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Review

# Animal and cellular models of microphthalmia

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***Abstract***

Microphthalmia is a rare developmental eye disorder affecting 1 in 7000 births. It is defined as a small (axial length ⩾2 standard deviations below the age-adjusted mean) underdeveloped eye, caused by disruption of ocular development through genetic or environmental factors in the first trimester of pregnancy. Clinical phenotypic heterogeneity exists amongst patients with varying levels of severity, and associated ocular and systemic features. Up to 11% of blind children are reported to have microphthalmia, yet currently no treatments are available. By identifying the aetiology of microphthalmia and understanding how the mechanisms of eye development are disrupted, we can gain a better understanding of the pathogenesis. Animal models, mainly mouse, zebrafish and *Xenopus*, have provided extensive information on the genetic regulation of oculogenesis, and how perturbation of these pathways leads to microphthalmia. However, differences exist between species, hence cellular models, such as patient-derived induced pluripotent stem cell (iPSC) optic vesicles, are now being used to provide greater insights into the human disease process. Progress in 3D cellular modelling techniques has enhanced the ability of researchers to study interactions of different cell types during eye development. Through improved molecular knowledge of microphthalmia, preventative or postnatal therapies may be developed, together with establishing genotype–phenotype correlations in order to provide patients with the appropriate prognosis, multidisciplinary care and informed genetic counselling. This review summarises some key discoveries from animal and cellular models of microphthalmia and discusses how innovative new models can be used to further our understanding in the future.

## Plain language summary

### Animal and Cellular Models of the Eye Disorder, Microphthalmia (Small Eye)

Microphthalmia, meaning a small, underdeveloped eye, is a rare disorder that children are born with. Genetic changes or variations in the environment during the first 3 months of pregnancy can disrupt early development of the eye, resulting in microphthalmia. Up to 11% of blind children have microphthalmia, yet currently no treatments are available. By understanding the genes necessary for eye development, we can determine how disruption by genetic changes or environmental factors can cause this condition. This helps us understand why microphthalmia occurs, and ensure patients are provided with the appropriate clinical care and genetic counselling advice. Additionally, by understanding the causes of microphthalmia, researchers can develop treatments to prevent or reduce the severity of this condition. Animal models, particularly mice, zebrafish and frogs, which can also develop small eyes due to the same genetic/environmental changes, have helped us understand the genes which are important for eye development and can cause birth eye defects when disrupted. Studying a patient's own cells grown in the laboratory can further help researchers understand how changes

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in genes affect their function. Both animal and cellular models can be used to develop and test new drugs, which could provide treatment options for patients living with microphthalmia. This review summarises the key discoveries from animal and cellular models of microphthalmia and discusses how innovative new models can be used to further our understanding in the future.

***Keywords:*** cells, development, eye, human, iPSC, microphthalmia, mouse, optic vesicles, organoids, *Xenopus*, zebrafish

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## Introduction

Microphthalmia describes a small underdeveloped eye and is defined as having a total axial length of <19 mm at 1 year of age or <21 mm in an adult measured on B-scan ultrasound, determined as being ⩾2 standard deviations below the age-adjusted mean.[1] It is a rare condition, with an estimated prevalence of 1 in 7000 live births,[2] resulting from disrupted eye development between 4–8 weeks gestation either due to genetic or environmental factors.[1,3–5] Currently no preventative or restorative treatments exist to improve vision.

Prospective UK incidence studies have indicated that environmental causes, such as maternal vitamin A deficiency or alcohol consumption, contribute to approximately 2% of microphthalmia cases.[2,3,6–8] There are over 90 identified monogenic causes, as well as large chromosomal aberrations.[2,3] The most common mutations associated with microphthalmia are in transcription factors that control correct gene expression during early eye development, such as *SOX2* and *OTX2* which account for 60% of severe bilateral microphthalmia,[9] along with *RAX*, *VSX2* and *PAX6*.[2,3] These transcription factors regulate signalling pathways (e.g. WNT, BMP, TGFβ and SHH) which stimulate morphogenic movements and specialisation of cells within the developing eye. Retinoic acid signalling is vital for early eye morphogenesis, and functional variants in this pathway frequently cause microphthalmia, including *STRA6*, *ALDH1A3*, *RAR*β and *RBP4*.[2,3] Inheritance patterns comprise autosomal dominant, recessive and X-linked, although germline mosaicism has been reported for multiple microphthalmia-associated variants, making deciphering inheritance patterns and providing appropriate genetic counselling challenging.[3,10–14] Most pathogenic mutations associated with non-syndromic cases arise *de novo* sporadically, and include missense, nonsense, frameshift and splice-site variants.[2,3,15] A molecular diagnosis can be obtained in approximately 70% of severe bilateral microphthalmia cases, but less than 10% of unilateral cases, which consists of the majority of patients.[2,3,16] This discrepancy may be the result of *de novo* mutations, mosaicism and haploinsufficiency in unilateral patients, or due to genetic/epigenetic modifiers, but has not been thoroughly investigated.[17–19]

Heterogeneity in clinical phenotype is observed amongst patients. Microphthalmia can manifest as an isolated condition with a continuum of severity and laterality, often in association with anophthalmia (in the contralateral eye) or ocular coloboma (in the same or contralateral eye), which are considered part of the same spectrum of ocular disorders (collectively known as MAC). An affected eye can display other ocular features (complex) such as cataract, anterior segment dysgenesis or retinal dystrophy, and 33–95% of patients exhibit systemic features (syndromic) (detailed by gene in Harding and Moosajee 2019).[20–22] Variable expressivity and non-penetrance have also been observed in microphthalmia probands.[3,12,23] The severity of microphthalmia on visual function depends on the stage in which eye development was disrupted, and so the degree to which ocular structure and cellular function is perturbed, as well as associated ocular malformations present.

Identification of causal microphthalmic genes and the pathways disrupted in eye development

will provide insight into pathogenesis as well as potential therapeutic targets to treat infants born with microphthalmia, through ocular delivery of drug compounds to stimulate postnatal growth and development.[3] Furthermore, uncovering environmental factors and genetic modifiers is key to begin understanding variable penetrance.[24,25] By recognising the effects of specific variants on clinical phenotype, we can establish genotype–phenotype correlations, provide important prognostic indicators and allow assembly of the correct multidisciplinary team and for families to receive informed genetic counselling and access to family planning advice.

As microphthalmia arises within the first few weeks of gestation, it is difficult to study eye development in humans, both morphologically and molecularly. Consequently, much of our understanding of microphthalmia derives from animal and cellular models.[26–29] This review highlights the key disease models used to study microphthalmia, as well as the innovative technologies which will further our understanding and aid the generation of pioneering treatments.

## Eye development and microphthalmia

Eye formation begins relatively early in vertebrate embryogenesis, from 3 weeks gestation in humans, embryonic day 8 (E8.0) in mice, 12 h post fertilisation (hpf) in zebrafish and embryonic stage 12.5 in *Xenopus* (Table 1). The molecular mechanisms of early eye development and the pathways relating to microphthalmia are reviewed in detail by Harding and Moosajee.[3] Briefly, the eye is initially specified in the anterior neural plate through the upregulation of eye field transcription factors (EFTFs), including *RAX*, *PAX6* and *SIX3*, which form a self-regulating network of genes coordinating eye development.[30–33] The eye field then splits in two as the cells migrate anteriorly away from the midline of the neural plate, evaginating towards the surface ectoderm.[30–32] Concurrently, the lens develops from a pre-placodal region within the surface ectoderm, and signalling from the evaginating optic vesicle stimulates the thickening of the lens placode, which then reciprocally induces invagination of the optic vesicle to form a bilayered optic cup.[30,34] The outer layer of the optic cup becomes the retinal pigmented epithelium (RPE), while the inner layer forms the presumptive neural retina (NR), which later differentiates into the specialised cell types of the retina.[32,35] The opening along the inferior surface of the optic cup (the optic fissure), which allows the hyaloid vasculature to support eye development, closes by week 7 in humans (Table 1).[26]

## Animal models of microphthalmia

Mature eye structure is similar across vertebrate species (Figure 1), with light entering *via* the pupil through the transparent cornea and lens, which refracts the light through ciliary muscle movement, before reaching the stratified, light-sensing NR at the back of the eye (whose function is supported by the RPE) where specialised retinal cells detect light (photoreceptors) and convert it into electrical signals, which are transmitted to the brain *via* the optic nerve. Investigating disease progression and molecular pathways in models with known genetic causes can help understand disease mechanisms. Due to their conserved ocular development and physiology, numerous mouse, zebrafish and *Xenopus* lines with a microphthalmia phenotype have been generated, many of which have overlapping causal molecular changes to patients (Table 2, Figure 2, Table S1). Microphthalmia has been studied in other animal models, including chick[47–49] and drosophila;[50] however, this review will only explore findings from mouse, zebrafish and *Xenopus*, as these are the most common models utilised to explore the molecular basis of microphthalmia due to their ease of experimental manipulation together with conserved genetics (Table 3).

