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Dominant variants in *PRR12* result in unilateral or bilateral complex microphthalmia

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Abstract

Complex microphthalmia is characterized by small eyes with additional abnormalities that may include anterior segment dysgenesis. While many genes are known, a genetic cause is identified in only 4–30% of microphthalmia, with the lowest rate in unilateral cases. We identified four novel pathogenic loss-of-function alleles in *PRR12* in families affected by complex microphthalmia and/or Peters anomaly, including two *de novo*, the first dominantly transmitted allele, as well as the first splicing variant. The ocular phenotypes were isolated with no additional systemic features observed in two unrelated families. Remarkably, ocular phenotypes were asymmetric in all individuals and unilateral (with structurally normal contralateral eye) in three. There are only three previously reported *PRR12* variants identified in probands with intellectual disability, neuropsychiatric disorders, and iris anomalies. While some overlap with previously reported cases is seen, non-syndromic developmental ocular anomalies are a novel phenotype for this gene. Additional phenotypic expansions included short stature and normal development/cognition, each noted in two individuals in this cohort, as well as absence of neuropsychiatric disorders in all. This

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study identifies new associations for *PRR12* disruption in humans and presents a genetic diagnosis resulting in unilateral ocular phenotypes in a significant proportion of cases.

Keywords

Peters anomaly; microphthalmia; unilateral; PRR12; developmental ocular disorder; exome

INTRODUCTION

Developmental ocular disorders encompass a wide range of ocular phenotypes, affecting a specific portion of the eye (e.g., iris hypoplasia), involving the whole eye (e.g., anophthalmia), or presenting with a combination of abnormal globe size along with other eye defects (e.g., complex microphthalmia). Pathogenic variants in over 100 genes have been reported to cause microphthalmia, anophthalmia, and coloboma (MAC) or anterior segment dysgenesis (ASD) spectrum phenotypes, with the highest proportion of cases explained by disruption of transcription factors/regulators [1–3]. While a greater number of genes are reported to result in MAC phenotypes, diagnostic rates for ASD conditions are typically higher, ranging from 25–60% for ASD vs 15–30% for MAC [3–6]. Rates of genetic diagnosis are lower in cases with unilateral ocular anomalies, typically 4–10% in unilateral MAC, for example [3,7], and these conditions are often regarded as less-likely to be due to a genetic etiology.

De novo loss-of-function variants in *PRR12* were recently reported in three probands with an overlapping phenotype consisting of eye anomalies, intellectual disability, and neuropsychiatric disorders such as anxiety, autism, or ADHD [8]. Variable additional anomalies and dysmorphic facial features were also noted. With regards to the eye, iris coloboma was present in two of the three, a stellate iris pattern was reported in all three, and myopia and exotropia were also seen. In one additional case, a *de novo* translocation was identified which resulted in a fusion protein predicted to cause frameshift and truncation of the *PRR12* sequence after the 11th exon (of 14); this individual was similarly affected with intellectual disability, anxiety/autistic like features, and myopia with strabismus, but had normal irises [9].

Here, we report novel pathogenic variants in *PRR12* in four families affected with unilateral or bilateral complex microphthalmia and/or Peters anomaly with or without additional non-ocular abnormalities.

MATERIALS AND METHODS

Variants were identified by exome or genome sequencing, undertaken through Perkin Elmer (Branford, CT) (Family 1), Psomagen (previously Axeq; Rockville, MD) (Families 2–4), or the University of Washington Center for Mendelian Genomics (Family 3) and analyzed as previously described using VarSeq (Golden Helix, Bozeman, MT) [10]. Exome data was reviewed from a total of 263 probands with MAC (182) or ASD with normal eye size (81) but without pathogenic variants in known ocular genes; in addition to exome analysis, genome data was also available for one proband (Individual 3). Bilateral eye anomalies were

present in 155, unilateral eye anomalies were noted in 74, and the remaining 34 had unknown laterality. In 149 individuals additional non-ocular anomalies were present, in 94- ocular anomalies were isolated, and in 17- information about non-ocular anomalies was not provided. For variant verification, Sanger sequencing of PCR products generated with region-specific primers (Supplemental Table 1) was performed. Splicing predictions were made using the Alternative Splice Site Predictor (ASSP) website [11]. The study adhered to the US Federal Policy for the Protection of Human Subjects and was approved by the Institutional Review Boards of Children's Wisconsin (Milwaukee, WI) and Einstein Medical Center (Philadelphia, PA) with written informed consent obtained for every participant, including photo publication, if applicable.

