Successes using ACE Exome Sequencing to Identify the Genetic Causes of Retinal Disorders

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Introduction

Retinal disorders are often Mendelian in nature, being caused by mutation of a single gene in a given individual. There is great interest in determining the genetic etiology of retinal disorders on a case-by-case basis in order to determine prognosis, inform counseling and risk assessment, and even determine therapies. However, identifying the genetic cause of retinal disorders is challenging due to a number of factors that confound diagnosis.

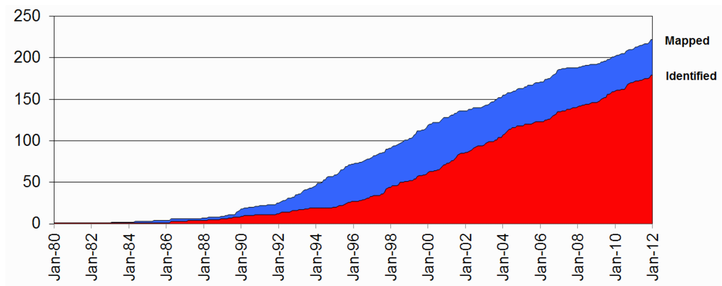
Allelic heterogeneity, where a number of different discrete mutations in the same gene cause the same phenotype, is common among eye disorders. For example, Stargardt disease, a form of juvenile macular degeneration that leads to blindness, can be caused by any of over 600 mutations in the *ABCA4* gene.

Locus heterogeneity, where the same phenotype is caused by mutations in different genes, is another substantial challenge. Retinitis pigmentosa, a degenerative retinal disorder that leads to severe vision impairment, can be caused by mutations in over 45 different genes.

Phenotypic heterogeneity, where mutations in the same gene can cause different phenotypes, is yet another common phenomenon in retinal disorders. For example, mutations in the *CRX* gene can cause retinitis pigmentosa (RP), Leber congenital amaurosis (LCA), and cone-rod dystrophy (CRD).

For these reasons, it can be very difficult to choose the correct single gene test or gene panel to use for any given case when it comes to retinal disorders. As a result, patients with a genetic basis for their retinal disorders often endure long diagnostic odysseys involving many single gene tests and panels.

Whole exome sequencing (WES) is a highly appealing alternative to panels and single gene tests because WES tests all genes at once. This is especially important with regards to retinal disorders, because the number of retinal disorder genes is currently increasing at a rate of about 25 new genes per year. Unsolved cases can be easily reassessed as new genes are identified with WES.



Mapped and Identified Retinal Disease Genes 1980 - 2012

However, a number of factors make WES prone to inaccuracies and missing coverage such as badly designed probes, poor performance over high GC regions, and difficult to enrich elements. To address these issues, we utilized Personalis Accuracy and Content Enhanced (ACE) exome sequencing, which significantly improves accuracy in regions WES typically misses. Coverage of known retinal genes was significantly improved by ACE sequencing, including coverage over regions that are completely missed by typical assays.

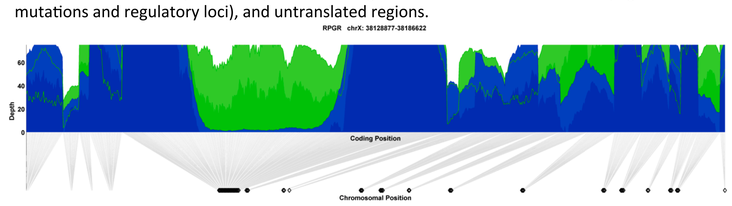
Methods

Case Series

Family	Diagnosis	Inheritance Pattern	Ethnicity
Family 1 (trio)	Cone-Rod Dystrophy	Sporadic	East Asian
Family 2 (trio)	Retinitis Pigmentosa	Autosomal Dominant, possibly X-Linked	French
Family 3 (parent-child)	Retinitis Pigmentosa, Cone-Rod Dystrophy	Autosomal Dominant	Mexican
Family 4 (trio)	Achromatopsia	Sporadic	Caucasian
Family 5 (sib pair)	Atypical Stickler Syndrome	Autosomal Recessive	Lebanese
Family 6 (proband)	Cone-Rod Dystrophy	Autosomal Recessive	Ashkenazi Jewish
Family 7 (trio)	Leber Congenital Amaurosis	Sporadic	Caucasian

