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Exome sequencing in patients with microphthalmia, anophthalmia, and coloboma (MAC) from a consanguineous population

Farrah Islam[#1], **Stephanie Htun**[#2], **Li-Wen Lai**[3], **Max Krall**[2], **Menitha Poranki**[2], **Pierre-Marie Martin**[4], **Nara Sobreira**[5], **Elizabeth S. Wohler**[5], **Jingwei Yu**[6], **Anthony T. Moore**[7], **Anne M. Slavotinek**[2]

[1]Department of Ophthalmology, Al-Shifa Eye Trust Hospital, Rawalpindi, Pakistan [2]Division of Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, California [3]Department of Pathology, University of Arizona, Tucson, Arizona [4]Institute of Human Genetics, University of California San Francisco, San Francisco, California [5]McKusick-Nathans Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland [6]Department of Laboratory Medicine, University of California San Francisco, San Francisco, California [7]Dept. Ophthalmology, University of California San Francisco, San Francisco, California

[#] These authors contributed equally to this work.

Abstract

Next-generation sequencing strategies have resulted in mutation detection rates of 21% to 61% in small cohorts of patients with microphthalmia, anophthalmia and coloboma (MAC), but despite progress in identifying novel causative genes, many patients remain without a genetic diagnosis. We studied a cohort of 19 patients with MAC who were ascertained from a population with high rates of consanguinity. Using single nucleotide polymorphism (SNP) arrays and whole exome sequencing (WES), we identified one pathogenic variant in *TENM3* in a patient with cataracts in addition to MAC. We also detected novel variants of unknown significance in genes that have previously been associated with MAC, including *KIF26B, MICU1* and *CDON*, and identified variants in candidate genes for MAC from the Wnt signaling pathway, comprising *LRP6, WNT2B* and *IQGAP1*, but our findings do not prove causality. Plausible variants were not found for many of the cases, indicating that our current understanding of the pathogenesis of MAC, a highly heterogeneous group of ocular defects, remains incomplete.

Correspondence: Anne M. Slavotinek, Division of Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, CA 94143-2711, USA. anne.slavotinek@ucsf.edu.

CONFLICT OF INTEREST

The authors have no potential conflict of interest.

DATA ACCESSIBILITY

Exome data is available through the Baylor-Hopkins Center for Mendelian Genomics.

DATA AVAILABILITY STATEMENT

Data availability statement: Exome data is available through the Baylor-Hopkins Center for Mendelian Genomics

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Keywords

Anophthalmia; cataract; *CDON*; Coloboma; Microphthalmia; *TENM3*

1 | INTRODUCTION

Microphthalmia, anophthalmia and coloboma (MAC) are structural eye defects with high importance because of the lifelong medical and social implications of reduced vision. Both environmental and genetic factors have been implicated in the pathogenesis of MAC and there are at least 82 known causative genes.[1] MAC can arise due to defects in early eye development, including the induction, proliferation, migration and differentiation of ocular tissues.[2] Pathogenic genes for MAC can be subdivided into transcription factors (*SOX2, OTX2, PAX6, VSX2, PITX3, RAX, SIX6, PAX6, FOXE3, SALL2, ATHO7*), genes involved in the retinoic acid signaling pathway (*ALDH1A3, STRA6, RARB*), genes from the TGFß/BMP signaling pathway (*BMP4, BMP7, GDF6, GDF3*), and other genes with known or unknown functions that do not fit into these categories (*SHH, ABCB6, MAB21L2, C12orf57, TENM3/ODZ3, PXDN, YAP1, HMGB3, CRIM1*).[3] In order to obtain data regarding causative genes and variants for MAC and to identify novel genes and variants, we performed single nucleotide polymorphism (SNP) arrays and trio whole exome sequencing (WES) on 19 patients with MAC that were recruited from a consanguineous population of Pakistani ethnicity. We have previously been successful in identifying causative variants for structural eye defects in a population with high rates of consanguinity.[4]