RESULTS

A splicing variant in *PRR12* was identified in Individual 1A and her daughter (1B), both affected with unilateral microphthalmia (Figure 1, Table 1); the daughter also has Peters anomaly in the affected eye, bilateral nystagmus, and mild developmental delay/learning difficulties. The c.5624–2A>G variant was present in 8/21 reads in exome data from Individual 1A, 60/143 reads in Individual 1B, and was not present in the unaffected father (mother not available) (Figure 2). ASSP analysis of the region did not identify any strong alternative acceptor sites, thus the variant is likely to result in skipping of the 98-bp exon 11 [12], resulting in frameshift with early truncation (p.Asp1875Glyfs*54). This prediction results in a stop codon within exon 13 (of 14), 85 nucleotides from the final exon-exon border, and thus would be expected to be subject to nonsense mediated decay.

Frameshift variants were identified in unrelated Individuals 2, 3, and 4 (Figure 1, Table 1). Parental samples were available for Individuals 2 and 3 and the variants were determined to be *de novo* in both (Figure 2). Individual 2 displays a significant ocular phenotype with bilateral iris coloboma, foveal hypoplasia, and nystagmus, left Peters anomaly/microcornea and right cataract, Persistent Fetal Vasculature (PFV) and other vascular defects, without any non-ocular abnormalities including normal growth and neurodevelopment. Review of Individual 2's exome data identified a c.2045delG p.Gly682Aspfs*44 variant, seen in 15/29 reads and not present in either unaffected parent. Individual 3 was diagnosed with bilateral Peters anomaly, left microphthalmia and right glaucoma, as well as non-ocular anomalies including severe cognitive impairment (non-verbal and required assistance with all activities of daily living) and short stature. Prior genetic testing identified a *de novo* 2.7 Mb deletion of 4q35.1, containing *TENM3, DCTD, ING2,* and *IRF2*, of uncertain significance. Review of Individual 3's genome data identified a c.677dupC p.Tyr227Leufs*41 variant, seen in 11/59 reads and not present in either unaffected parent. Subsequent re-review of prior exome data for the same individual found that the variant was similarly skewed, identified in 4/18 reads, suggesting possible mosaicism in this individual; the results from Sanger sequencing were also consistent with mosaicism, showing lower peaks for the mutant allele (Supplemental Figure 1). Finally, Individual 4 is affected with unilateral microphthalmia, global delays and short stature; family history and parental samples are not available. Exome data review revealed a c.2353_2360delGCCGGGGG p.Ala785Profs*2 variant in 8/14 reads. These three frameshift variants all occur in exon 4 (of 14) and thus all three would be expected to be subject to nonsense mediated decay. All four variants were novel (absent in

gnomAD) and met ACMG criteria to be considered pathogenic (PVS1 and PM2 for all; plus PP1 for Individual 1, PS2 for Individuals 2 and 3, and PP3 for Individual 4); all were confirmed by Sanger sequencing of the corresponding *PRR12* regions (Supplemental Table 1).

DISCUSSION

PRR12 encodes a Proline-Rich Protein 12 of unknown function. Current evidence suggests that PRR12 functions as a cofactor in transcriptional regulation within the nucleus [8]. The gene is predicted to be highly intolerant of loss-of-function variants based on gnomAD data [13]. All four novel variants are predicted to result in loss-of-function alleles via nonsense-mediated decay (NMD) or, if escaped, generation of severely truncated and thus functionally deficient proteins; if it escapes NMD, the splicing variant in Family 1 would be expected to produce the most functional protein, containing 92% of the normal protein sequence. Including the previously reported cases, nine individuals with loss-of-function variants in *PRR12* have been identified, with highly variable phenotypes. The current report includes the most N-terminal (frameshift in Individual 3) as well as the most C-terminal (splicing defect in Individuals 1A and B) variants.

