Considerations in multi-gene panel testing in pediatric ophthalmology

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Multi-gene panel testing is used increasingly in ophthalmology practice as an efficient and cost-effective method for diagnosing inherited eye conditions. Panel testing is a powerful diagnostic tool, and it has the potential to reveal syndromic information in patients with seemingly isolated eye findings. This case series highlights our experience with 4 children in 3 families who were referred for evaluation of an isolated retinal degeneration and diagnosed with neuronal ceroid lipofuscinosis on panel testing. These cases are important reminders that several neurodegenerative conditions can present initially with isolated eye findings in childhood and pretest genetic counseling is critical.

Neuronal ceroid lipofuscinoses (NCL) are a group of inherited lysosomal storage disorders that cause progressive neurodegeneration and early death.[1] Vision loss is a common feature and, in some cases, the first presenting symptom.[2] We report the clinical presentations of 4 children with seemingly isolated retinal pathology who were molecularly diagnosed with NCL when they were neurologically asymptomatic.

Case Reports

Family 1 (Patient 1)

A 6-year-old girl with reduced visual acuity for one year was referred to the National Eye Institute (NEI) for evaluation of bull's eye maculopathy. On examination, her best-corrected visual acuity was 20/320 in the right eye and 20/250 in the left, with significant color discrimination deficits and unrecordable electroretinogram (ERG) amplitudes. The retina was atrophic, with retinal vascular attenuation, and photoreceptor loss was evident on optical coherence tomography (OCT). She had no other medical concerns, although her mother noted a recent change in behavior that was attributed to the start of the school year. Inherited retinal dystrophy (IRD) panel testing revealed two previously reported pathogenic mutations in *CLN3*, the gene associated with juvenile-onset NCL: 1kb deletion (paternally inherited) and a nonsense mutation c.833G>T; p.Glu295Ter (maternally inherited). See eTable 1. At 3 years' follow-up, her best-corrected visual acuity had decreased to light perception in each eye, and OCT showed diffuse photoreceptor atrophy. She reported having had one seizure, but her health was otherwise unchanged.

Family 2 (Patients 2 and 3)

A 9-year-old boy with reduced visual acuity for 1 month was referred for evaluation of suspected Stargardt disease. His best-corrected visual acuity was 20/200 in each eye. He had large central scotomas that corresponded to areas of macular atrophy. Bidirectional sequence analysis of the *ABCA4*, *PRPH2*, and *ELOVL4* genes was negative. The following year, the proband's 7-year-old sister presented with reduced visual acuity of 20/125 in each eye, with large central scotomas corresponding to areas of macular atrophy. Neither sibling had any neurologic or behavioral issues at presentation. IRD panel testing in the proband revealed a previously reported pathogenic mutation and a previously reported likely pathogenic variant in *CLN3*: 1kb deletion (inherited maternally) and c.1056+3A>C (inherited paternally). Targeted testing in the sister confirmed the presence of these variants.

At follow-up 3 years later, the proband's visual acuity was 20/800 in each eye, with significant visual field loss, a diffuse retinal degeneration and optic atrophy. His parents noted that he had difficulty with word finding, anxiety, and demonstrated more aggressive behavior. His sister's visual acuity decreased to 20/320, and her funduscopic examination and OCT demonstrated progression of the macular atrophy. She developed seizures but did not have evidence of cognitive regression.

Family 3 (Patient 4)

A 10-year-old boy with reduced visual acuity (20/50) and macular cysts was referred to the NEI for evaluation of X-linked retinoschisis. His fundus examination revealed granular pigmentation of the peripheral retina, attenuated vessels and rare pigment spicules (Figure 1A, B), and he was noted to have peripheral field constriction on visual field testing. OCT revealed cysts in the central macula, with ellipsoid zone loss (Figure 1C). ERG testing demonstrated severe rod and moderate cone dysfunction. He noted nyctalopia for the last 1 year and had a diagnosis of attention deficit hyperactivity disorder (ADHD). IRD panel testing identified a previously reported nonsense mutation and a novel intronic variant in *PPT1*, the gene associated with infantile-onset NCL (CLN1 disease): c.451C>T; p.Arg151Ter (maternally inherited) and c.234+4A>C (absent maternally).

