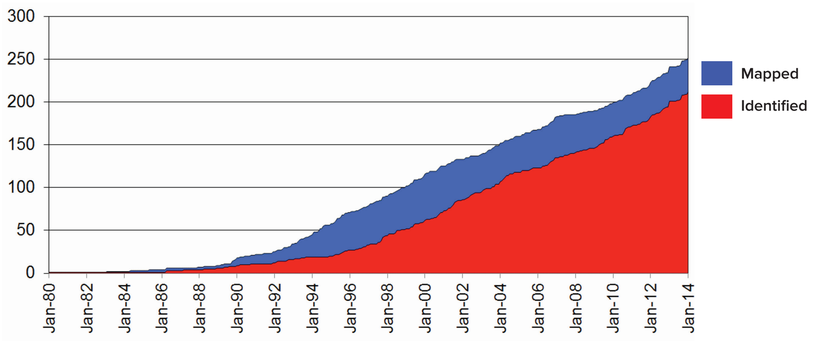
Introduction

Identifying the genetic etiology for retinal disorders, like many other Mendelian 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, with a genetic etiology remaining undetermined in a large percentage of cases (30-50%).

Whole exome sequencing (WES) is a highly appealing alternative to panels which require frequent revision as new causative genes are discovered (Figure 1); however, incomplete coverage of relevant genes means standard WES is also non-ideal. To address these limitations, we developed an augmented exome (ACE Exome), which improves sensitivity to detect variants by enhancing coverage over genes of biomedical relevance.

FIGURE 1: Growth of Mapped and Identified Retinal Disease Genes (1980 - 2014)



Mapped and Identified Retinal Disease Genes 1980 - 2014

Methods

Case Series

We conducted ACE Exome sequencing for members of eleven families with undiagnosed retinal disorders. Personalis ACE Exome™ sequencing was performed in-house, variants were called and annotated with the Personalis ACE pipeline, and the Personalis Annotation and Ranking Engine (PARE) was used to identify candidate variants.

All participants provided written informed consent as part of their enrollment in existing research studies at the participating collaborator sites with Institutional Review Board approval. Personalis, Inc. also employed an external Institutional Review Board to approve our involvement in the data analysis.

Results

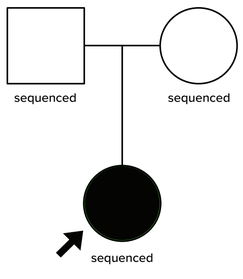
Likely causative variants were reported in 10 of the 11 analyzed cases.

Family	Diagnosis	Presumed Inheritance Pattern	Ethnicity	Gene
Family 1 (trio)	Cone-Rod Dystrophy Retinitis Pigmentosa	Sporadic	East Asian	***CRX***, p.Ser185Valfs*51
Family 2 (trio)	Retinitis Pigmentosa	Autosomal dominant, possibly X-Linked	French	***CRX***, p.Arg40Trp
Family 3 (parent-child)	Retinitis Pigmentosa, Cone-Rod Dystrophy	Autosomal dominant	Mexican	***CRX***, p.Pro197Argfs*22
Family 4 (trio)	Achromatopsia	Sporadic	Caucasian	***CNGB3***, p.Thr383Ilefs*13
Family 5 (proband)	Cone-Rod Dystrophy	Autosomal recessive	Ashkenazi Jewish	***CACNA1F***, p.Arg978*
Family 6 (sib pair)	Atypical Stickler Syndrome	Autosomal recessive	Lebanese	***LEPREL1***, p.Leu466Pro
Family 7 (trio)	Leber Congenital Amaurosis	Sporadic	Caucasian	***NMNAT1***, p.Arg188Trp
Family 8 (three affected individuals)	Retinal degeneration	Autosomal dominant	Caucasian	(novel gene, embargoed for publication)
Family 9 (three affected individuals)	Retinitis Pigmentosa	Autosomal dominant	Caucasian	***PRPF31***, p.Glu325*
Family 10 (proband)	Retinitis Pigmentosa	Autosomal recessive or X-linked	Caucasian	***RPGR***, p.Glu809Glyfs*25
Family 11 (three affected individuals)	Retinitis Pigmentosa	Autosomal recessive	Greek	(negative)

Discussion of Selected Cases

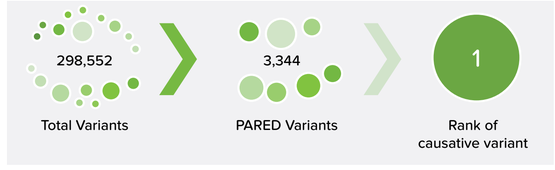
Family 1 – Cone-Rod Dystrophy

A trio consisting of a female proband affected with **cone-rod dystrophy** and her unaffected parents.



Variant Identification

In total, **298,552** variants were identified in the family by ACE exome sequencing. Of those, **3,344** passed frequency, effect, and quality filters and fit expected modes of inheritance. After filtering, ranking, scoring, and adjustment, the causative variant was ranked **1**.