The ocular phenotypes in our cohort were more severe than previously reported, with microphthalmia (4) or coloboma (1) seen in all five individuals, Peters anomaly in three, and cataract, foveal hypoplasia, abnormal vascular development, glaucoma and hyperopia in one case each. Remarkably, ocular phenotypes were asymmetric in all affected individuals and unilateral (with structurally normal contralateral eye) in three (Individuals 1A, 1B, and 4); iris coloboma was also unilateral in one previously reported case. While genes such as *SOX2* and *OTX2* have been occasionally reported to cause unilateral ocular anomalies [14,15], unilateral developmental ocular disorders historically have a low rate of genetic diagnosis [7,16]. At the same time, no neuropsychiatric diagnoses were present within our cohort and two of the five affected individuals had completely normal development and cognition, while intellectual disability ranging from mild to moderate and neuropsychiatric diagnoses were seen in all four previously reported individuals. Individual 3 in our study displayed more severe cognitive impairment, however, there may be a contribution from the *de novo* deletion in this individual. Additionally, short stature was present in two unrelated individuals in our study, which may indicate another new variable feature associated with *PRR12* disruption.

Given the variability observed in reported individuals thus far, it seems likely that additional phenotypic variation will be identified as more *PRR12* variants are discovered. In this cohort, the apparent loss-of-function variants in *PRR12* explained 4/182 (2.2%) of unsolved MAC including 2/57 (3.5%) cases with unilateral MAC. All variants reported thus far are predicted to result in protein truncation and are likely subject to nonsense mediated decay, thus haploinsufficiency represents the most likely disease mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY:

There are no other data associated with this manuscript

REFERENCES

1. Reis LM, Semina EV. Conserved genetic pathways associated with microphthalmia, anophthalmia, and coloboma. Birth defects researchPart C, Embryo today : reviews. 2015;105(2):96–113.
2. Reis LM, Semina EV. Genetics of anterior segment dysgenesis disorders. Current opinion in ophthalmology. 2011;22(5):314–324. [PubMed: 21730847]
3. Patel A, Hayward JD, Tailor V, et al. The Oculome Panel Test: Next-Generation Sequencing to Diagnose a Diverse Range of Genetic Developmental Eye Disorders. Ophthalmology. 2019;126(6):888–907. [PubMed: 30653986]
4. Jackson D, Malka S, Harding P, Palma J, Dunbar H, Moosajee M. Molecular diagnostic challenges for non-retinal developmental eye disorders in the United Kingdom. Am J Med Genet C Semin Med Genet. 2020.
5. Ma A, Yousoof S, Grigg JR, et al. Revealing hidden genetic diagnoses in the ocular anterior segment disorders. Genet Med. 2020.
6. Thanikachalam S, Hodapp E, Chang TC, et al. Spectrum of Genetic Variants Associated with Anterior Segment Dysgenesis in South Florida. Genes (Basel). 2020;11(4).
7. Chassaing N, Causse A, Vigouroux A, et al. Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. Clinical genetics. 2014;86(4):326–334. [PubMed: 24033328]
8. Leduc MS, McGuire M, Madan-Khetarpal S, et al. De novo apparent loss-of-function mutations in PRR12 in three patients with intellectual disability and iris abnormalities. Hum Genet. 2018;137(3):257–264. [PubMed: 29556724]
9. Cordova-Fletes C, Dominguez MG, Delint-Ramirez I, et al. A de novo t(10;19)(q22.3;q13.33) leads to ZMIZ1/PRR12 reciprocal fusion transcripts in a girl with intellectual disability and neuropsychiatric alterations. Neurogenetics. 2015;16(4):287–298. [PubMed: 26163108]
10. Reis LM, Sorokina EA, Thompson S, et al. De Novo Missense Variants in WDR37 Cause a Severe Multisystemic Syndrome. Am J Hum Genet. 2019;105(2):425–433. [PubMed: 31327510]
11. Wang M, Marin A. Characterization and prediction of alternative splice sites. Gene. 2006;366(2):219–227. [PubMed: 16226402]
12. Anna A, Monika G. Splicing mutations in human genetic disorders: examples, detection, and confirmation. J Appl Genet. 2018;59(3):253–268. [PubMed: 29680930]
13. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581(7809):434–443. [PubMed: 32461654]
14. Schilter KF, Schneider A, Bardakjian T, et al. OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype. Clinical genetics. 2011;79(2):158–168. [PubMed: 20486942]
15. Schneider A, Bardakjian T, Reis LM, Tyler RC, Semina EV. Novel SOX2 mutations and genotype-phenotype correlation in anophthalmia and microphthalmia. American journal of medical geneticsPart A. 2009;149A(12):2706–2715.