### *Mouse*

*Advantages of mouse models of ocular development.* Mice are the most common animal model for studying development and disease.[155,156] They are easy to manage in a laboratory environment, being small with a short generation time, and are relatively cost effective.[157] The mouse is the best characterised mammalian model system for hereditary disease, and 99% of its genome is conserved compared with humans.[25,158] Eye development between humans and mice is similar (Table 1), and the mature ocular structure resembles the human, albeit lacking the cone-rich macula, instead with few cone photoreceptor cells distributed evenly throughout the retina (Figure 1).[159] The size of the mouse eye permits morphological analysis without the need for advanced technical equipment.[25,38]

**Table 1.** Stages of early eye development in humans and animal models.

| Species | EFTF expression | Splitting of eye field | Optic vesicle evagination | Optic cup invagination | Closure of the optic fissure | References |
|---|---|---|---|---|---|---|
| Human | 22 days | 22 days | 27 days | 28–35 days | 35–49 days | Harding and Moosajee[3]; Richardson *et al.*[26]; Patel and Sowden[36] |
| Mouse | E8.0 | E8.5 | E9.0–E9.5 | E10–E12.5 | E11–E13 | Patel and Sowden[36]; Cvekl *et al.*[37]; Graw[38]; Reis and Semina[39] |
| Zebrafish | 12 hpf | 12–14 hpf | 14 hpf | 15–28 hpf | 48–56 hpf | Richardson *et al.*[26]; Deml *et al.*[40]; Chhetri *et al.*[41]; Kimmel *et al.*[42] |
| | 6–10 somite stage | 10–12 somite stage | 12 somite stage | 12–15 somite stage | Hatching period (long pec stage) | |
| *Xenopus* | Stage 12.5–15 | Stage 16–18 | Stage 18–26 | Stage 27–34 | Stage 38–46 | Zuber[27]; Zuber *et al.*[33]; Ledford *et al.*[43]; Holt[44]; Feldman[45]; Henry *et al.*[46] |

Days, days gestation; E, embryonic day; EFTF, eye field transcription factor; hpf, hours post fertilisation; stage, *Xenopus* Nieuwkoop and Faber developmental stage.

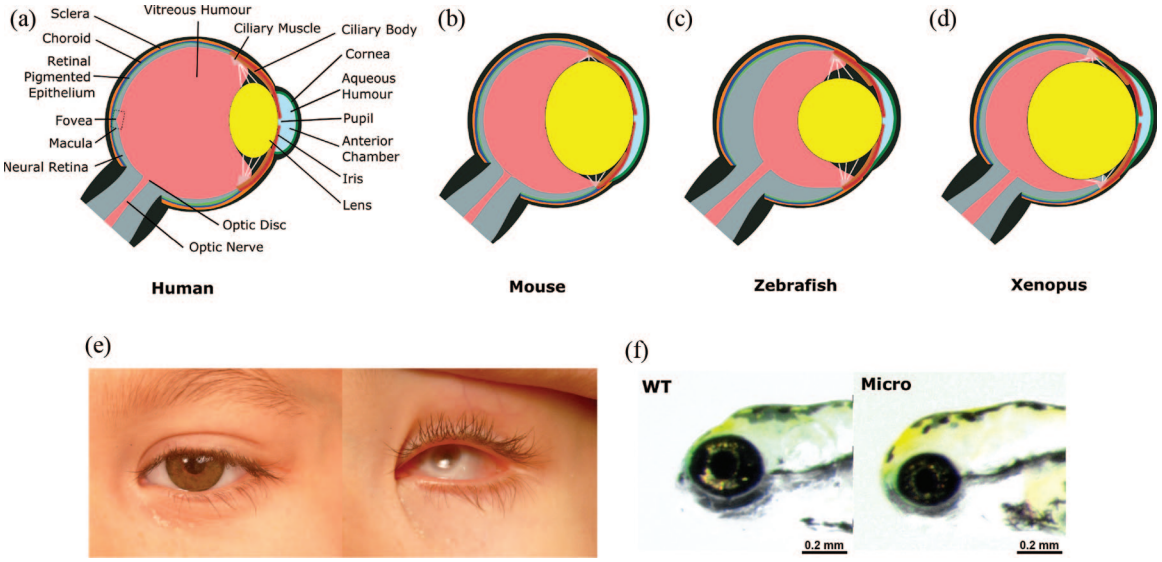


**Figure 1.** Diagrams of mature eye structure in human, mouse, zebrafish and *Xenopus*, and images of microphthalmic eyes in human and zebrafish. (a) Human eye with a cone-rich macula responsible for central vision, and a small lens which refracts light, along with the cornea. (b) Mouse eye with an enlarged lens compared with humans and lacking a cone-rich macula, with cones instead dispersed throughout the retina. (c) Zebrafish eye with thick neural retina layer and spherical lens which alone is responsible for focusing light. (d) *Xenopus* eye with a large, spherical lens encompassing most of the vitreous. (e) Clinical image of patient with unilateral left microphthalmia with and without prosthetic shell. (f) Wildtype and microphthalmic zebrafish at 76 h post fertilisation (hpf).

*Generation of microphthalmic mice.* Historically, forward genetics was used to create phenotypes randomly in animals, and those of interest were screened to identify genetic mutations.[25,156] However, recent advances in genome editing technologies means it is now more common to directly modify specific genes to create mouse models using targeted or conditional mutagenesis, thereby using reverse genetics to generate specific mutants.[25,156] Mouse phenotyping centres, such as the International Mouse Phenotyping Consortium (IMPC, https://www.mousephenotype.org/) are used to screen targeted mutants for ocular phenotypes.[25,155] Many mutant mouse lines have been generated (Table 2), and 269 genes or loci have been linked to microphthalmia, from which key discoveries have been made and are described in Graw[25] (Figure 2, Table S1).

*Drawbacks of mouse ocular models.* Despite the shared genetics of mice and humans, discrepancy in ocular phenotypes implies divergence in molecular regulatory mechanisms between the species. For example, common microphthalmia-associated genes involved in retinoic acid signalling, such as *ALDH1A3* and *RARβ*, do not produce a microphthalmic phenotype in mouse models, which instead suffer from ocular disorders including lens and retinal anomalies.[3,25,160,161] On the other hand, mutations in some genes produce a more severe ocular phenotype in mice than typically observed in humans, such as *Pax6* which was first studied as a classical anophthalmia model as many mutants display no eyes, while microphthalmic models often develop many additional eye defects (Table 2).[25] A further problem with mouse models is that inbred strains can be associated with background ocular disorders; for example, 5–10% of C57BL/6 mice and related strains develop sporadic microphthalmia/anophthalmia, depending on age, environment and additional induced mutations, most likely as the result of a polygenic disease basis.[156] Therefore, choice of strain is important for translating results in mice to understanding human eye development and disease, as well as when testing novel therapies.

*Understanding molecular pathways in microphthalmia.* Conservation of genetics makes mice a practical model for investigation of genetic pathways in microphthalmia development. Through transcriptome and proteome analysis of knockout mouse models, downstream targets of disease-causing genes can be identified to resolve complex molecular networks. Transcription factor *Pitx3* is known to regulate a large number of molecular elements which are important for eye development.[25] Homozygous deletion of the promoter region of *Pitx3* or homozygous nonsense mutations resulting in overexpression of truncated protein lead to severe microphthalmia and aphakia due to halted lens development.[123,162–164] Investigation of molecular targets through EMSA and ChIP assays demonstrated *Pitx3* binds to an evolutionarily conserved region of *Foxe3*, resulting in increased transcriptional activity.[165] *Foxe3* mutants display a similar phenotype to *Pitx3* including microphthalmia and aphakia (Table 2), reflecting the phenotypic similarity of patients with pathogenic mutations in *PITX3* causing anterior segment dysgenesis 2 (OMIM #610256) and *FOXE3* producing Cataract 11 (OMIM #610623), both of which result in microphthalmia, cataract, anterior segment disorders and sclerocornea, indicating conservation of molecular regulatory mechanisms.[16,166] Understanding the interaction and shared pathways of genes in eye development helps to clarify genotype–phenotype correlations in patients, as well as identify potentially effective therapeutic targets.