2 | MATERIALS AND METHODS

Written, informed consent was obtained in Urdu using a protocol approved by the Committee for Human Research at the University of California, San Francisco (UCSF; protocol 15-17 275). Clinical information and ophthalmological examination findings were collected as part of standard clinic practice. SNP-based cytogenomic arrays were performed in 16 patients using the Illumina CytoSNP-850 K Platform with genome build hg19 to detect genome-wide genomic copy number changes (CNVs) and regions of homozygosity (ROH) and reported as for standard clinical practice by the UCSF Cytogenetics laboratory (Supplemental File 1). We used the software program “Firefly” together with the search terms “coloboma”, “microphthalmia” and “microcornea” to interrogate ROHs from the SNP arrays for causative genes. WES was performed by the Baylor Hopkins Center for Mendelian Genomics (BHCMG; see Supplemental File 1). We utilized the web-based, BHCMG PhenodB tool to examine the data for deleterious variants in known genes for MAC.[5] We also examined the .vcf files for variants using Opal Clinical (Fabric Genomics) and Moon Diploid and compared variants in patients with those in biological parents to search for causes of Mendelian genetic disease (de novo, homozygous, compound heterozygous and inherited heterozygous disease-causing variants). We used the guidelines published by the American College of Medical Genetics (ACMG)[6] to evaluate variants.

The ophthalmological findings and extraocular features of the patients are summarized in Table 1 and pedigrees from each family are shown in Figure S1. Of the 19 families, 15 were

consanguineous, with 12 sets of parents who were first cousins and one set of parents who were second cousins. All patients had ocular colobomas, with 15 patients having iris colobomas, 16 with chorioretinal colobomas, 12 with optic nerve colobomas, and one patient with a macular coloboma. Additional eye findings comprised microphthalmia (12 patients), microcornea (13 patients), retinal dystrophy (8 patients), cataract (4 patients), vitreous syneresis (3 patients), and single patients each had lens subluxation or retinal detachment. Extraocular findings were rare, but single patients were diagnosed with hearing loss, anterior glottic web and hearing loss, nephrotic syndrome, cleft lip, or global developmental delays (Table 1).

SNP array results on 16 patients confirmed parental consanguinity, with ROHs ranging from 16 megabases (Mb) to 503 Mb and coefficients of inbreeding of 1/64 to 11/64 (Table 2). All families underwent WES and variants in genes related to MAC have been listed in Table 3.

3 | RESULTS

Patient EG16_1, with bilateral iris and chorioretinal colobomas, microphthalmia and cataracts, had a large ROH on chromosome 4q [4q34.1q35.2(175936490_187,257 576)×2 hmz]. WES identified the homozygous variant c.1558C>T:p.(Arg520*) in *TENM3*, a gene contained within this ROH. Given the association between biallelic loss of function variants in *TENM3* and MAC,[7–10] the variant was considered to be pathogenic. The presence of cataracts potentially expands the ocular phenotype associated with loss of function variants in *TENM3*, as cataracts have not previously been described. We also identified a variant with a classification of likely pathogenic in *POLR2A* (Table 3), but this gene is not known to be associated with MAC.[11]

We found several variants of unknown significance (VUSs) in these patients. In EG38_1, with unilateral iris and chorioretinal colobomas, a homozygous missense variant, c.2285G>A:p.(Arg762Gln) was detected in *KIF26B.* A heterozygous, 22 bp deletion affecting exon 12 of *KIF26B* was previously described in a patient with renal coloboma syndrome who had mild, bilateral optic nerve colobomas and underwent renal transplant.[12] In EG40_1 with unilateral macular coloboma, microphthalmia and microcornea, cataract and lens subluxation, homozygosity for c.886T>G:p.(Phe296Val) was identified in *MICU1*, a gene previously associated with cataracts, but only in one patient with a partial, homozygous gene deletion.[13] In patient EG37_1, with coloboma of the iris, chorioretinal structures and optic nerve, microphthalmia, cataract and retinal dystrophy, a homozygous variant, c.863A>G:p.(Tyr288Cys), was identified in *CDON.* A homozygous, truncating variant, c.622C>T:p.(Arg208Ter), was described in *CDON* in a female with bilateral optic nerve colobomas, unilateral retinal coloboma, growth and developmental delays, hypotonia and facial anomalies.[14] The reported patient also had a homozygous missense variant in *MAPRE*, c.344G>A:p.(Arg115Gln), but *MAPRE* is not associated with MAC.[14] Heterozygous, missense variants in *CDON* have been reported in patients with holoprosencephaly spectrum and pituitary stalk interruption syndrome, but only congenital convergent strabismus was reported in these patients.[15] Cdon is a multifunctional, cell surface protein of the immunoglobulin superfamily that is expressed in the dorsal eye during early ocular development of the mouse and functions as a co-receptor for Shh in early