ACE Exome Sequencing

Taking in a series of retinal disorder cases that had tested negative for suspected genetic causes by panel and single gene tests, we performed **Accuracy and Content Enhanced (ACE) exome sequencing** to 12Gb of total sequence on the proband and certain family members where available. ACE exome increases coverage over all biomedical regions of the genome including coding exons for over **7,800 genes**, all non-coding yet disease-associated variants (e.g. intronic mutations and regulatory loci), and untranslated regions.



ACE exome sequencing coverage over the RPGR gene, a notoriously hard to sequence gene causative for retinitis pigmentosa, cone-rod dystrophy, and macular degeneration. Y-axis represents depth (up to a maximum of 80x), X-axis represents coding position in RPGR. The blue histogram shows depth by standard exome sequencing. The green histogram shows depth by ACE exome sequencing. Note that substantial portions of RPGR have low coverage by standard exome, but high coverage by ACE exome. ACE similarly improves coverage of over 7,800 biomedical genes, including many eye genes.

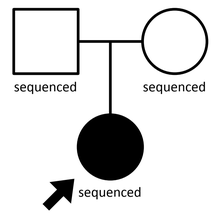
Personalis Annotation and Ranking Engine (PARE)

We utilized the Personalis Annotation and Ranking Engine (PARE) to rapidly and accurately identify the most likely candidate mutations detected by ACE exome sequencing for each set of samples. PARE takes into account the clinical features reported to Personalis by the clinician and scores variants based on their association with those clinical features. Genes associated with a greater number of clinical features in the literature are scored more highly. Variants are also ranked based on their consistency with expected modes of inheritance. Variants matching a more likely mode of inheritance pattern for the family in question are ranked more highly. PARE then filters variants based on extensive annotations such as population frequencies, predicted impact of mutational effect, and mutation type. It also modifies the variant rank based on previous presence in databases of known disease-causing variants like ClinVar, HGMD, and OMIM. Together, these scores and ranks are used to prioritize manual variant review by a team of genomic counselors and bioinformatics experts. Variants are then reviewed manually and once a "hit" is identified, it is validated by orthogonal methods and a report is written up describing the finding in detail.

Case Series Analyses

Family 1 – Cone-Rod Dystrophy

A trio consisting of a female proband affected with **cone-rod dystrophy** and her unaffected parents. Based on family history, the genetic etiology is expected to be a **sporadic *de novo*** variant.

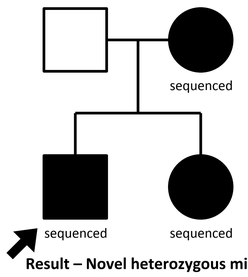


Variant Identification
In total, **298,552** variants were identified in the trio by ACE exome sequencing. Of those, **3,344** fit acceptable models of inheritance, passed population frequency and were predicted to have some potential effect on protein coding sequences. Ranks based on variant consistency with inheritance pattern and scores based on correlation of genes to phenotypes were then applied. These ranks were adjusted based on presence in known mutational databases. After filtering, ranking, scoring, and adjustment, **1** variant required manual review before the causative variant was identified and subsequently validated.

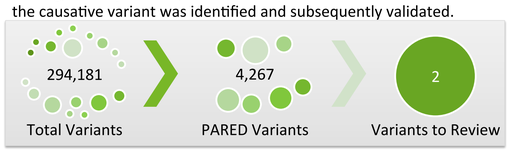
Result – *De novo* variant in *CRX*
A potentially causative ***de novo* mutation in the *CRX* gene** was identified in the proband. This mutation was not present in either parental sample, has not been reported in the literature, and is not present in any of our population frequency datasets. Multiple frameshift mutations 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. Based on family history, the genetic etiology is expected to be **autosomal or x-linked dominant**.