*Modelling variable ocular and syndromic phenotypes.* One of the earliest mouse models of microphthalmia was an 'eyeless' mouse ($Rax^{ey}$ – MGI: 3809647) in which 10% of mice were reported to develop 'small' or 'medium' sized eyes, later determined to be the result of a point mutation in transcriptional regulator *Rax*, creating a hypomorphic mutant protein with a partial loss-of-function.[25,56] Variation in eye size observed in patient cohorts and animal models [like *Rax* and *Pax6* mutant mice (Table 2)] is the result of microphthalmia and anophthalmia (no eye) sharing the same clinical spectrum and genetic basis, which may be the result of dose-dependent gene function.[3] The availability of allelic series of mouse mutants with a wide range of ocular disorders makes them an ideal model to study the effect of gene dosage on eye development (Table 2). For example, *Sox2* mutants with a range of pathogenic variants display a spectrum of disease severity which correlates to the expression level of *Sox2* in the progenitor cells of the NR, validating a role of dosage sensitivity in microphthalmia (Table 2).[51] *Mitf* variants have differing effects on gene function: (i) semi-dominant mutations affecting the DNA-binding or transcriptional activation domains yield proteins which do not bind to DNA but still dimerise to other proteins, thereby impairing their functional DNA-binding ability; (ii) recessive variants

**Table 2.** Animal models of known human microphthalmia genes (mouse, zebrafish and *Xenopus*).

| Human gene | Animal | Genotype/allele ID | Genotype | Predominant ocular phenotype | Reference(s) |
|---|---|---|---|---|---|
| SOX2 | Mouse | MGI:3625924 | $Sox2^{tm1Lpev}/Sox2^{tm3Lpev}$ | Mi, An, RD | Taranova *et al.*[51] |
| | | MGI:3625925 | $Sox2^{tm1Lpev}/Sox2^{tm4Lpev}$ | Mi, An, ONH, RD | Taranova *et al.*[51] |
| OTX2 | Mouse | MGI:5573220 | $Otx2^{tm12.1Asim}/Otx2^{tm12.1Asim}$ | Mi, An, ONH, RD | Bernard *et al.*[52] |
| | | MGI:2172552 | $Otx2^{tm1Sia}/Otx2^{+}$ | Mi, An, RD, Ak, AC, ASD, A | Matsuo *et al.*[53] |
| | Zebrafish | ZDB-ALT-100412-1 | $otx2b^{hu3625}/otx2b^{hu3625}$ | Mi, RD | Bando *et al.*[54] |
| RAX | Mouse | MGI:5494276 | $Rax^{tm1.1(rTA,tetO-cre)Lan}/?$ | Mi | Plageman annd Lang[55] |
| | | MGI:3809647 | $Rax^{ey1}/Rax^{ey1}$ | Mi, An, ONH, LS | Chase[56] |
| | Zebrafish | ZDB-ALT-020514-4 | $rx3^{s399}/rx3^{s399}$ | Mi, An, LS | Yin *et al.*[57]; Loosli *et al.*[58] |
| VSX2 | Mouse | MGI:3799537 | $Vsx2^{or-2J}/Vsx2^{or-2J}$ | Mi, LA | Prochazka *et al.*[59] |
| | | MGI:5449361 | $Vsx2^{or-J}/Vsx2^{or-J}$ | Mi, OHN, RD, LS | Zou and Levine[60] |
| | | MGI:4358055 | $Vsx2^{or}/Vsx2^{or}$ | Mi, ONH, RD, LA | Truslove[61] |
| | | MGI:5449360 | $Vsx2^{tm1.1Eml}/Vsx2^{tm1.1Eml}$ | Mi, RD, LS | Zou and Levine[60] |
| | | MGI:5449358 | $Vsx2^{tm1.1Itl}/Vsx2^{tm1.1Itl}$ | Mi, RD, LS | Zou and Levine[60] |
| | | MGI:5449362 | $Vsx2^{or-J}/Vsx2^{tm1.1Eml}$ | Mi | Zou and Levine[60] |
| PAX6 | Mouse | MGI:2680573 | $Pax6^{1Jrt}/Pax6^{+}$ | Mi, An, RD, LA, CO, IH | Rossant[62] |
| | | MGI:3613473 | $Pax6^{2Neu}/Pax6^{+}$ | Mi, Cat | Favor *et al.*[63]; Favor and Neuhäuser-Klaus[64] |
| | | MGI:3590307 | $Pax6^{3Neu}/Pax6^{+}$ | Mi, Cat | Favor *et al.*[63]; Favor and Neuhäuser-Klaus[64] |
| | | MGI:4943211 | $Pax6^{3Neu}/Pax6^{+}$ | Mi, Cat | Favor *et al.*[65] |
| | | MGI:3590308 | $Pax6^{4Neu}/Pax6^{+}$ | Mi, Cat, IH | Favor *et al.*[63]; Favor and Neuhäuser-Klaus[64] |
| | | MGI:3613474 | $Pax6^{5Neu}/Pax6^{+}$ | Mi, Cat | Favor *et al.*[63]; Favor and Neuhäuser-Klaus[64] |
| | | MGI:3588509 | $Pax6^{6Neu}/Pax6^{+}$ | Mi, Cat | Favor *et al.*[63]; Favor and Neuhäuser-Klaus[64] |
| | | MGI:3613467 | $Pax6^{7Neu}/Pax6^{+}$ | Mi, Cat, IH | Favor *et al.*[63]; Favor and Neuhäuser-Klaus[64] |
| | | MGI:3613475 | $Pax6^{8Neu}/Pax6^{+}$ | Mi | Favor *et al.*[63] |
| | | MGI:3613476 | $Pax6^{9Neu}/Pax6^{+}$ | Mi | Favor *et al.*[63] |
| | | MGI:3613477 | $Pax6^{10Neu}/Pax6^{+}$ | Mi | Favor *et al.*[63] |
| | | MGI:3707321 | $Pax6^{132\text{-}14Neu}/Pax6^{132\text{-}14Neu}$ | Mi, Col, LA, ASD | Favor *et al.*[66] |
| | | MGI:3611340 | $Pax6^{ADD4802}/Pax6^{+}$ | Mi, LA, Cat, CO | Graw *et al.*[67] |
| | | MGI:5511023 | $Pax6^{Aey80}/Pax6^{+}$ | Mi, LS | Puk *et al.*[68] |