16. Patel N, Anand D, Monies D, et al. Novel phenotypes and loci identified through clinical genomics approaches to pediatric cataract. Human genetics. 2016.

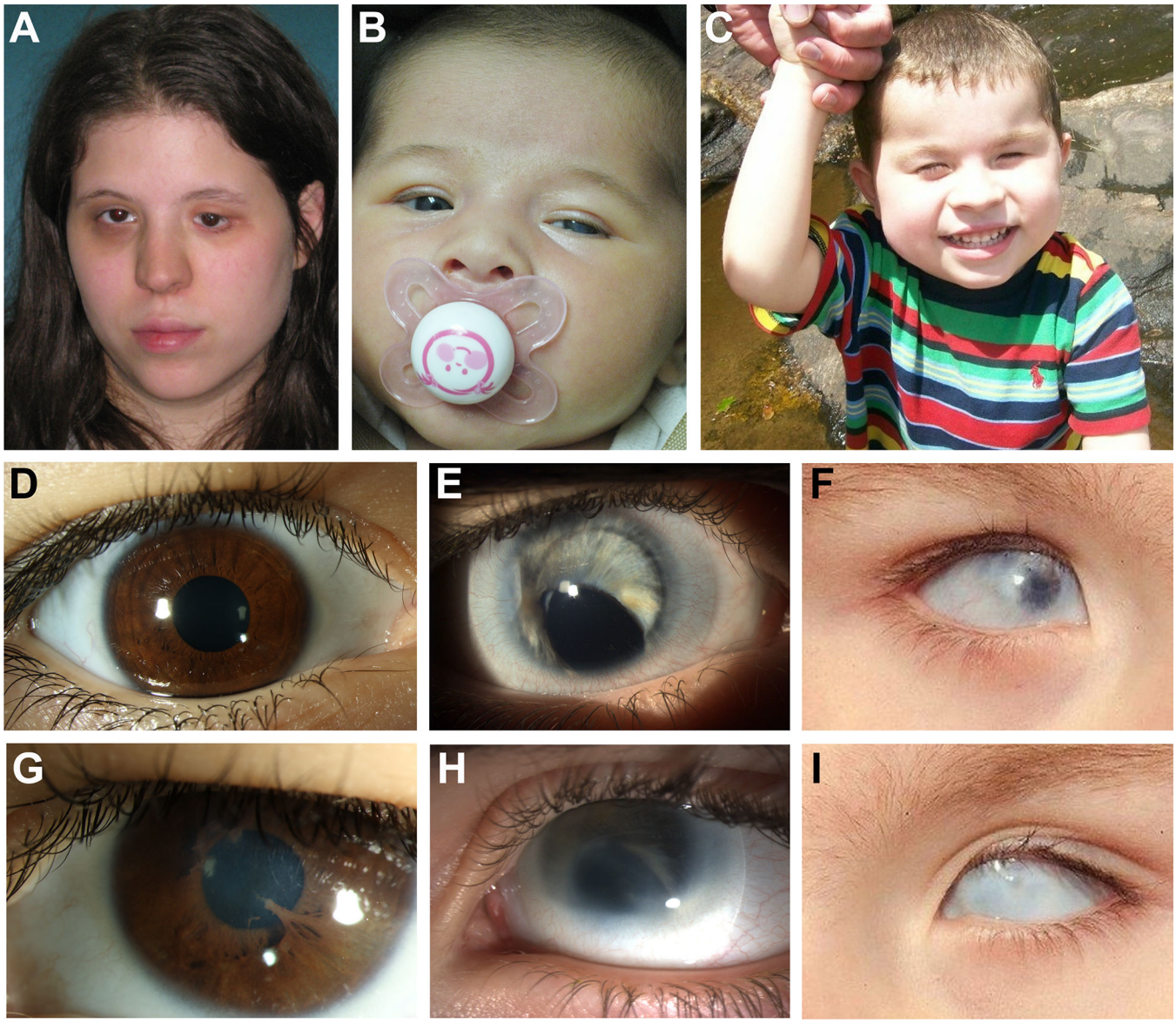

Figure 1: Images from individuals with *PRR12* variants.