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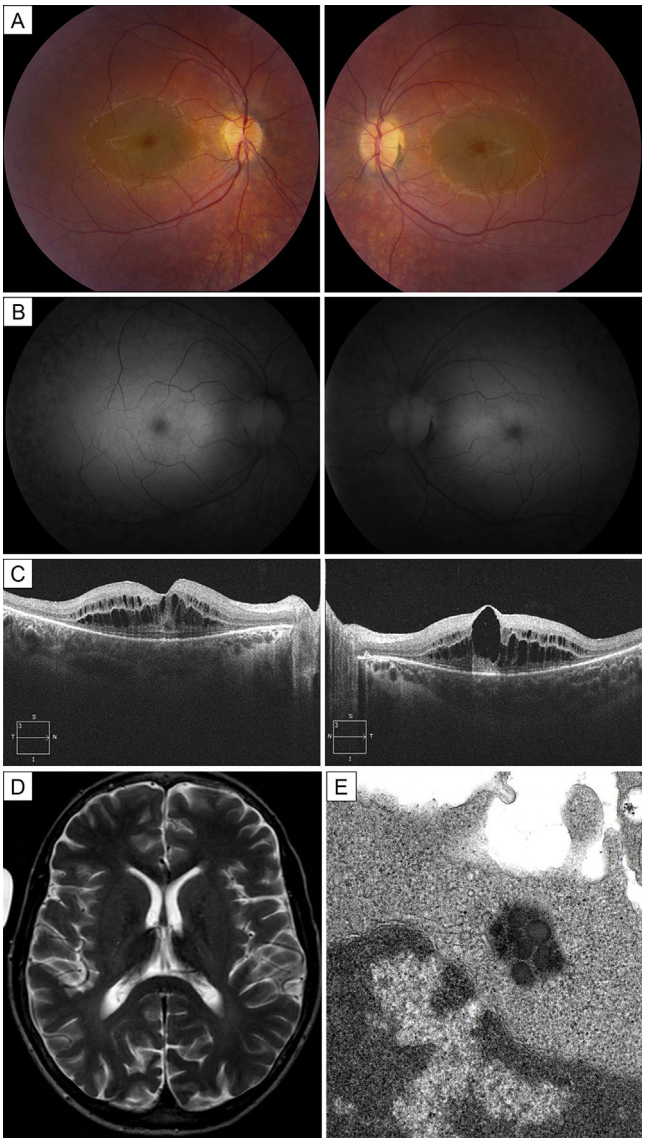

FIG 1. 10-year-old boy with PPT1-related neuronal ceroid lipofuscinoses. Fundus photographs demonstrating retinal vascular attenuation and retinal pigment epithelium granularity (A), with corresponding areas of hypoautofluorescence (B). Optical coherence tomography showing cysts in the central macula and ellipsoid zone loss (C). Although the family reported no neurologic symptoms or regression, he was found to have encoding and short-term memory difficulties, visuomotor integration issues on neurocognitive testing, and cortical atrophy on magnetic resonance imaging (D). Diagnosis was confirmed with electron microscopy, showing accumulation of dense material, and skin biopsy, which revealed granular osmophilic deposits in the cytoplasm of a lymphocyte (E).

Unknown at the initial evaluation, the patient was performing at a first-grade level as a fourth grader, which had been attributed to his vision loss and attention deficit hyperactivity disorder. He returned to the National Institutes of Health Clinical Center for neurology consultation, neuropsychology testing, and neuroimaging. Cognitive testing documented more cognitive decline than could be accounted for by inter-rater and inter-testing variability, when compared to testing performed for his individualized education plan. Brain MRI suggested mild cerebral volume loss relative to normal for the patient's age (Figure 1D). A skin biopsy and peripheral venous blood sample were obtained for electron microscopy and identified granular osmophilic deposits consistent with CLN1 disease (Figure 1E). Enzyme testing revealed deficient PPT1 enzyme activity, although less deficient than observed in cases of classic infantile-onset NCL.

Discussion

In this case series, 4 children diagnosed with NCL prior to the onset of neurologic symptoms had ophthalmic findings ranging from bull's eye maculopathy to optic atrophy with diffuse retinal degeneration. Neurodegenerative disease should be considered in all pediatric patients presenting for evaluation of retinal degeneration, because visual complaints may be the first manifestation,[1,2] and pretest genetic counseling must discuss this possibility. History of recent behavioral or personality changes, hyperactivity, or changes in school performance could be initial signs of neurologic decline and should increase suspicion of NCL in a child with retinal degeneration.

While genotype-phenotype correlations exist,[3] diagnosis of NCL in a child with retinal degeneration alone comes with a significant amount of uncertainty. The presence of a variant of uncertain significance and atypical *PPT1*-disease in patient 4 resulted in significant doubt for the family. With increased use of next-generation sequencing in ophthalmology practice, the phenotypic spectrum of genetic diseases continues to broaden and the identification of uncertain variants is likely.[4-6] The presence of variants in genes associated with NCL warrants systemic evaluation and monitoring over time, especially in children.