*(Continued)*

**Table 2.** (Continued)

| Human gene | Animal | Genotype/allele ID | Genotype | Predominant ocular phenotype | Reference(s) |
|---|---|---|---|---|---|
| | | MGI:2175199 | $Pax6^{Coop}/Pax6^{+}$ | Mi, CO, IH | Lyon *et al.*[69] |
| | | MGI:2687018 | $Pax6^{Leca1}/Pax6^{Leca1}$ | Mi, LA | Thaung *et al.*[70] |
| | | MGI:2687019 | $Pax6^{Leca2}/Pax6^{Leca2}$ | Mi, LA | Thaung *et al.*[70] |
| | | MGI:2687020 | $Pax6^{Leca3}/Pax6^{Leca3}$ | Mi, LA | Thaung *et al.*[70] |
| | | MGI:2687021 | $Pax6^{Leca4}/Pax6^{Leca4}$ | Mi, LA | Thaung *et al.*[70] |
| | | MGI:3611468 | $Pax6^{Mhdaaey11}/Pax6^{Mhdaaey11}$ | Mi, Cat, CO | Graw *et al.*[67] |
| | | MGI:3611342 | $Pax6^{Mhdaaey18}/Pax6^{+}$ | Mi, Cat, CO | Graw *et al.*[67] |
| | | MGI:3526886 | $Pax6^{Mhdaaey18}/Pax6^{+}$ | Mi, Cat | European Mouse Mutant Archive[71] |
| | | MGI:3798480 | $Pax6^{Rgsc20}/Pax6^{+}$ | Mi, Cat | RBCGS Center[72] |
| | | MGI:3798889 | $Pax6^{Rgsc123}/Pax6^{+}$ | Mi, Cat | RBCGS Center[72] |
| | | MGI:3799164 | $Pax6^{Rgsc242}/Pax6^{+}$ | Mi, Cat | RBCGS Center[72] |
| | | MGI:2175204 | $Pax6^{Sey\text{-}Dey}/Pax6^{+}$ | Mi, Col, RD, LA, LS, Cat, IH, A, | Theiler *et al.*[73] |
| | | MGI:2175206 | $Pax6^{Sey\text{-}H}/Pax6^{+}$ | Mi, Col | Hogan *et al.*[74] |
| | | MGI:2175208 | $Pax6^{Sey\text{-}Neu}/Pax6^{+}$ | Mi, LA, ASD, IH | Ramaesh *et al.*[75] |
| | | MGI:3771036 | $Pax6^{Sey}/Pax6^{+}$ | Mi | Hill *et al.*[76] |
| | | MGI:2170872 | $Pax6^{Sey}/Pax6^{+}$ | Mi, ONH, RD, LA, ASD | Hill *et al.*[76] |
| | | MGI:5567085 | $Pax6^{tm1.1Zkoz}/Pax6^{tm1.1Zkoz}$ | Mi, RD, LA | Klimova and Kozmik[77] |
| | | MGI:5317872 | $Pax6^{tm1.2Xzh}/Pax6^{tm1.2Xzh}$ | Mi, Col, LA | Carbe *et al.*[78] |
| | | MGI:4821786 | $Pax6^{tm2Pgr}/Pax6^{+}$ | Mi, OHN, LS, ASD | Kroeber *et al.*[79] |
| | | MGI:4366458 | $Pax6^{tm2Pgr}/Pax6^{tm2Pgr}$ | Mi, LS, LA, Cat | Shaham *et al.*[80] |
| | | MGI:4358211 | $Pax6^{tm2Pgr}/Pax6^{tm2Pgr}$ | Mi, RD, LA, LS, Cat, ASD, IH, | Davis *et al.*[81] |
| | Zebrafish | ZDB-ALT-980203-1333 | $pax6b^{tq253a}/pax6b^{tq253a}$ (sri) | Mi, LA, ASD | Kleinjan *et al.*[82] |
| | Xenopus | – | $Pax6^{-/-}$ | Mi, RD, Ak | Nakayama *et al.*[83] |
| | | – | $Pax6^{-}/*$ | Mi, Cat, CO, A | Nakayama *et al.*[83] |
| STRA6 | Mouse | MGI:5490888 | $Stra6^{tm1Nbg}/Stra6^{tm1Nbg}$ | Mi, RH | Ruiz *et al.*[84] |
| | Zebrafish | ZDB-ALT-180521-1 | $stra6l^{musc97}/stra6l^{musc97}$ | Mi | Shi *et al.*[85] |
| FOXE3 | Mouse | MGI:2175026 | $Foxe3^{dyl}/Foxe3^{dyl}$ | Mi, LA, LS, Cat, CO, ASD | Sanyal and Hawkins[86] |
| | | MGI:3604813 | $Foxe3^{tm1Mjam}/Foxe3^{tm1Mjam}$ | Mi, RD, ASD, LA, | Medina-Martinez *et al.*[87] |

*(Continued)*

**Table 2.** (Continued)

| Human gene | Animal | Genotype/allele ID | Genotype | Predominant ocular phenotype | Reference(s) |
|---|---|---|---|---|---|
| | Zebrafish | ZDB-ALT-181015-1 | Foxe3$^{s4001}$/Foxe3$^{s4001}$ | Mi, LA, LS | Krall *et al.*[88] |
| BMP4 | Mouse | MGI:3711773 | Bmp4$^{tm1Blh}$/Bmp4$^{+}$ | Mi, An, ONH, RD, Cat, CO, AC, ASD, IH, | Dunn *et al.*[89] |
| BMP7 | Mouse | MGI:3629218 | Bmp7$^{tm2Rob}$/Bmp7$^{tm4(Bmp4)Rob}$ | Mi | Zouvelou *et al.*[90] |
| | | MGI:2451062 | Bmp7$^{tm1Rob}$/Bmp7$^{tm1Rob}$ | Mi, An | Dudley *et al.*[91] |
| | | MGI:3847892 | Bmp7$^{tm1.2Dgra}$/Bmp7$^{tm1.2Dgra}$ | Mi, An, RD, LA | Oxburgh *et al.*[92] |
| GDF6 | Zebrafish | ZDB-ALT-980203-555 | gdf6a$^{s327}$/$^{s327}$ (dark half) | Mi | French *et al.*[93]; Pant *et al.*[94] |
| | | ZDB-ALT-050617-10 | gdf6a$^{m233}$/$^{m233}$ (out) | Mi, An | den Hollander *et al.*[95] |
| SMOC1 | Mouse | MGI:4941783 | Smoc1$^{Tn(sb-lacZ,GFP)PV384Jtak}$/ Smoc1$^{Tn(sblacZ,GFP)PV384Jtak}$ | Mi, ONH, RD | Okada *et al.*[96] |
| SHH | Mouse | MGI:3759227 | Shh$^{tm1Amc}$/Shh$^{tm2Amc}$ | Mi, ONH, RD | Wang *et al.*[97]; Dakubo *et al.*[98] |
| | | MGI:3812210 | Shh$^{tm1Chg}$/Shh$^{+}$ | Mi, An | Ratzka *et al.*[99] |
| | | MGI:3589447 | Shh$^{tm1Chg}$/Shh$^{+}$ | Mi, Ak | Grobe *et al.*[100] |
| | | MGI:3042780 | Shh$^{tm1Chg}$/Shh$^{tm1Chg}$ | Mi | Bulgakov *et al.*[101] |
| | | MGI:3851497 | Shh$^{tm1.1Rseg}$/Shh$^{tm1.1Rseg}$ | Mi | Chan *et al.*[102] |
| | | MGI:3851498 | Shh$^{tm1Amc}$/Shh$^{tm1.1Rseg}$ | Mi | Chan *et al.*[102] |
| | Zebrafish | ZDB-ALT-980413-636 | shha$^{tq252}$/shha$^{tq252}$ (syu) | Mi, RD | Brand *et al.*[103]; Stenkamp *et al.*[104] |
| MAB21L2 | Zebrafish | ZDB-ALT-140130-18 | mab21l2 $^{au10}$/mab21l2 $^{au10}$ | Mi, Col, LA, ASD | Gath and Gross[105] |
| | | ZDB-ALT-150611-1 | mab21l2$^{Q48Sfs*5}$/ mab21l2$^{Q48Sfs*5}$ | Mi, Col, CO | Deml *et al.*[40] |
| | | ZDB-ALT-150611-2 | mab21l2$^{R51_F52del}$/ mab21l2$^{R51_F52del}$ | Mi, An, Col, RD, ASD | Deml *et al.*[40] |
| PORCN | Mouse | MGI:6368187 | Porcn$^{tm1.1Lcm}$/Porcn$^{+}$ | Mi, Col, RD | Bankhead *et al.*[106] |
| FRAS1 | Mouse | MGI:2657302 | Fras1$^{bl}$/Fras1$^{bl}$ | Mi | Phillips[107] |
| FREM1 | Mouse | MGI:3026630 | Frem1$^{crf11}$/Frem1$^{crf11}$ | Mi | Kile *et al.*[108]; Beck *et al.*[109] |
| | | MGI:5473606 | Frem1$^{eyes2}$/Frem1$^{eyes2}$ | Mi | Beck *et al.*[110] |
| TCTN2 | Mouse | MGI:5292219 | Tctn2$^{tm1.1Reit}$/Tctn2$^{tm1.1Reit}$ | Mi | Sang *et al.*[111] |
| COL4A1 | Mouse | MGI:4822250 | Col4a1$^{D456}$/Col4a1$^{+}$ | Mi, LA, Cat | Favor *et al.*[112] |
| | | MGI:5308056 | Col4a1$^{deltaex40}$/Col4a1$^{+}$ | Mi, ONH, RD | Labelle-Dumais *et al.*[113] |
| | | MGI:4822242 | Col4a1$^{ENU911}$/Col4a1$^{+}$ | Mi, LA, Cat, CO | Favor *et al.*[112] |
| PTCH2 | Zebrafish | – | ptch2$^{uta4}$/ptch2$^{uta4}$ | Mi, LA, Cat, ASD | Lee *et al.*[114] |
| | | – | ptch2$^{uta5}$/ptch2$^{uta5}$ | Mi, RD | Lee *et al.*[114] |