Facial photographs of Individual 1A (**A**) with left ocular prosthesis, Individual 1B (**B**), and Individual 3 (**C**). Ocular images of Individual 1B showing normal right eye (**D**) and left eye (**G**) with mild microphthalmia and Peters anomaly with corneal opacity and iridocorneal adhesion; Individual 2 (**E, H,** wearing contacts) with bilateral iris coloboma and left Peters anomaly and Individual 3 (**F, I**) with bilateral Peters anomaly and left microphthalmia.

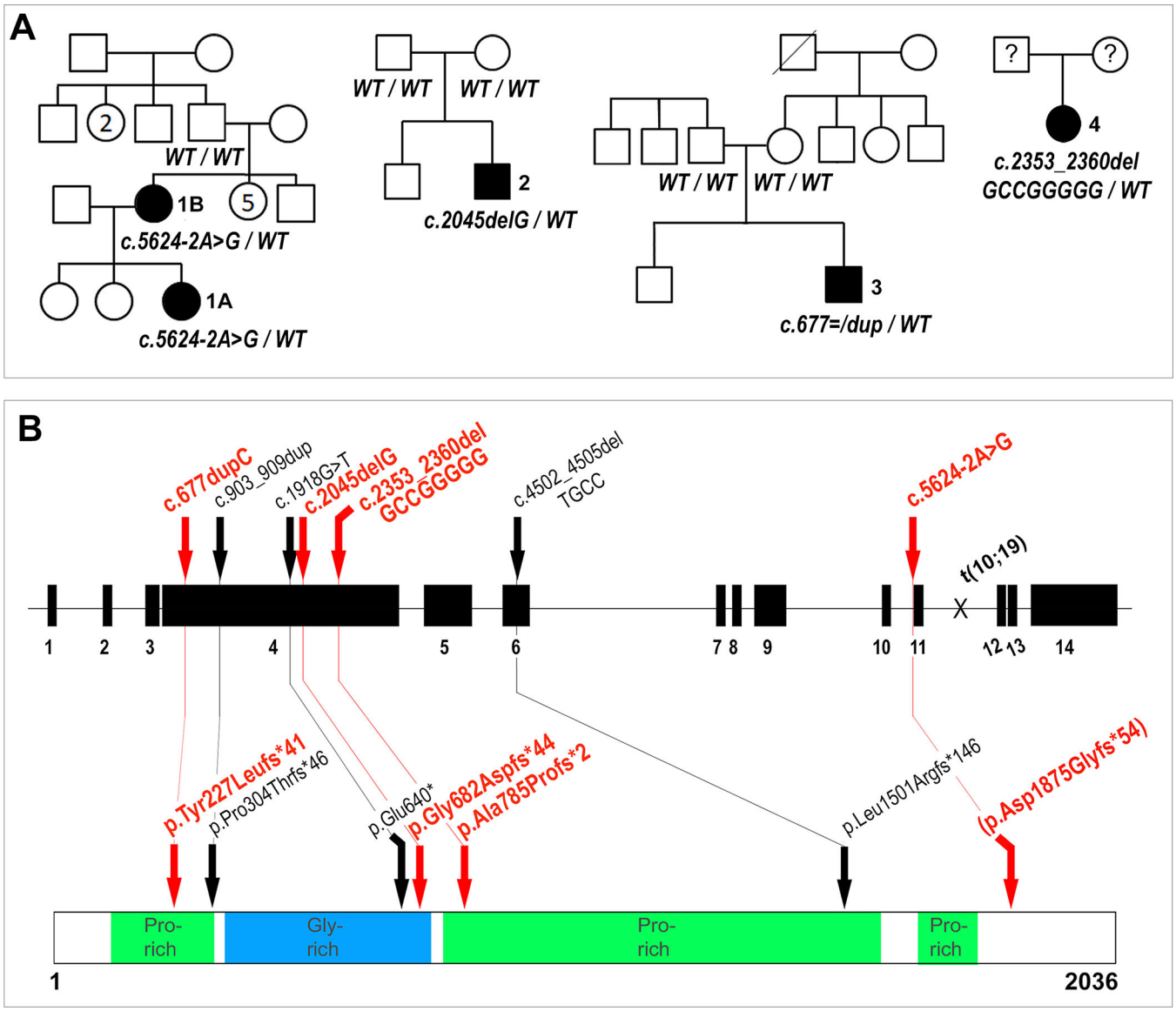

Figure 2: Pedigrees and *PRR12* variant details.

A. Pedigrees of Families 1–4 with *PRR12* genotyping information; Individuals 1A, 1B, 2, 3, 4 are noted on each pedigree and affected individuals are indicated with solid symbols. **B**. Schematic of *PRR12* showing new variants (red arrows) as well as previously reported alterations (black arrows for intragenic variants and X for translocation breakpoint). Genomic structure (exons 1–14, numbered) is displayed above with protein structure indicated below. Protein domains are denoted based on Uniprot Q9ULL5 (Pro-rich 161 – 306; Gly-rich 335 – 685; Pro-rich 764 – 1545; Pro-rich 1666 – 1737).

Table 1:

Summary of novel and previously reported pathogenic *PRR12* variants.

ID	Age; ancestry	DNA change[a]	Predicted Effect	Exon	gnomAD	Inheritance	Eye	Neuro	Other
Individual 1A	31y; White	c.5624–2A>G	splicing defect; (p.Asp1875Glyfs*54)	11	NP	NP in father; mother N/A	L severe MI	-	Fragile hair
Individual 1B	7y; White	c.5624–2A>G	splicing defect; (p.Asp1875Glyfs*54)	11	NP	Inherited from 1A	L PA and MI; R hyperopia; B nystagmus	Mild DD/LD	-