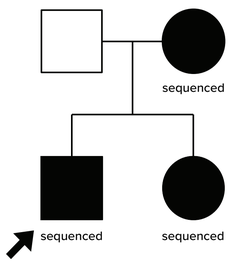


Result - *De novo* Variant in *CRX*

A potentially causative ***de novo* variant in the *CRX* gene** was identified in the proband. This variant was not present in either parental sample, has not been reported in the literature, and is not present in any of our population frequency datasets. Multiple frameshift variants associated with CRD have been reported in the third exon of *CRX*.

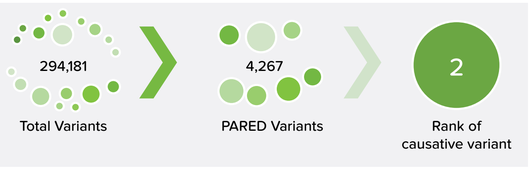
Family 2 – Retinitis Pigmentosa

A trio consisting of a male proband affected with retinitis pigmentosa and his affected mother and sister.



Variant Identification

In total, **294,181** variants were identified in the family by ACE exome sequencing. Of those, **4,267** passed frequency, effect, and quality filters and fit expected models of inheritance. After filtering, ranking, scoring, and adjustment, the causative variant was ranked **2**.

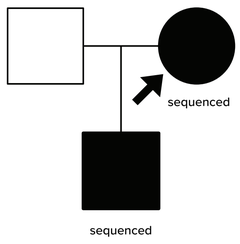


Result - Novel Heterozygous Missense Variant in *CRX*

A **novel heterozygous missense variant in the *CRX* gene** was identified in all three affected individuals. This variant causes an amino acid change predicted to be pathogenic by multiple *in silico* models at a highly conserved site. This variant is not present in any of the major population databases we routinely check (1000 Genomes, NHLBI GO-ESP, HapMap, UK10K Healthy Genomes). variants at an immediately adjacent amino acid have been described in patients with CRD and RP.

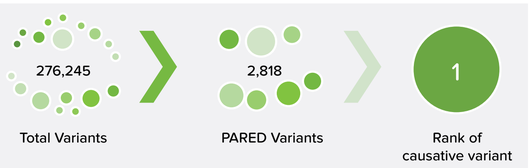
Family 3 – Retinitis Pigmentosa / Cone-Rod Dystrophy

A pair of affected individuals from a family segregating an **apparent autosomal dominant eye disorder**. The affected proband is diagnosed with **retinitis pigmentosa** while her son has **cone-rod dystrophy**.



Variant Identification

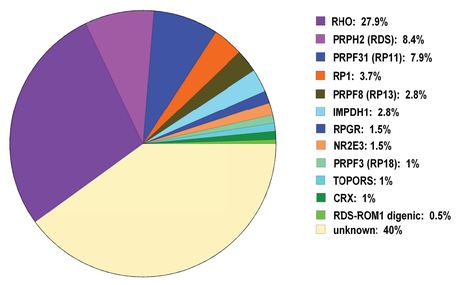
In total, **276,245** variants were identified in the mother and son by ACE exome sequencing. Of those, **2,818** passed frequency, effect, and quality filters and fit expected models of inheritance. After filtering, ranking, scoring, and adjustment, **1** variant required manual review before the causative variant was identified and validated.



Result - Novel Heterozygous Deletion in *CRX*

A **novel heterozygous deletion causing a frameshift variant in the *CRX* gene** was identified in both affected individuals. This variant is not present in any of the major population databases we routinely check (1000 Genomes, NHLBI GO-ESP, HapMap, UK10K Healthy Genomes). A 1bp insertion at the same amino acid residue has been described in individuals with CRD, and multiple frameshift variants in the third exon of CRX have been associated with CRD as well.

Regarding Testing *for* *CRX* Variants

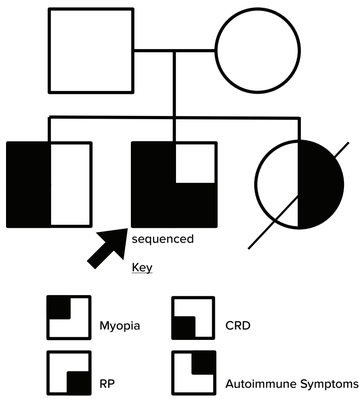


In the context of retinal disorders as a whole, variants in ***CRX*** are responsible for a very small number of total cases. Variants in ***CRX*** are only responsible for 1% of all cases of retinitis pigmentosa.

CRX is a prime example of the many confounding issues facing genetic diagnosis of retinal disorders. Due to the low rate at which variants in ***CRX*** cause retinal disorders, and the phenotypic, locus, and allelic heterogeneity of retinal disorders, and the prevalence of novel ***CRX*** variants, it is not typically the first or even one of the more commonly tested genes. The use of an enhanced exome allows ***CRX*** to be assessed alongside other common and rare causes of retinal disorders.