*(Continued)*

**Table 2.** (Continued)

| Human gene | Animal | Genotype/allele ID | Genotype | Predominant ocular phenotype | Reference(s) |
|---|---|---|---|---|---|
| | | – | ptch2$^{uta6}$/ptch2$^{uta6}$ | Mi, RD | Lee *et al.*[114] |
| | | – | ptch2$^{uta16}$/ptch2$^{uta16}$ | Mi, LA | Lee *et al.*[114] |
| | | – | ptch2$^{uta17}$/ptch2$^{uta17}$ | Mi | Lee *et al.*[114] |
| | | – | ptch2$^{uta19}$/ptch2$^{uta19}$ | Mi, Cat | Lee *et al.*[114] |
| | | – | ptch2$^{uta20}$/ptch2$^{uta20}$ | Mi, Cat | Lee *et al.*[114] |
| | | – | ptch2$^{uta22}$/ptch2$^{uta22}$ | Mi, Cat | Lee *et al.*[114] |
| TBC1D32 | Mouse | MGI:5560506 | Tbc1d32$^{b2b2596Clo}$/ Tbc1d32$^{b2b2596Clo}$ | Mi, An | Lo[115] |
| MFRP | Zebrafish | ZDB-ALT-180816-9 | Mfrp$^{mw78}$/mfrp$^{mw78}$ | Mi, RD | Collery *et al.*[116] |
| PRSS56 | Mouse | MGI:5444191 | Prss56$^{glcr4}$/Prss56$^{glcr4}$ | Mi, ONH, RD, ASD | Nair *et al.*[117] |
| PXDN | Mouse | MGI:5584292 | Pxdn$^{mhdakta048}$/ Pxdn$^{mhdakta048}$ | Mi, ONH, RD, LA, ASD, IH | Yan *et al.*[118] |
| PITX2 | Mouse | MGI:1857844 | Pitx2$^{tm1Sac}$/Pitx2$^{+}$ | Mi, Cat | Gage *et al.*[119] |
| | | MGI:1857846 | Pitx2$^{tm2Sac}$/Pitx2$^{tm2Sac}$ | Mi, LA | Gage *et al.*[119] |
| | | MGI:2445429 | Pitx2$^{tm4(cre)Jfm}$/Pitx2$^{+}$ | Mi, LA, LS, CO, IH | Liu and Johnson[120] |
| | Zebrafish | ZDB-ALT-180731-2 | pitx2$^{M64*}$/pitx2$^{M64*}$ | Mi, An, ASD, IH | Hendee *et al.*[121] |
| PITX3 | Mouse | MGI:4429423 | Pitx3$^{eyl}$/Pitx3$^{eyl}$ | Mi, RD, Ak | Rosemann *et al.*[122] |
| | | MGI:3042029 | Pitx3$^{ak}$/Pitx3$^{ak}$ | Mi, RD, LA, ASD, IH | Varnum and Stevens[123] |
| MITF | Mouse | MGI:2662939 | Mitf$^{Mi-Crc}$/Mitf$^{Mi-Crc}$ | Mi | Hetherington[124] |
| | | MGI:3525852 | Mitf$^{mi-ce}$/Mitf$^{mi-ce}$ | Mi, RD, LA, Cat | Zimring *et al.*[125] |
| | | MGI:4455018 | Mitf$^{Mi}$/Mitf$^{Mi}$ | Mi | Steingrímsson *et al.*[126] |
| | | MGI:2663064 | Mitf$^{Mi-wh}$/Mitf$^{mi-x}$ | Mi | Munford[127] |
| | | MGI:4442409 | Mitf$^{mi-x39}$/Mitf$^{mi-x39}$ | Mi | Hallsson *et al.*[128] |
| | | MGI:3630349 | Mitf$^{Mi-ws}$/Mitf$^{Mi-ws}$ | Mi, RD | Hollander[129] |
| | | MGI:3762342 | Mitf$^{Mi-wh}$/Mitf$^{Mi-wh}$ | Mi, Col, ONH, RD | Packer *et al.*[130] |
| | | MGI:4455017 | Mitfmi-$^{vga9}$/Mitf$^{mi-vga9}$ | Mi, RD | Steingrímsson *et al.*[126] |
| | | MGI:4356490 | Mitf$^{mi-tg}$/Mitf$^{mi-tg}$ | Mi, IH | Krakowsky *et al.*[131] |
| | | MGI:4410320 | Mitf$^{mi-rw}$/Mitf$^{mi-rw}$ | Mi, RD | Southard[132] |
| | | MGI:4356528 | Mitf$^{Mi-Or}$/Mitf$^{Mi-Or}$ | Mi, An, RD | Steingrímsson *et al.*[126]; Stelzner [133] |
| | | MGI:3041536 | Mitf$^{mi-Mhdabcc2}$/Mitf$^{mi-Mhdabcc2}$ | Mi | Hansdottir *et al.*[134] |
| | | MGI:5307227 | Mitf$^{Mi}$/Mitf$^{Mi-J}$ | Mi, RD | Silvers *et al.*[135] |
| | | MGI:4455020 | Mitf$^{mi-ew}$/Mitf$^{mi-ew}$ | Mi | Steingrímsson *et al.*[126] |

*(Continued)*

**Table 2.** (Continued)

| Human gene | Animal | Genotype/allele ID | Genotype | Predominant ocular phenotype | Reference(s) |
|---|---|---|---|---|---|
| | | MGI:4442432 | $Mitf^{mi\text{-}enu198}/Mitf^{mi\text{-}enu198}$ | Mi | Hallsson *et al.*[128] |
| | | MGI:3587635 | $Mitf^{mi\text{-}enu122}/Mitf^{mi\text{-}enu122}$ | Mi, RD | Steingrímsson *et al.*[126] |
| | | MGI:3041533 | $Mitf^{mi\text{-}enu5}/Mitf^{mi\text{-}enu5}$ | Mi | Hansdottir *et al.*[134] |
| | | MGI:3522321 | $Mitf^{mi\text{-}di}/Mitf^{mi\text{-}di}$ | Mi, RD | West *et al.*[136] |
| FOXC1 | Mouse | MGI:3802472 | $Foxc1^{hith}/Foxc1^{hith}$ | Mi, LA, ASD, IH | Zarbalis *et al.*[137] |
| CRYAA | Mouse | MGI:3690118 | $Cryaa^{2J}/Cryaa^{2J}$ | Mi, LS, Cat | Xia *et al.*[138] |
| | | MGI:2653233 | $Cryaa^{Aey7}/Cryaa^{Aey7}$ | Mi, LA, Cat | Graw *et al.*[139] |
| | | MGI:3784583 | $Cryaa^{tm1.1Ady}/Cryaa^{tm1.1Ady}$ | Mi, LA, LS, Cat | Xi *et al.*[140] |
| | | MGI:2175799 | $Cryaa^{tm1Wawr}/Cryaa^{tm1Wawr}$ | Mi, LS, Cat | Brady *et al.*[141] |
| | | MGI:2653234 | $Cryaa^{Aey7}/Cryaa^{+}$ | Mi, LA, Cat | Graw *et al.*[139] |
| FREM2 | Mouse | MGI:5618921 | $Frem2^{ne}/Frem2^{ne}$ | Mi | Lo[115] |
| | | MGI:3603819 | $Frem2^{my\text{-}F11}/Frem2^{my\text{-}F11}$ | Mi, An | Timmer *et al.*[142] |
| | | MGI:3796628 | $Frem2^{b2b3270Clo}/Frem2^{b2b3270Clo}$ | Mi, An | Curtain and Donahue[143] |
| RPGRIP1L | Mouse | MGI:3716631 | $Rpgrip1l^{tm1Urt}/Rpgrip1l^{tm1Urt}$ | Mi | Vierkotten *et al.*[144]; Delous *et al.*[145] |
| SMG9 | Mouse | MGI:5776357 | $Smg9^{em1(IMPC)J}/Smg9^{em1(IMPC)J}$ | Mi | Shaheen *et al.*[146] |
| SIX3 | Zebrafish | ZDB-ALT-160421-3, ZDB-ALT-071211-1 | $six3a^{vu129}/six3a^{vu129}$, $six3b^{vu87}/six3b^{vu87}$ | Mi, RD | Samuel *et al.*[147] |
| SNX3 | Mouse | MGI:5767809 | $Snx3^{tm1.1(KOMP)Vlcg}/Snx3^{tm1.1(KOMP)Vlcg}$ | Mi, An | Mouse Genome Informatics and the International Mouse Phenotyping Consortium[148] |
| DAG1 | Mouse | MGI:4440460 | $Dag1^{tm2Kcam}/Dag1^{tm2Kcam}$ | Mi, Bu, CO | Satz *et al.*[149] |
| HMX1 | Mouse | MGI:3838401 | $Hmx1^{dmbo}/Hmx1^{dmbo}$ | Mi | Munroe *et al.*[150] |
| RERE | Mouse | MGI:3577769 | $Rere^{eyes3}/Rere^{eyes3}$ | Mi | Kim *et al.*[151] |
| | | MGI:5503952 | $Rere^{eyes3}/Rere^{om}$ | Mi | |
| | Zebrafish | ZDB-ALT-980203-1102, ZDB-ALT-980203-311 | $rerea^{tb210}/rerea^{tw220c}$ | Mi, ONH, RD | Plaster *et al.*[152]; Schilling *et al.*[153] |
| RAB18 | Mouse | MGI:5698703 | $Rab18^{m1Hongc}/Rab18^{m1Hongc}$ | Mi, ONH | Cheng *et al.*[154] |