Individual 2	13y; White	c.2045delG	p.Gly682Aspfs*44	4	NP	*de novo*	B iris coloboma, nystagmus and foveal hypoplasia; L PA, microcornea; R PFV, cataract, abnormal blood vessels in iris and cornea	-	-
Individual 3	16y; White	c.677dupC	p.Tyr227Leufs*41	4	NP	*de novo*	B PA, L MI, R glaucoma	Severe ID, moderate periventricular leukomalacia	Short stature, dysmorphic facial features, 4q35.1 del
Individual 4	UN; UN	c.2353_2360delGCCGGGGG	p.Ala785Profs*2	4	NP	unknown	U MI	Global delays	Short stature
Leduc Pt 1	4y; White	c.1918G>T	p.Glu640*	4	NP	*de novo*	L iris coloboma, Brushfield spots, stellate pattern, myopia, exotropia	Moderate DD, autism, anxiety	Pectus excavatum, dysmorphic facial features, brachydactyly, 2,3 toe syndactyly
Leduc Pt 2	8y; UN	c.4502_4505delTGCC	p.Leu1501Argfs*146	6	NP	*de novo*	Stellate iris pattern, exotropia	Mild to moderate ID, autism, ADHD	Dysmorphic facial features, L hearing loss
Leduc Pt 3	3y; UN	c.903_909dup	p.Pro304Thrfs*46	4	NP	*de novo*	B iris and lenticular coloboma, stellate iris, myopia, exotropia	Mild ID, ADHD, anxiety	Sacral dimple, pes planus, umbilical hernia, PFO, dysmorphic facial features
Córdova-Fletes	11y; UN	t(10;19); gene fusion	frameshift and truncation	intron 11	-	*de novo*	Myopia, strabismus	ID	Pes varus, long philtrum

[a]NM_020719.2; B bilateral; U unilateral; L left; R right; DD developmental delay; LD learning difficulties; ID intellectual disability; MI microphthalmia; N/A not available; NP not present; PA Peters Anomaly; PFV Persistent Fetal Vasculature; UN unknown; - none reported