Family 5 – High Myopia and Cone-Rod Dystrophy

The male proband displays **high myopia** and **cone-rod dystrophy** as well as features of a **peripheral neuropathy with autoimmune-like symptoms**. His brother is reported to have myopia and CRD, and his sister to have RP and lupus. As well, both parents have histories of eye disorders.



Variant Identification

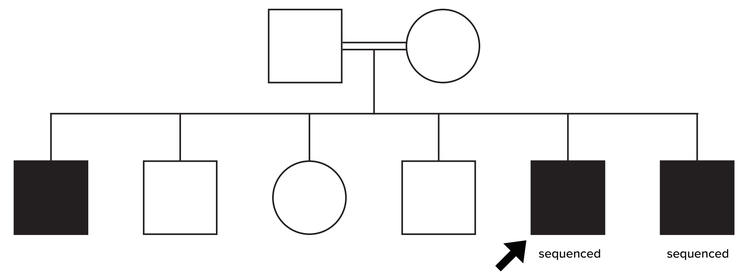
ACE exome sequencing identified **214,574** variants in the proband. After processing with PARE, the causative variant was ranked **21** (as X-linked inheritance was ranked as an unlikely explanation for disease in this family).

Result - Novel Nonsense X-linked Variation in *CACNA1F*

A **novel nonsense variant in *CACNA1F*** was identified in the proband.
A note about X-linked inheritance in this family: Upon further case review the sister was determined to not to have retinitis pigmentosa.

Family 6 – "Atypical" Stickler Syndrome

Two brothers affected with **cataracts** and **early onset retinal detachments** were tested by ACE exome sequencing. This family is from Lebanon and were reported **consanguineous** (parents reported to be first cousins). The brothers also have a third affected brother.



While a diagnosis of "atypical Stickler syndrome" was made, no genetic testing was performed due to the unusual presentation.

"Atypical" Stickler Syndrome

- Myopia
- Congenital cataracts
- Vitreous degeneration
- Retinal detachment
- Subluxated lenses

Variant Identification

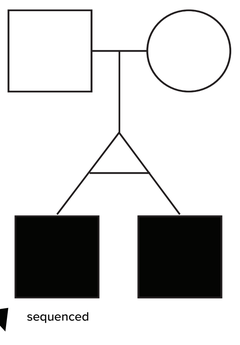
As with the previously described cases, while the total number of variants detected between the two brothers was quite high at **259,182** variants, after processing with PARE, the causative variant was ranked **1.**

Result - Homozygous Missense Variant in the *LEPREL1* Gene in Each Brother

A **homozygous variant** in the ***LEPREL1* gene** was identified in both brothers. Another variant (p.G508V) in this gene has been previously associated with autosomal recessive myopia, early-onset cataracts, vitreoretinal degeneration, and subluxated lenses in a large Israeli family (Mordechai *et al.*, 2011). This particular variant is predicted to be deleterious and has been seen in only one European-American individual in the NHLBI Exome Sequencing Project, but has not been reported anywhere else, and has never been associated with genetic disease.

Family 10 – Infantile-onset Retinitis pigmentosa

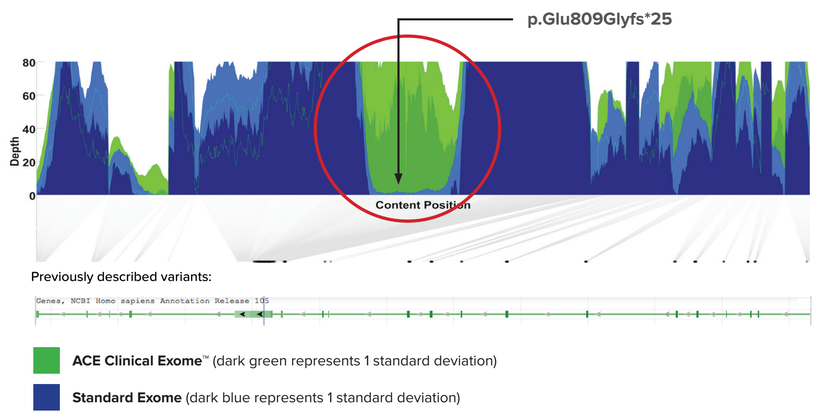
Two identical twin brothers affected **infantile-onset rod-cone demonstrated retinitis pigmentosa and high myopia** were tested by ACE exome sequencing.



Result - Heterozygous Frameshifting Indel in *RPGR*

Frameshift variant identified in ***RPGR*** in ORF15 (p.Glu809Glyfs*25 (c.2426_2427del). ORF15 is poorly using a standard exome but well covered by the ACE Exome.

FIGURE 2: Depth coverage plot of *RPGR*



Conclusions

For ten of the eleven analyzed 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 cause disease only rarely, *e.g.*, variants in ***CRX***, which is associated with only 1% of cases of retinitis pigmentosa (RP). Two 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 family has a variant in a new gene, previously considered a candidate gene for RP. Another family was found with variation in ***NMNAT1***, a recently described gene not available on panels at the time the individual had negative panel testing. Finally, one family has an X-linked genetic 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.

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