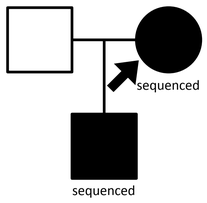
Variant Identification
In total, **294,181** variants were identified in the family by ACE exome sequencing. Of those, **4,267** passed frequency, effect, and quality filters and fit expected models of inheritance. After filtering, ranking, scoring, and adjustment, **2** variants required manual review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



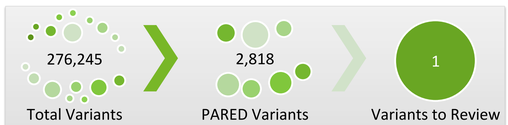
Result – Novel heterozygous missense variant in *CRX*
A **novel heterozygous missense variant in the *CRX* gene** was identified in all three affected individuals. This mutation causes an amino acid change predicted to be pathogenic by multiple *in silico* models at a highly conserved site. This variant is not present in any of the major population databases we routinely check (1000 Genomes, NHLBI GO-ESP, HapMap, UK10K Healthy Genomes). Mutations 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 appears to have **retinitis pigmentosa** while her son has **cone-rod dystrophy**.

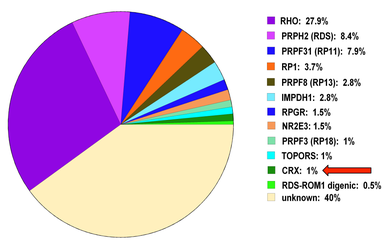


Variant Identification
In total, **276,245** variants were identified in the mother and son by ACE exome sequencing. Of those, **2,818** passed frequency, effect, and quality filters and fit expected models of inheritance. After filtering, ranking, scoring, and adjustment, **1** variant required manual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



Result – Novel het. del. in *CRX*
A **novel heterozygous deletion causing a frameshift mutation in the *CRX* gene** was identified in all three 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 mutations in the third exon of *CRX* have been associated with CRD as well.

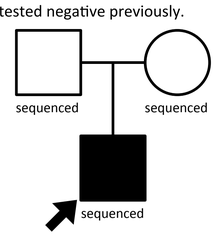
Regarding Testing for CRX Mutations



In the context of retinal disorders as a whole, mutations in *CRX* are responsible for a very small number of total cases. Mutations 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 mutations in *CRX* cause retinal disorders, the phenotypic, locus, and allelic heterogeneity of *CRX* mutations, and the prevalence of novel *CRX* mutations, it is not typically the first or even one of the more commonly tested genes. ACE exome, however, completely assesses it.

Family 4 - Achromatopsia

A male proband with **achromatopsia** and unaffected parents. Achromatopsia type 3 is an **autosomal recessive** condition, and that mode of inheritance is consistent with this family's pattern. Although causative mutations can be identified in >70% of achromatopsia type 3 by standard testing, this family tested negative previously.



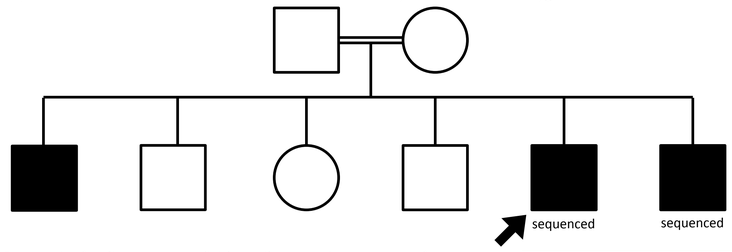
Variant Identification
In total, **304,493** variants were identified in the mother and son by ACE exome sequencing. Of those, **1,486** passed frequency, effect, and quality filters and fit expected models of inheritance. After filtering, ranking, scoring, and adjustment, **1** variant required manual review before the causative variant was identified and subsequently validated.

Result – Homozygous frameshift mutation in *CNBG3*
The proband was **homozygous** for a **well-known frameshift mutation** in ***CNBG3***, which he did indeed inherit one copy of from each parent. It is unknown why previous testing missed this variant.