Mouse genotype ID and phenotypic data was taken from the Mouse Genome Informatics database (http://www.informatics.jax.org/). Zebrafish allele ID was taken from The Zebrafish Information Network (ZFIN) database (https://zfin.org/). *Xenopus* data was taken from Xenbase (http://www.xenbase.org). Data from December 2020.
A, aniridia; AC, absent cornea; Ak, aphakia; An, anophthalmia; ASD, anterior segment dysgenesis; Bu, buphthalmos; Cat, cataract; CO, corneal opacity; Col, coloboma; IH, iris hypoplasia; LA, lens abnormalities; LS, small lens; Mi, microphthalmia; ONH, optic nerve hypoplasia; RD, retina dysplasia.

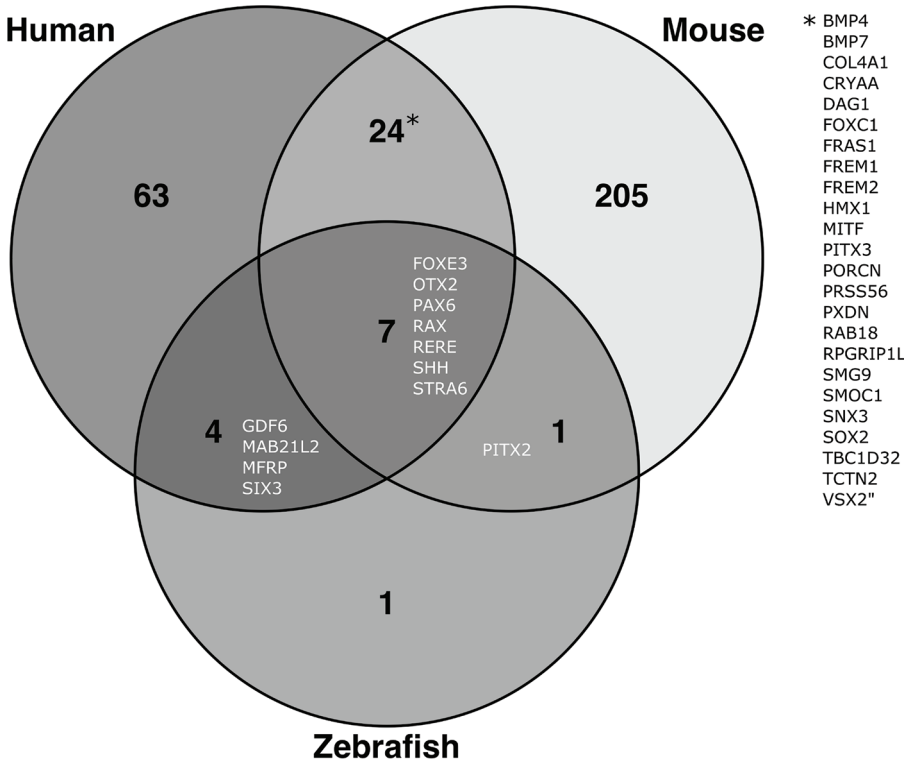


**Figure 2.** Genes identified to cause microphthalmia in mouse, zebrafish and humans based on database and literature search, with overlapping genes listed.
Mouse data from Mouse Genome Informatics database (http://www.informatics.jax.org/). Zebrafish data from Zebrafish Information Network (ZFIN) database (https://zfin.org/). Data from December 2020. Full list of genes in Supplemental Table 1.

affect *Mitf* transcription or produce mutant proteins which do not dimerise, and hence do not interfere with DNA binding of other proteins.[167] This results in a variable ocular phenotype between heterozygotes, homozygotes and compound heterozygotes (Table 2). Similarly, patients with biallelic *MITF* pathogenic mutations exhibit COMMAD (coloboma, osteopetrosis, microphthalmia, macrocephaly, albinism, and deafness) syndrome (OMIM #617306), but haploinsufficient heterozygotes display more mild symptoms of Waardenburg syndrome (OMIM #193510), and patients with semi-dominant heterozygous mutations have the more severe overlapping disorder Tietz albinism-deafness syndrome (OMIM #103500), neither of which include microphthalmia.[167] Consequently multiple models are required to understand the full spectrum of ocular and systemic features which can be caused by disruption of an individual gene.[25]

Beyond the ocular phenotype, mutant mice with comprehensive phenotype annotation can be used to study any systemic involvement associated with candidate genes. For example, *Otx2*$^{+/-}$ mice display microphthalmia and otocephaly, alongside reduced fertility in males, reflective of the abnormal development of the hypothalamic–pituitary–gonadal axis seen in humans with *OTX2* mutations causing syndromic microphthalmia 5 (MCOPS5 – OMIM #610125).[53,168–172] These features coincide as, in addition to controlling oculogenesis, *Otx2* regulates the expression of genes involved in pituitary development, such as *Hesx1*.[168,173,174] Investigation of extraocular phenotypes in mice can provide information on the effect of different variants and genetic/environmental factors on systemic involvement, thus unlocking genotype–phenotype relationships.

*Identification of novel variants through mouse studies.* Forward genetic approaches and phenotypic screening to generate and catalogue eye phenotypes lead to successful discovery of many disease-causing microphthalmia genes, including *Mitf*, first identified in the early *mi* mouse line, and subsequently in a multitude of different lines with a range of ocular defects including a small eye (Table 2).[25,167,175–178] Targeted mutagenesis is

**Table 3.** Advantages and disadvantages of mouse, zebrafish, *Xenopus* and 2D/3D cellular models of microphthalmia.

| Model | Size of ocular structure | Availability of material | Cost | Time to develop mature ocular structure | Genetic conservation with humans | Morphological similarity to humans | Availability of genetic/ phenotypic data |
|---|---|---|---|---|---|---|---|
| Mouse | Large (3 mm) | Breed in medium numbers (5–10 pups per litter) | High | 1–2 months | High | High | Very good |
| Zebrafish | Medium (1–2 mm) | Breed in large numbers (>100 fertilised eggs/ clutch) | Medium | 3–5 days | Low | High | Good |
| *Xenopus* | Large (3–6 mm) | Breed in large numbers (>100 fertilised eggs/ clutch) | Medium | 3–5 days | Medium | High | Poor |
| Cellular – 2D | N/A | Easy to expand (although can be difficult to obtain primary patient/ embryonic tissue) | Medium | N/A | Human | N/A | Very good |
| Cellular – 3D | Small (100–500 µm) | Protocols to obtain mature structures can be inefficient (and difficulty obtaining primary patient/embryonic tissue) | High | 2–6 months (plus 2–3 months to reprogramme primary cells to iPSCs if required) | Human | Medium (mature structure does not contain vasculature etc) | Very good |

now performed more frequently, allowing validation and further exploration into novel candidate genes, although it is time-consuming and costly to test the effect of genes of uncertain significance, unless performed by large consortiums such as IMPC.[25,155]

*Genetic modifiers in microphthalmia aetiology.* The vast number of mouse lines with a microphthalmic phenotype means the effect of the genetic background can be investigated by inducing multiple mutations in the same mouse model, or the same mutation in multiple strains. *Cx50*$^{-/-}$ mice have microphthalmia with nuclear cataracts.[179,180] When studying *Cx50* knockouts in both 129S6 and C57BL/6J strains, genetic modifiers were found to influence cataract severity due to differentially altered solubility of crystallin proteins, while eye growth was unaffected by genetic background.[181] Understanding oligogenic effects on ocular development using these techniques could aid in understanding the variation of MAC spectrum and additional ocular/extraocular features observed within and between families with the same molecular diagnosis.