forebrain development.[16–17] Mice lacking *Cdon* display coloboma, failure to form a proper boundary between the retinal pigmented epithelium and optic stalk, defective lens formation, failure of the lens to separate from the surface ectoderm, and microphthalmia.[16–17] In cardiomyocytes from *Cdon* homozygous null mice, hyperactivity of Wnt signaling has been observed[18] and this mechanism may also be relevant to the eye defects observed with bialleic variants in this gene. This patient has a broader spectrum of eye defects and is the third to be reported with retinal coloboma in association with biallelic variants in *CDON*, as whilst this paper was in review, two patients with ocular colobomas involving the iris, retina and choroid who were compound heterozygotes for c.928 +1G>A and c.2650 +1G>T in this gene were published.[19]

We noted several variants in genes that are members of the Wnt signaling pathway or putatively involved in Wnt signaling. Patient EG22_1 with colobomas of the iris, chorioretinal structures and optic nerve, microphthalmia, microcornea and retinal dystrophy had a heterozygous missense variant, c.802C>T:p.(Arg268Cys), in *WNT2B.* This gene was expressed in the anterior epithelium of the lens, anterior rim of the optic vesicle and in the retinal pigment epithelium in animal studies and inhibits differentiation of the progenitor cells in the marginal retina by downregulating the expression of proneural genes.[20–22] The gene is an excellent candidate for structural eye defects and the variant had a CADD score of 33, but heterozygosity for this *WNT2B* variant was present in 10/251396 individuals in the gnomAD database. In EG28_1 with unilateral chorioretinal coloboma and coloboma of the optic nerve, we detected heterozygosity for c.1031T>C:p. (Leu344Ser) in *LRP6*, a member of the low-density lipoprotein receptor family. Murine embryos that were homozygous for a *Lrp6* loss-of-function allele displayed microphthalmia and colobomas involving the retina and the optic nerve that varied in laterality and severity, but were fully penetrant.[23–24] Patient EG21_1 has colobomas of the iris, chorioretina, and optic nerve and was homozygous for c.2524A>C:p. (Ile842Leu) in *IQGAP1.* This gene encodes a multidomain, scaffolding protein that interacts with numerous signaling molecules, including calmodulin, MAPK, PI3K, AKT, and forkhead box protein O1.[25] IQGAP1 interacts with β-catenin in the Wnt signaling pathway in the retinal pigment epithelium,[26] but there is no data on eye defects in association with this gene in humans or animal models.

We also detected several variants in genes associated with MAC, but that we could not conclude were pathogenic because of zygosity or inheritance from a reportedly unaffected parent. These variants included a heterozygous variant, c.2675G>A:p.(Arg892Gln), in *DHX37*, a gene recently associated with chorioretinal lacunae and optic nerve coloboma,[27] c.4268A>G:p.(His1423Arg) in *MYO10* in patient with iris and chorioretinal colobomas, microcornea and unilateral retinal dystrophy and a heterozygous, missense variant in *RERE*, c.4087C>T:p.(Pro1363Ser), that was paternally inherited.

Only one CNV, a heterozygous, 47.6 kilobase deletion at chromosome 6q21 containing exons 2 to 10 of the *FIG4* gene was considered likely to be pathogenic by the reporting laboratory. FIG4 is a lipid phosphatase and although biallelic, deleterious variants in this gene are associated with Yunis-Varon syndrome,[28] haploinsufficiency for *FIG4* is not known to cause MAC. We therefore consider that this deletion is of unknown significance. No further variants in this gene were found with WES.

Our yield of pathogenic variants (1/19; 5%) was much lower than two recent studies employing WES in patients with anophthalmia and microphthalmia (60%)[29] and in microphthalmia and posterior microphthalmia (61%).[1] However, low rates of diagnosis have previously been reported in patients with MAC who underwent exome sequencing (11%).[30] Other factors that may have influenced variant detection rate in our work include incomplete phenotypic information and a lack of direct patient contact after sequencing, which hindered our ability to re-phenotype the patients and to perform further clinical investigations to evaluate candidate variants. Despite the low yield, this study has identified plausible candidate variants and genes for MAC that can be further evaluated with entry into gene matchmaking sites and functional research.