Case Series Analyses

Family 5 – "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 to have some **consanguinity** (parents are reported to be first cousins). The brothers also have a third affected brother. For these reasons, the expected inheritance pattern is **autosomal recessive** likely with a **rare homozygous variant** involved.



Due to the family structure of this pedigree and the nature of phenotype segregation among the siblings, an underlying genetic condition was suspected. 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, only **1** variant required manual review before the causative variant was identified and subsequently validated.

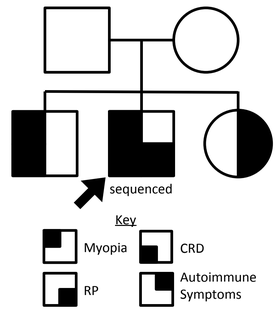
Result – Homozygous missense variant in the *LEPREL1* gene in each brother
A **homozygous variant** in the ***LEPREL1* gene** was identified in both brothers. Another mutation (p.G508V) in this gene has been previously associated with autosomal recessive myopia, early-onset cataracts, vitreoretinal degeneration, 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.

There are no available single gene or gene panel tests for variants in *LEPREL1*. While there are tests for many of the genes we identified in this study, *LEPREL1* is not one of them.

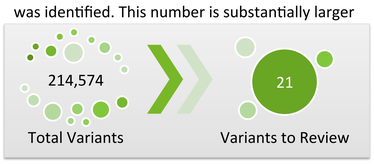
This is likely because this family represents only the **second** identified case of this particular disorder. Again, the issue of **locus heterogeneity** makes this particular form of retinal disorder hard to correctly diagnose. Moreover, mutations in *LEPREL1* may account for a very small percent of overall cases, but likely a greater number than we currently recognize. This is due to an **ascertainment bias**—since we do not routinely test for *LEPREL1* mutations, we do not know how commonly it is mutated in retinal disorders. **ACE Exome and PARE, however, always detect *LEPREL1* and other more obscure genes.**

Family 6 – 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. This therefore suggests possibly an autosomal recessive condition.



Variant Identification
ACE exome sequencing identified **214,574** variants in the proband. After processing with PARE, **21** variants needed to undergo manual follow-up before the presumed causative variant was identified. This number is substantially larger than usual because of the variable phenotype and because only the proband (rather than the trio including the parents or the proband plus siblings or the whole family) was sequenced.



Result – novel nonsense X-linked variant in *CACNA1F*
A **novel nonsense variant in *CACNA1F*** was identified in the proband. Although this particular variant is novel, over 60 unique *CACNA1F* mutations, including nonsense variants, have been described to date. This variant is predicted by *in silico* prediction algorithms to be deleterious.
A note about X-linked inheritance in this family: Although there is an affected sister with RP in this family, skewed X-inactivation may explain her phenotype. There is evidence in the literature that female carriers of *CACNA1F* mutations may display severe congenital stationary night blindness-like phenotypes and intellectual disability.
Regarding the autoimmune symptoms: The *CACNA1F* mutation does not explain the peripheral neuropathy or autoimmune symptoms. It is possible this is a second, unrelated phenotype.

Family 7 – Leber Congenital Amaurosis

In one final trio family consisting of an **affected proband with LCA and unaffected parents**, the proband was found to be a **compound heterozygote for two missense variants in *NMNAT1***, one inherited from each parent. These were our top ranked candidate variants. *NMNAT1* is a newly described LCA gene.

Conclusions

The genetic diagnosis of retinal disorders is facilitated in a much more comprehensive and accurate way by performing whole exome sequencing. In this case series, we identified mutations in genes that are very rarely the cause of retinal disorders (*CRX*), 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*). Through the use of ACE exome sequencing and the PARE method, we were able to identify the causative variant with manual review of just one variant in five of our seven cases, and with just two in one more case. These experiences demonstrate the success of Personalis ACE Clinical Exome for diagnosis of retinal disorders.