Historically, a diagnosis of NCL was typically delayed until neurologic findings were present and NCL-specific molecular or biochemical testing was clinically indicated. Earlier diagnosis enables patients and families to access disease-specific multidisciplinary care and support, prepare for the future, and understand recurrence risks. In a study of 179 parents of children with NCL, 54% pursued presymptomatic genetic testing of at-risk siblings.[7] While these families have firsthand experience with NCL to help inform this choice, families with children presenting with retinal degeneration alone likely have very little context to consider such a profound theoretical finding. Pretest genetic counseling is critical to convey the possible outcomes of multi-gene panel testing, to help families begin to consider the implications of genetic information, and to facilitate informed decisions about genetic testing.

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Computed tomography–based 3D modeling to provide custom 3D-printed glasses for children with craniofacial abnormalities

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Children with craniofacial malformations frequently require spectacles but have difficulty finding an acceptable fit with current offerings of pediatric spectacle frames. We describe a novel method for creating custom 3D-printed spectacle frames based on a 3D reconstruction of a prior computed tomography scan. This method offers the ability to create better-fitting spectacles to children who are not served by "off the rack" frames.

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Craniofacial anomalies encompass a wide range of congenital malformations, ranging from isolated craniosynostosis to larger syndromic abnormalities. Craniosynostosis with at least one suture involved occurs in 1 in 2000 live births.[1] A range of ophthalmic problems can occur as a result of craniofacial anomalies, including strabismus, refractive error, exposure keratopathy, proptosis, nasolacrimal duct obstruction, and optic nerve atrophy.[2,3] An underrecognized but important additional challenge faced by these patients is the difficulty in spectacle wear. Because of their uniquely irregular anatomy, commercially available spectacles fit poorly, and families often resort to homemade adaptations with straps to improve fit, with mixed results.[4] This problem is especially significant because these patients have an increased incidence of high and asymmetric refractive error with concomitant risk of amblyopia.[5] We developed a novel method for producing custom spectacles for children with craniofacial malformations that leverages existing imaging and 3D-printing technology.

Methods

A single patient from our practice was selected based on her inability to wear conventional spectacles due to skull deformity, ear asymmetry, midface hypoplasia, and a flattened nasal bridge. She is a 3-year-old girl with congenital glaucoma, bilateral anterior segment dysgenesis, and chorioretinal colobomas. At age 3 months, she underwent multiple surgeries, including Ahmed valve placement, penetrating keratoplasty, and lensectomy in both eyes. In addition, she has absent corpus callosum, coronal synostosis (following cranial vault reconstruction with fronto-orbital advancement due to shallow orbits with bilateral proptosis), developmental delay, and conductive hearing loss. She requires aphakic spectacle correction and a bone anchored hearing device coupled with a soft band on the left ear. Attempts had been made to augment her glasses fit using additional straps, but she did not tolerate this ad hoc solution.

First a 3D model of her superficial head anatomy was created from a computed tomography (CT) scan obtained 6 months prior for a nonophthalmic indication. Optical designers from JINS eyewear (Maebashi, Japan) designed custom spectacle frames for our patient's unique anatomy. The spectacle design was then 3D printed with standard lenses edged to fit the glasses.

Because the patient was unable to tolerate measurement of her facial anatomy in clinic, a 3D head model was created using the most recently acquired craniofacial images using a GE Revolution CT scanner (GE Healthcare, Waukesha WI). After the

eTable 1. Results of genetic testing[a]

	Inherited retinal dystrophy panel	Gene	Variant 1	Variant 2	Configuration
Family 1					
Patient 1	Version 5 (211 genes)	*CLN3*	1.02kb deletion including exons 7-8[b]	NM_001042432.1: c.883G>T; NP_001035897.1: p.Glu295Ter[b]	Trans
Family 2					
Patient 2	Version 8 (233 genes)	*CLN3*	1.02kb deletion including exons 7-8[b]	NM_001042432.1: c.1056+3A>C[c]	Trans
Patient 3	Site-directed	*CLN3*	1.02kb deletion including exons 7-8[b]	NM_001042432.1: c.1056+3A>C[c]	Trans
Family 3					
Patient 4	Version 4 (183 genes)	*PPT1*	NM_000310.3: c.451C>T; NP_000301.1: p.Arg151Ter[b]	NM_000310.3: c.234+4A>C[d]	Trans

[a]Genetic testing for all patients in this series was performed at the Molecular Vision Laboratory, Hilsboro, OR (CLIA Lab ID: 38D2059762).
[b]Previously reported mutation.
[c]Previously reported likely pathogenic variant.
[d]Novel intronic variant.