In summary, we studied 19 patients with MAC from a population with high rates of consanguineous unions to increase the probability of finding causative, autosomal recessive variants. We identified a pathogenic variant in *TENM3* and identified VUSs in genes that have previously been associated with structural eye defects, including *KIF26B, MICU1* and *CDON.* We also identified candidate variants in *WNT2B, LRP6* and *IQGAP1*, genes that are related to the Wnt signaling pathway, that require further investigation. However, the lack of pathogenic variants in many patients emphasizes the high heterogeneity of MAC and suggests that previously unknown variants or novel genes will be relevant to the etiology of eye defects in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Patel N, Khan AO, Alsahli S, et al. Genetic investigation of 93 families with microphthalmia or posterior microphthalmos. Clin Genet. 2018;93:1210–1222. [PubMed: 29450879]
2. Graw J Eye development. Curr Top Dev Biol. 2010;90:343–386. [PubMed: 20691855]
3. Slavotinek A Genetics of anophthalmia and microphthalmia. Part 2: syndromes associated with anophthalmia-microphthalmia. Hum Genet. 2019;138:831–846. [PubMed: 30374660]
4. Ullah E, Nadeem Saqib MA, Sajid S, et al. Genetic analysis of consanguineous families presenting with congenital ocular defects. Exp Eye Res. 2016;146:163–171. [PubMed: 26995144]
5. Sobreira N, Schiettecatte F, Boehm C, Valle D, Hamosh A. New tools for Mendelian disease gene identification: PhenoDB variant analysis module; and GeneMatcher, a web-based tool for linking investigators with an interest in the same gene. Hum Mutat. 2015;36:425–431. [PubMed: 25684268]
6. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and

Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424. [PubMed: 25741868]

7. Aldahmesh MA, Mohammed JY, Al-Hazzaa S, et al. Homozygous null mutation in ODZ3 causes microphthalmia in humans. Genet Med 2012;14:900–904. [PubMed: 22766609]
8. Williamson KA, FitzPatrick DR. The genetic architecture of microphthalmia, anophthalmia and coloboma. Eur J Med Genet. 2014;57: 369–380. [PubMed: 24859618]
9. Chassaing N, Ragge N, Plaisancié J, et al. Confirmation of TENM3 involvement in autosomal recessive colobomatous microphthalmia. Am J Med Genet A. 2016;170:1895–1898. [PubMed: 27103084]
10. Singh B, Srivastava P, Phadke SR. Sequence variations in TENM3 gene causing eye anomalies with intellectual disability: expanding the phenotypic spectrum. Eur J Med Genet. 2019;62:61–64. [PubMed: 29753094]
11. Haijes HA, Koster MJE, Rehmann H, et al. De novo heterozygous POLR2A variants cause a neurodevelopmental syndrome with profound infantile-onset Hypotonia. Am J Hum Genet. 2019;105:283–301. [PubMed: 31353023]
12. Okumura T, Furuichi K, Higashide T, et al. Association of PAX2 and other gene mutations with the clinical manifestations of renal coloboma syndrome. PLoS One. 2015;10:e0142843. [PubMed: 26571382]
13. Lewis-Smith D, Kamer KJ, Griffin H, et al. Homozygous deletion in MICU1 presenting with fatigue and lethargy in childhood. Neurol Genet. 2016;2:e59. [PubMed: 27123478]
14. Berkun L, Slae M, Mor-Shaked H, Koplewitz B, Eventov-Friedman S, Harel T. Homozygous variants in MAPRE2 and CDON in individual with skin folds, growth delay, retinal coloboma, and pyloric stenosis. Am J Med Genet A. 2019;179:2454–2458. [PubMed: 31502381]
15. Bashamboo A, Bignon-Topalovic J, Rouba H, McElreavey K, Brauner R. A nonsense mutation in the hedgehog receptor CDON associated with pituitary stalk interruption syndrome. J Clin Endocrinol Metab. 2016;101:12–15. [PubMed: 26529631]
16. Zhang W, Mulieri PJ, Gaio U, Bae GU, Krauss RS, Kang JS. Ocular abnormalities in mice lacking the immunoglobulin superfamily member Cdo. FEBS J. 2009;276:5998–6010. [PubMed: 19754878]
17. Cardozo MJ, Sánchez-Arrones L, Sandonis A, et al. Cdon acts as a hedgehog decoy receptor during proximal-distal patterning of the optic vesicle. Nat Commun. 2014;5:4272. [PubMed: 25001599]
18. Jeong MH, Kim HJ, Pyun JH, et al. Cdon deficiency causes cardiac remodeling through hyperactivation of WNT/ β-catenin signaling. Proc Natl Acad Sci U S A. 2017;114:E1345–E1354. [PubMed: 28154134]
19. Reis LM, Basel D, McCarrier J, et al. Compound heterozygous splicing CDON variants result in isolated ocular coloboma. Clin Genet. 2020;98:486–492. [PubMed: 32729136]
20. Kubo F, Takeichi M, Nakagawa S. Wnt2b inhibits differentiation of retinal progenitor cells in the absence of notch activity by downregulating the expression of proneural genes. Development. 2005;132:2759–2770. [PubMed: 15901663]
21. Fokina VM, Frolova EI. Expression patterns of wnt genes during developmemnt of an anterior part of the chicken eye. Dev Dyn. 2006; 235:496–505. [PubMed: 16258938]
22. Iwai-Takekoshi L, Balasubramanian R, Sitko A, et al. Activation of Wnt signaling reduces ipsilaterally projecting retinal ganglion cells in pigmented retina. Development. 2018;145(21):163212.
23. Zhou CJ, Molotkov A, Song L, et al. Ocular coloboma and dorsoventral neuroretinal patterning defects in Lrp6 mutant eyes. Dev Dyn. 2008;237:3681–3689. [PubMed: 18985738]
24. Zhou CJ, Wang YZ, Yamagami T, Zhao T, Song L, Wang K. Generation of Lrp6 conditional gene-targeting mouse line for modeling and dissecting multiple birth defects/congenital anomalies. Dev Dyn. 2010;239:318–326. [PubMed: 19653321]
25. Erickson HL, Anakk S. Identification of IQ motif-containing GTPase-activating protein 1 as a regulator of long-term ketosis. JCI Insight. 2018;3:e99866.