*Modelling environmental causes of microphthalmia in mice.* Only 2% of microphthalmia cases were attributed to environmental factors in a UK prospective incidence study; however, this varies in different regions of the world.[2,3,6–8] These include maternal vitamin A deficiency, *in utero* exposure to toxic/teratogenic substances such as alcohol, and certain infections, for example rubella.[8,182–186] For decades, mice have been used to study the effect of the maternal environment on eye development on embryos, due to their *in utero* gestation, for example, induction of microphthalmia through maternal exposure to toxic trypan blue at 7 days' gestation.[187,188] More recently, a study of maternal diabetes showed embryonic glucose exposure mimicking hyperglycaemia and diminished expression of Wnt-PCP pathway genes, resulting in altered cytoskeletal organisation, cell shape and

cell polarity in the optic vesicle and ultimately ocular defects including anophthalmia and microphthalmia.[189] Likewise, the effect of maternal diet, including folic acid deficiency,[190] alcohol consumption,[191,192] pharmaceuticals[193] and infection[194,195] has also been explored in relation to mouse eye development. Studies on the environmental influences on ocular development are vital to understanding pathogenesis and providing appropriate clinical guidance and care during pregnancy.

### *Zebrafish*

*Advantages of zebrafish models of ocular development.* Zebrafish (*Danio rerio*) are a popular organism for studying vertebrate eye development and related disorders.[26,41] Zebrafish are easily maintained and breed in large numbers at low cost, with a generation time of 2–4 months.[24,26,41] They have many advantages over other organisms such as mice, including external fertilisation, transparency of embryos permitting direct visualisation of organogenesis, rapid eye development leading to adult-like patternation by 72 hpf and a highly organised heterotypical photoreceptor mosaic, which is cone-rich similar to humans, unlike mice.[24,26,41,196] Overall, zebrafish eye development closely resembles that of humans, although there are some distinctions. Hollow optic vesicles extend from the forebrain at 27 days' gestation in humans, while in contrast zebrafish optic vesicles begin as a solid mass of cells, which undergo cavitation by 14 hpf (Table 1).[24,26,197] The thickening of the highly proliferating NR and thinning of the RPE through cell flattening occurs earlier in zebrafish development, and ultimately a wider NR layer is present in the adult zebrafish with squamous epithelial cells in the RPE, where cells in the mature human RPE maintain a cuboidal shape (Figure 1).[24,41,198,199] Beyond this, the mature eye is remarkably similar between humans and zebrafish, except that in the fish, like many aquatic vertebrates, the larger, spherical lens is solely responsible for focusing light, without contribution from the cornea (Figure 1).[24,198]

*Generation of microphthalmic zebrafish.* There is significant genetic conservation between humans and zebrafish, with 70% of human genes corresponding to at least one zebrafish orthologue, and 84% of known human disease-causing genes having a zebrafish counterpart, providing potential to model a wide scope of human conditions.[26,200] Moreover, zebrafish are highly amenable to genetic manipulation, meaning mutations can be induced easily. Injection of genome modification tools at the single-cell stage of the fertilised egg allows induction of genetic changes which display a phenotype in the F0 generation.[24,26] Knockdown morphants can be generated by injection of an antisense oligonucleotide morpholino, which is complementary to the mRNA of interest, and leads to transient gene knockdown in the embryo for up to 5 days post fertilisation (dpf).[1] Injection of TALENs or CRISPR/Cas9 gene editing tools can be used to generate specific mutations in models, and establish stable mutant lines, which prevent mosaicism and are important to carry out a more complete investigation of phenotypes.[24,201]

*Drawbacks of zebrafish ocular models.* Due to a whole-genome duplication which occurred in zebrafish ancestry, many orthologues of mammalian genes have two copies. Consequently, careful experimental planning is required when undertaking any genetic manipulation to avoid genetic compensation/redundancy. Role-sharing between multiple orthologous genes can lead to variations in phenotypic severity, e.g. missense mutations in the sunrise (sri) *pax6b* homozygous line replicate the milder microphthalmia phenotype observed in patients with some missense *PAX6* mutations, while morpholino-induced knockdown of *pax6a* shows more extreme phenotypes, including reduced body size and abnormal brain development, in addition to microphthalmia.[26,82,202,203] Moreover, morpholinos can produce variable phenotypes, particularly regarding eye morphology (often spanning the MAC spectrum), and concerns have been raised with regards to their reliability, given disparities between morpholino and mutant phenotypes.[26,204–206] However, off-target effects can be controlled for by co-injecting with p53 morpholino to mitigate non-specific phenotypes.[207]

*Understanding molecular pathways in microphthalmia.* The shared molecular basis of human and zebrafish eye development means complex genetic networks underpinning microphthalmia can be resolved using transgenic/mutant zebrafish lines to establish the function of genes during eye development (Table 2). The functional role of the *shh* signalling pathway in retinal cell proliferation and survival was established using *syu* mutants, which have homozygous *shha* deletions causing reduced eye size due to decreased mitosis and

increased apoptosis in the retina.[103,104] *rx3*$^{-/-}$ mutants display an eyeless phenotype and expanded forebrain, similar to isolated microphthalmia 3 (OMIM #611038) in patients with biallelic *RAX* mutations.[57] Transcriptome analysis of these mutants showed downregulation of transcription factors regulating eye development (such as *mab21l2*) and retinoic acid signalling pathway components (including *aldh1a3*), with upregulation of Wnt signalling pathway components which function in brain development and are associated with microphthalmia and multiple neural disorders. Investigation of *mab21l2* morpholino-induced knockdown showed a similar phenotype to *rx3*$^{-/-}$ mutants, validating the downstream role of *mab21l2* in the *rx3* ocular regulatory network, and its role in microphthalmia development.[208] Other genetic knockdowns inducing microphthalmia include: transcription factors *otx2*,[39] *rax*,[209] *six6*[210,211] and *alx1*;[212] retinoic acid signalling components *rarβ*[213] and *aldh1a3*;[160] TGFβ signalling component *gdf6*;[214] and SHH signalling component *ptch1*.[215] There are few established microphthalmic mutant zebrafish lines (Table 2), and most exist from ENU mutagenesis screens.

*Modelling variable ocular and syndromic phenotypes.* Heterogenous ocular and systemic features observed in patient cohorts are mirrored in genetically modified zebrafish, allowing for further analysis of the sources of phenotype variation, whether genetic, epigenetic or environmental. Functional knockdown using *vsx2* morpholinos shows concentration-dependent reduction in eye size. This dosage effect of *vsx2* may explain the variable MAC phenotype observed in *VSX2* patients with isolated microphthalmia 2 (OMIM #610093) or colobomatous microphthalmia 3 (MCOPCB3 – OMIM #610092).[216] Loss-of-function biallelic variants in human *STRA6* leads to syndromic microphthalmia 9 (MCOPS9/Matthew-Wood Syndrome – OMIM #601186), where severe systemic features include pulmonary, diaphragmatic and cardiac defects, resulting in death usually within the first 2 years of life.[2,217–219] This phenotype is recapitulated by morpholino-induced knockdown, which exhibits microphthalmia, curved body axis, cardiac oedema and craniofacial defects due to disrupted retinoic acid signalling in the developing eye.[220] A less severe phenotype was observed when an alternative morpholino was used where a small concentration of RNA was still detectable, indicating a dose-dependent mechanism which may explain the milder or isolated microphthalmia/coloboma phenotype (MCOPCB8) observed in some patients with homozygous *STRA6* mutations, including certain missense variants.[9,217] Ocular and cardiac malformations in *stra6*-knockdowns were partially rescued by reduction of retinoic acid binding protein 4 (*rbp4*) using morpholino knockdown or 1-phenyl-2-thio-urea (PTU)-mediated inhibition (which downregulates *rbp4* mRNA expression at larval stages), demonstrating potential avenues for treatment *via* targeting of retinoic acid signalling pathways.[220–222]

*Identification of novel variants through zebrafish studies.* Where a new microphthalmia candidate gene or variant of unknown pathogenic significance is identified in a family through genetic investigation, zebrafish can be used to provide evidence of pathogenicity through expression studies in the early developing eye and through rapid gene knockdown in F0 fish and with validation of resulting phenotype. A novel association of *TMX3* with microphthalmia was validated with morpholinos targeting the *tmx3* zebrafish orthologue *zgc:110025*, resulting in significantly smaller eye size at 2 dpf.[17] This phenotype was rescued by injection of human wildtype *TMX3* mRNA, but not by injection of the patient mutant mRNA (p.Arg39Gln), confirming a functional effect of the *TMX3* variant on eye growth.

