Exome Sequencing in 20 Probands with Developmental Eye Defects Identifies Causative Mutations in Five Cases and Demonstrates Genetic Heterogeneity

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Introduction

Anophthalmia (absent eyes)/microphthalmia (small eyes) (A/M) is found in 3 per 10,000 live births and is a clinically heterogeneous birth defect for which the genetic etiology remains incompletely understood. A/M can occur as an isolated malformation (simplex A/M), or with additional ocular abnormalities affecting the anterior or posterior segments of the eye (complex A/M). Complex A/M can include defects such as coloboma, or failure of the optic fissure to close, cataracts and anterior segment dysgenesis (ASD), an eye defect with maldevelopment of the anterior ocular structures behind the cornea and in front of the lens, including the iris, ciliary body and trabecular meshwork.

Case Reports

(n=20, 2 patients in which causative variant(s) were found and 1 with plausible variant are highlighted below)

Child with *PNPT1* mutations:

- 13 month old male
- Prenatal onset growth retardation (weight 1900g at 35 wks)
- Severe developmental delays
- Cortical visual impairment with chorioretinal defect of L eye and optic atrophy; ERG showed a mild reduction in outer retinal function affecting rods.
- Daily seizures described as myoclonic epilepsy
- EEG showed infantile spasms and hypsarrhythmia
- Examination revealed microcephaly (OFC 39 cm; 50th centile for 2-3 mths) and truncal hypotonia
- Metabolic investigations and an array were normal
- MRI of the brain: diminished white matter, simplified gyri, a thin corpus callosum and thinning of the optic tracts

Child with *COL4A1* mutation:

- IUGR noted from 28 wks; C-section at 34 wks
- 5 wk NICU stay complicated by apnea and bradycardia
- Red reflex absent; bilateral cataracts diagnosed at 5 wks
- Baby arrested with ventricular fibrillation and asystole during lensectomies
- Initial ECHO showed good ventricular function
- Subsequent ECHO showed hypertrophic cardiomyopathy
- Baby was resuscitated with ECMO
- Developed seizures and multiorgan failure and the parents opted for palliative care
- Autopsy confirmed cataracts and cardiomyopathy and muscle pathology was consistent with a mitochondrial myopathy
- A diagnosis of Senger syndrome was suggested, but sequencing of the *AGK* gene was negative

Child with *GCNT2* mutations:

- Baby born after a normal pregnancy
- Bilateral cataracts were diagnosed after absent red reflex
- Required surgical and medical treatment
- Also had R microtia with an absent auditory canal and R conductive deafness
- Previously reported array finding of 1q41 copy gain containing *DISP1*, *TLR5*, *SUSD4*. Significance unknown.
- 3 yr old sister had bilateral cataracts and aphakic glaucoma
- Parents were of Hispanic ethnicity and there was no known consanguinity

Materials and Methods

We used the ACE Exome™ (Personalis, Inc.) to sequence 20 children with A/M or other developmental eye defects for mutations in causative genes, both known and novel. Five patients were ascertained through UCSF and 15 through the International Children's Anophthalmia Network (ICAN). The majority had complex A/M with varied extraocular findings (13 patients), two had coloboma, two had cataracts and single patients each had simplex A/M, anterior segment dysgenesis or a chorioretinal defect. Short clinical descriptions have been provided for 3 mutation positive patients (see case reports).

The ACE Exome™ constitutes an exome sequencing platform in which coverage is enhanced for >7800 biomedically relevant genes. The platform also incorporates genome-wide structural variant detection.

Results

We found clearly causative mutations in 5/20 (25%) patients (two patients had mutations in *STRA6*, one had a previously reported mutation in *GDF6*, one had 2 mutations in *PNPT1* and one was homozygous for a mutation in *GCNT2*) (Table 1). Two additional patients had plausible mutations in genes with phenotypic spectra that were previously more narrowly defined (*COL4A1* and *SKI*).

Conclusion

Our results illustrate significant genetic heterogeneity for eye defects and demonstrate the utility of exome sequencing in birth defects for identifying rare sequence variants that would likely have remained unidentified using previous technologies.

Discussion

In *PNPT1*, we found a maternally inherited splice site mutation and a paternally inherited missense mutation, p.Ala507Ser, in a patient with a chorioretinal defect. *PNPT1* is critical for mRNA import into mitochondria and had not previously been associated with ocular findings. Two papers have previously reported mutations in this gene associated with severe bilateral hearing impairment and respiratory chain deficiency (Vedrenne et al., 2012; Von Ameln et al., 2012). Our patient has clinical findings that are consistent with a mitochondrial presentation (see case report).

Two female siblings with bilateral congenital cataracts were both found to be homozygous for p.Tyr347Cys in *GCNT2* (see case report). This gene has previously been associated with cataracts and failure to replace the i antigen with the I antigen on red blood cells. The previously reported microarray finding was determined to be present in the unaffected mother during the course of this study, contextualizing it as a likely benign variant.

A female with cataracts and cardiomyopathy had a *de novo* mutation in *COL4A1*, p.Gly773Arg (see case report). Cataracts, microcornea and anterior segment dysgenesis have previously been associated with *COL4A1* mutations, but the finding of cardiomyopathy is new, although valvular heart disease has been described (Kuo et al., 2012).

Finally, a female with anophthalmia, cleft lip/palate and features of Goldenhar syndrome had a *de novo* missense mutation in *SKI*, p.Arg216Leu. The significance of this alteration is, however, unknown and some of the predictive software programs have classified it as likely to be benign.

References

Kuo et al: Hum Mol Genet 2012;21(R1):R97.

Vedrenne et al: Am J Hum Genet 2012;91:912.

Von Ameln et al: Am J Hum Genet 2012;91:919.

Table 1: Phenotypic Features and Mutations in Selected A/M Patients

(n=20, 5 patients in which causative variant(s) were found and 1 with plausible variant are highlighted below)

Patient	Eyes	Other	Gene	Mutation	Zygosity
Female	Microphthalmia	Vascular ring	*STRA6*	p.Arg399Glu	Het.
Male	Microphthalmia	-	*STRA6*	Splice/p.Tyr18fs*	Het./Het.
Male	Anophthalmia	-	*GDF6*	p.Ala249Glu	Het. (de novo)
Female	Cataracts	See case report	*GCNT2*	p.Tyr347Cys	Hom.
Male	Chorioretinal defect	See case report	*PNPT1*	Splice/p.Ala507Ser	Het./Het.
Female	Cataracts	See case report	*COL4A1*	p.Gly773Arg	Het. (de novo)

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Further Information from Personalis:

- **Presentation: Friday, March 28, 2014, 12:30 pm – 1:00 pm, Theater 1**
- Church et al., **Impacts of Updating the Reference Assembly on Genome Interpretation,** poster #259
- Tirch et al., **User-Friendly Genomic Results: Leveraging a Novel Approach that has the Potential to Decrease Turn-Around Time and Preserve Opportunities for Novel Discoveries,** poster #561
- Chervitz et al., **Accurate Structural Variant Calling for Comprehensive Clinical Interpretation,** poster #291
- Chen et al., **Approaches to Increase Diagnostic Yield for Clinical Genomic Sequencing,** poster #286
- Garcia et al., **The Clinical Exome: Personalis' Experience Using an Enhanced Exome and Genome-wide Structural Variant Detection for the Diagnosis of Diseases of Unknown Genetic Etiology,** poster #192
- Clark et al., **Successes Using ACE Exome Sequencing to Identify the Genetic Cause of Retinal Disorders in a Case Series,** poster #280
- **Personalis Booth #312**