26. Wang H, Han X, Bretz CA, et al. Retinal pigment epithelial cell expression of active Rap1a by scAAV2 inhibits choroidal neovascularization. Mol Ther Methods Clin Dev. 2016;3:16056. [PubMed: 27606349]

27. Paine I, Posey JE, Grochowski CM, et al. Paralog studies augment gene discovery: DDX and DHX genes. Am J Hum Genet. 2019;105:302–316. [PubMed: 31256877]

28. Campeau PM, Lenk GM, Lu JT, et al. Yunis-Varón syndrome is caused by mutations in FIG4, encoding a phosphoinositide phosphatase. Am J Hum Genet. 2013;92:781–791. [PubMed: 23623387]

29. Matías-Pérez D, García-Montaño LA, Cruz-Aguilar M, et al. Identification of novel pathogenic variants and novel gene-phenotype correlations in Mexican subjects with microphthalmia and/or anophthalmia by next-generation sequencing. J Hum Genet. 2018;63:1169–1180. [PubMed: 30181649]

30. Deml B, Reis LM, Maheshwari M, Griffis C, Bick D, Semina EV. Whole exome analysis identifies dominant COL4A1 mutations in patients with complex ocular phenotypes involving microphthalmia. Clin Genet. 2014;86:475–481. [PubMed: 24628545]

TABLE 1

Ocular findings in 19 patients with structural eye defects, together with exome results

Patient	Sex	Consanguinity	Eye phenotype: coloboma	Eye phenotype: globe	Eye phenotype: other	Extraocular findings	Gene variants discussed in text
EG14_1	M	No	Iris; Chorioretina	L microphthalmia; microcornea	L retinal detachment; L cataract	Nephrotic syndrome	
EG15_1	F	Yes; degree unspecified	Chorioretina; Optic nerve	R microphthalmia; microcornea	Retinal dystrophy; L syneresis; cataract; microspherophakia	Hearing loss	
EG16_1	M	first cousins	Iris; Chorioretina	L microphthalmia	Cataract; syneresis	–	*TENM3* c.886T>G:p.(Phe296Val)
EG17_1	M	first cousins	Chorioretina; Optic nerve	R microphthalmia; microcornea	–	–	
EG18_1	F	first cousins	Iris; Chorioretina; Optic disc	R microphthalmia; microcornea	–	Global delays	
EG20_1	M	first cousins	Iris; Chorioretina; Optic nerve	Microphthalmia; Microcornea	R retinal dystrophy	–	
EG21_1	F	first cousins	Iris; Chorioretina; Optic nerve	–	–	–	*IQGAP1* c.2524A>C:p.(Ile842Leu)
EG22_1	F	first cousins	Iris; Chorioretina; Optic nerve	L microphthalmia; microcornea	R retinal dystrophy	–	*WNT2B* c.802C>T:p.(Arg268Cys)
EG25_1	F	No	Iris; Optic nerve	Microcornea	Retinal dystrophy	Hearing loss; Anterior glottic web	*POLR2A* c.2887C>T: p.(Arg963Trp)
EG26_1	M	Yes; degree unspecified	Iris; Chorioretina; Optic nerve	–	–	–	
EG28_1	M	No	Chorioretina; Optic nerve	–	–	–	*LRP6* c.1031T>C:p.(Leu344Ser)
EG29_1	F	first cousins	Iris; Chorioretina; Optic disc	Microcornea	R retinal dystrophy	–	
EG37_1	M	second cousins	Iris; Chorioretina; Optic nerve	Microphthalmia; microcornea	Retinal dystrophy; R syneresis; Cataract	–	*CDON* c.863A>G:p.(Tyr288Cys)
EG38_1	F	first cousins	Iris; Chorioretina	–	–	–	*KIF26B* c.2285G>A: p.(Arg762Gln)
EG40_1	M	first cousins	Chorioretina	R microphthalmia; microcornea	Cataract; lens subluxation; microspherophakia	–	*MICU1* c.886T>G:p.(Phe296Val)
EG41_1	M	first cousins	Chorioretina; Optic disc	L microphthalmia; icrocornea	R retinal dystrophy	–	
EG42_1	F	first cousins	Iris; Chorioretina; Optic nerve	L microphthalmia; microcornea	–	–	
EG46_1	M	No	Iris	L microphthalmia	R retinal dystrophy		

Patient	Sex	Consanguinity	Eye phenotype: coloboma	Eye phenotype: globe	Eye phenotype: other	Extraocular findings	Gene variants discussed in text
EG47_1	F	first cousins	Iris; Chorioretina; Optic nerve	–	–	Cleft lip	

TABLE 2

Single nucleotide polymorphism (SNP) array results in 16 patients with microphthalmia, anophthalmia and coloboma (MAC)

Patient	Consan- guinity	Coefficient of in-breeding (F)	ROH (Mb)	Copy number variants GRCh37/hg19	CNV size	Interpretation by testing laboratory	Protein-coding genes (OMIM)
EG15_1	Yes; degree unspecified	–	16	–	–	–	–
EG16_1	first cousins	3/32	288	6q21(110023322_110070936)×1	47.6 kb	Likely pathogenic	*FIG4* exons 2–10
EG17_1	first cousins	11/64	503	–	–	–	–
EG18_1	first cousins	1/64	64	Xq21.1(80855919_81855868)×3	1 Mb	Likely benign	–
EG20_1	first cousins	1/32	112	–	–	–	–
EG21_1	first cousins	1/16	166	3p12.2(82316849_83126828)×3	0.81 Mb	Likely benign	–
EG22_1	first cousins	3/32	255	–	–	–	–
EG25_1	No	–	–	6q26 (162696517_162792379)×1/17p11.2p11.1 (21470731_22213908)×3	96 kb/0.743 Mb	VUS/ VUS, Likely benign	–
EG26_1	Yes; degree unspecified	3/32	263	11p15. (4309990_4712353)×1	0.402 Mb	Likely benign	*TRIM21, TRIM6, OR51E1, R51E2*
EG29_1	first cousins	1/32	94	Xq21.31q21.32(91546790_92620821)×3	1.074 Mb	VUS	*PCDH11X*
EG37_1	second cousins	1/64	58	–	–	–	–
EG38_1	first cousins	1/32	111	–	–	–	–
EG40_1	first cousins	1/16	178	–	–	–	–
EG41_1	first cousins	1/32	112	–	–	–	–
EG42_1	first cousins	3/32	271	2q14.3(122777669_123102720) ×1/9q11(27801814_28492962)×3	0.325 Mb/0.691 Mb	VUS, Likely benign/VUS, Likely benign	–
EG47_1	first cousins	1/32	103	–	–	–	–

Abbreviations: CNV, copy number variant; kb, kilobases; Mb, megabases; OMIM, Online Mendelian Inheritance in Man; ROH, regions of homozygosity; VUS, variant of unknown significance.

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TABLE 3

Variants in genes associated with microphthalmia, anophthalmia and coloboma (MAC)

Sample	Gene name	Human genome variation society (HGVS) nomenclature	Zygosity	gnomAD minor allele frequency	Polyphen-2	Mutation Taster	CADD score[a]	Conservation	ACMG Classification[6]
	pathogenic/likely pathogenic variants								
EG16_1	*TENM3* chr4:183601421C>T	NM_001080477.4: c.1558C>T: p.(Arg520*)	Homozygous; biparental	Absent	–	DC[b]; 1.0	38	–	**P**[c] (PVS1, PM2, PP3)
EG25_1	*POLR2A* chr17:7406570C>T	NM_00937.4: c.2887C>T: p.(Arg963Trp)	Heterozygous; de novo	Absent	Prob. D[d]; 1.0	DC; 0.999	27.7	Pt[e]/ Mmus[f]/Dr[g]/Dm[h]/Ce[i]/Xt[j]	**LP**[k] (PM2; PS2, PP3)
	Variants of unknown significance								
EG22_1	*WNT2B* chr1:113059863C>T	NM_024494.3: c.802C>T: p.(Arg268Cys)	Heterozygous; maternal	10/251396 No Hz[l]	Prob. D; 0.999	DC; 0.999	33	Pt/Mm[m]/Fc[n]/Mmus	**VUS**[o] (PP3)
EG41_1	*DHX37* chr12:125438446C>T	NM_032656.3: c.2675G>A: p.(Arg892Gln)	Heterozygous; paternal	5/241454 No HZ	Prob. D; 1.0	DC; 0.999	32	Mm/Fc/ Mmus/Gg[p]/Tr[q]/Dr/Dm/Ce	**VUS** (PP3)
EG38_1	*KIF26B* chr1:245847561G>A	NM_018012:c.2285G>A: p.(Arg762Gln)	Homozygous; biparental	5/248968 No HZ[c]	Prob. D[d]; 0.976	DC; 0.999	31	Pt[e]/ Mmus[f]/Gg[g]/Tr[h]/Dr[i]/ CeVXt[k]	**VUS** (PM2, PP3)
EG40_1	*MICU1* chr10:74234905A>C	NM_001195518.2: c.886 T>G: p.(Phe296Val)	Homozygous; biparental	Absent	Prob. D; 1.0	DC; 0.999	29.5	Pt/Mm[l]/Fc[m]/ Mmus/Gg/Tr/Dr/Dm[n]/Ce/Xt	**VUS** (PM2, PP3)
EG41_1	*ADAMTS9* chr3:64536590C>A	NM_182920.1: c.4847G>T: p.(Trp1616Leu)	Heterozygous; maternal	2/251360 No HZ	Prob. D; 0.989	DC; 0.999	29.5	Pt/Mm/ Mmus/Gg/Dr/Ce/Xt	**VUS** (PP3)
EG28_1	*LRP6* chr12:12334319G>A	NM_002336.3: c.1031 T>C: p.(Leu344Ser)	Heterozygous; paternal	Absent	Prob. D; 1.0	DC; 1.0	28.8	Pt/Mm/ Mmus/Gg/Dr/Dm/Xt	**VUS** (PM2, PP3)
EG25_1	*WFS1* chr4:6303728G>A	NM_00114853: c.2206G>A: p.(Gly736Ser)	Heterozygous; paternal	8/245860 No HZ	Prob. D; 1.0	DC; 0.999	28.7	Mm/Fc/ Mmus/Dr/Dm/Xt	**VUS** (PP3)
EG15_1	*RERE* chr1:8418508G>A	NM_012102.3: c.4087C>T: p.(Pro1363Ser)	Heterozygous; paternal	Absent	Prob. D; 0.969	DC; 1.0	26.2	Pt/Mm/Fc/ Mmus/Gg/Dr/Xt	**VUS** (PM2, PP3)
EG37_1	*CDON* chr11:125887048 T>C	NM_016952: c.863A>G: p.(Tyr288Cys)	Homozygous; biparental	1/251320 No HZ	Prob. D; 1.0	DC; 0.999	25.9	Pt/Mm/Fc/ Mmus/Dr/Dm	**VUS** (PP3, PP4)

Sample	Gene name	Human genome variation society (HGVS) nomenclature	Zygosity	gnomAD minor allele frequency	Polyphen-2	Mutation Taster	CADD score[a]	Conservation	ACMG Classification[6]
EG21_1	*IQGAP1* chr15:91017314A>C	NM_0038704: c.2524A>C: p.(Ile842Leu)	Homozygous; biparental	3/250952 No HZ	Poss. D[r]; 0.924	DC; 0.999	25.4	Pt/Mm/Fc/ Mmus/Gg/Tr/Dr/Ce/Xt	**VUS** (PP3)
EG42_1	*WDR37* chr10:1118109G>A	NM_014023.3: c.14G>A: p.(Ser5Asn)	Homozygous; biparental	2/251418 No HZ	Benign; 0.004	DC; 0.999	23.4	Pt/Mm/Fc/ Mmus/Gg/Dr/Xt	**VUS** (PP3)
EG17_1	*CASK* chr23:41446185C>T	NM_001126054: c.1289G>A: p.(Arg430His)	Hemizygous; maternal	47/203108 No HZ	–	DC; 0.999	23.4	Pt/Mm/Fc/ Mmus/Gg/Tr/Dr/Dm/ Xt	**VUS** (PP3)
EG14_1	*COL4A1* chr13:110835413C>A	NM_001845.4: c.2022G>T: p.(Arg674Ser)	Hemizygous; Maternal	2/246948 No HZ	Prob. D; 0.990	DC; 0.999	22.6	Pt/Mm/Fc/ Mmus/Gg/Xt	**VUS** (PP3)
EG14_1	*FAT1* chr4:187540896C>A	NM_005245.4: c.6844G>A: p. (Val2282Met)	Heterozygous; paternal	Absent	Benign; 0.245	DC; 0.999	22.3	Pt/Mm/ Mmus/Gg/Tr/Dr/Xt	**VUS** (PM2)
EG29_1	*MYO10* chr5:16681534 T>C	NM_012334.3: c.4268A>G: p. (His1423Arg)	Heterozygous; paternal	Absent	Benign; 0.044	DC; 1.0	21.2	Pt/Mm/ Mmus/Gg/Tr/Dr/Xt	**VUS** (PM2)
EG42_1	*POMT1* chr9:134396833G>A	NM_007171.3: c.1865G>A: p.(Arg622Gln)	Hemizygous; maternal	9/282808 No HZ	Poss D; 0.612	DC; 0.999	21.1	Pt/Mm/ Mmus/Gg/Tr/Dr/Xt	**VUS** (PP3)
EG42_1	*POMT1* chr9:134385176G>T	NM_007171.3:c.586G>T: p.(Ala196Ser)	Hemizygous; paternal	Absent	Benign; 0.094	PM[s]; 0.999	20.2	Pt/Mm/Gg	**VUS** (PM2)
EG46_1	*GDF6* chr8:97157179G>T	NM_001001557.2: c.980C>A: p.(Pro327His)	Heterozygous; paternal	77/149532 No HZ	Benign; 1.0	DC: 0.057	16.8	Pt/Mm/Fc/Mmus	**VUS** (PP5)
EG28_1	*KDM6A* chr23:44941845_ 44941847delCAT	ENST00000382899.4 c.3190_3192delCAT: p. (His1064del)	Hemizygous; maternal	–	–	DC; 0.999	–	Pt/Mm/Fc/Mmus/Gg	**VUS** (PM4; PM2)

[a] Genes are ordered from highest to lowest CADD scores in each category.

[b] DC, disease-causing.

[c] P, pathogenic; abbreviations from reference 6.

[d] Prob. D, probably damaging.

[e] Pt, *Pan troglodytes.*

[f] Mmus, *Mus* musculus.

[g] Dr, *Danio rerio.*

[h] Dm, *Drosophila melanogaster.*

[i] Ce, *Caenorhabditis elegans.*

[j] Xt, *Xenopus tropicalis.*

[k] LP, likely pathogenic.

[l] HZ, homozygous.

[m] Mm, *Macaca mulatta.*

[n] Fc, *Felis catus.*

[o] VUS, variant of unknown significance.

[p] Gg, *Gallus gallus.*

[q] Tr, *Takifugu rubripes.*

[r] Poss. D, possibly damaging.

[s] PM, polymorphism.