Equally, phenotypic annotation of zebrafish knockdowns can be used to identify putative novel genes to screen unsolved patient cohorts. For example, *bco1* encodes a key enzyme for vitamin A formation and causes microphthalmia when knocked-down at the larval stage.[223] Similarly, *rbm24a* has been found to positively control the mRNA stability of *sox2* transcripts, with gene knockdowns resulting in a small-eye phenotype.[224–227] So far, no pathogenic mutations have been successfully detected in human orthologues *BCO1* or *RBM24* in microphthalmic patients, although disease-causing variants of *RBM24* are known to cause cardiomyopathy. Nevertheless, examining these genes for functional mutations in patients without a known genetic cause through next-generation sequencing could improve molecular diagnosis rates by broadening the mutational spectrum and inclusion in future panel-based diagnostic testing.[228]

*Genetic modifiers in microphthalmia aetiology.* Rapid generation of phenotypes in F0 fish provides

an efficient method examine gene combinations to decipher epistatic interactions and oligogenic inheritance, aiding the investigation of multigenic factors in microphthalmia pathogenesis.[24] Patients with pathogenic mutations in transcription factor *TFAP2A* display a variable ocular phenotype including microphthalmia, coloboma and cataract as part of branchio-oculo-facial syndrome (BOFS – OMIM #113620), but homozygous loss-of-function *tfap2a* zebrafish mutants and morpholino-induced knockdown display no ocular phenotype.[229,230] Heterozygous mutations in *BMP4* cause syndromic microphthalmia 4 (OMIM #607932), but *bmp4*$^{-/-}$ zebrafish have normal eye morphology. Transcription factor *tcf7l1a* plays a role in the Wnt signalling, but zebrafish *tcf7l1a*$^{-/+}$ and *tcf7l1a*$^{-/-}$ mutants do not have a disrupted ocular phenotype. However, injection of *tfap2a* morpholinos *tcf7l1a*$^{-/+}$ and *tcf7l1a*$^{-/-}$ mutants results in coloboma/anophthalmia, respectively, while injection into *bmp4*$^{-/-}$ mutants causes microphthalmia and/or coloboma.[229] *tcf7l1a* and *bmp4* variants sensitise the developing eye to the effects of additional deleterious mutations, implying human hypomorphic *TFAP2A* variants may contribute to developmental eye disorders when on a background with additional mutations, potentially explaining phenotypic variability. More severe microphthalmia has also been noted in *tfc7l1a*$^{-/-}$ fish combined with mutations which when in isolation show no ocular phenotype (e.g. in *hesx1*) or a mild reduction in eye size (e.g. in *cct5* or *gdf6a*).[24,231]

Zebrafish studies show genetic interactions also influence syndromic heterogeneity, as *otx2* morpholino knockdowns display mild microphthalmia and shortening of the pharyngeal skeleton, but the combination of *otx2* and other otocephaly gene knockdowns (including *pgap1*, *prrx1* and *msx1*) result in more severe mandibular malformations, similar to craniofacial anomalies in patients with *OTX2*-associated otocephaly-dysgnathia complex.[39,172] This work demonstrates that *otx2* interacts with other genetic loci to regulate development throughout the body, which may explain the high systemic variation observed in patients with *OTX2*-associated microphthalmia.[3,172] Further investigation of multigenic factors in syndromic microphthalmia using zebrafish could clarify variability observed within families.

*Modelling environmental causes of microphthalmia in zebrafish.* Relatively few studies of environmental factors influencing eye growth have been performed; however, phenotypic variability observed within families indicates environmental factors could account for certain cases of variable penetrance and expressivity. Fertilisation and development of zebrafish *ex vivo* allows for easy modification of the embryonic environment. Vitamin A deprivation through pharmacological inhibition of enzyme retinaldehyde dehydrogenase in early wildtype zebrafish embryos results in a dose-dependent reduction in eye size, with high doses causing systemic features reminiscent of the MCOPS9 phenotype including cardiac oedema and mortality within the first days after treatment.[217,221] Variable severity of MAC observed between siblings with retinoic signalling component *STRA6*, the molecular cause underlying MCOPS9, indicates environmental factors such as maternal retinoic acid intake may be the cause of clinical heterogeneity.[217] Modelling these factors in zebrafish, where external conditions can be manipulated, can help explain the role of environmental factors in variable ocular and systemic phenotypes.

### *Xenopus*

*Advantages and drawbacks of* Xenopus *models of ocular development.* *Xenopus* have similar advantages as disease models to zebrafish, including external fertilisation and development and low cost.[232,233] Unlike zebrafish, *Xenopus* are tetrapods, hence are evolutionarily more similar to humans, with *Xenopus tropicalis* sharing 79% of their genes with humans.[234–237] *Xenopus* embryos are also larger in size, and able to tolerate extensive surgical manipulation, with transplantation of single cells to other parts of embryos in order to understand the role of interacting tissues and environments in development.[234] However, like zebrafish, *Xenopus* genomes can contain duplicated genes, therefore clear understanding of compensation and subfunctionalisation is important when evaluating data.[238] For example, genetic manipulation of *six6* in *Xenopus* shows diverged functionality of the duplicated genes, where knockdown of *six6.L* results in a more severe microphthalmia phenotype than knockdown of *six6.S*.[238]

Much of the early understanding of eye field specification, cell fate determination and the key regulators of oculogenesis were obtained from *Xenopus* studies.[33,233,239] The development and mature eye structure is extremely similar between humans

and *Xenopus*; nevertheless, the main difference is that *Xenopus*, like many amphibians and fish, can regenerate certain eye structures beyond embryogenesis. *Xenopus* have especially high capacity for ocular regeneration, and can produce new retinal cells through functional stem cell populations, and restore lost/damaged lens through transdifferentiation of the corneal epithelium.[46,233,240] Overall, conservation of cellular and developmental processes, as well as genomic synteny with mammals, makes *Xenopus* a valuable resource for studying eye development and microphthalmia.[233,234,241]

*Generation of microphthalmic* Xenopus*.* Microphthalmic phenotypes can be generated in the F0 generation using morpholino-induced knockdowns or injection of genome editing tools at the single-cell stage, without the need for time-consuming crosses.[232,235,242,243] For example, over 85% of TALENS-injected embryos to induce targeted gene disruption of *pax6a* and *pax6b* reveal perturbed eye formation and a spectrum of anophthalmia/microphthalmia phenotypes.[26,83,233,242] Gain-of-function experiments have often been performed in *Xenopus* to understand molecular networks, as embryos tolerate injection with mRNA.[234]

*Understanding molecular pathways in microphthalmia.* Size, external development and regenerative properties of *Xenopus* embryos allows surgical manipulations to be performed which can provide new insights into the molecular pathways at the initiation of eye development. Early transplantation experiments were invaluable to establishing the timing of eye induction.[30,33] Following this work, ectopic expression of EFTFs showed eye field initiation can only occur in the presence of *Otx2*, demonstrating a permissive role in regulating early eye development.[33,239] Fluorescent tissue induced to express EFTFs and *Otx2* transplanted to different regions of host embryos form functional, organised eye-like structures, demonstrating the need for these factors alone to stimulate and coordinate oculogenesis.[27,244] This understanding of the early regulators of eye development gleaned from *Xenopus* has been essential for extricating the molecular pathways underlying microphthalmia.

*Modelling variable ocular and syndromic phenotypes.* Developmental and genetic conservation with humans means *Xenopus* can be used to study both ocular and systemic phenotypes caused by microphthalmia-associated gene disruption. Overexpression of the epigenetic regulator *SMCHD1* through injection of wildtype or mutant mRNA results in craniofacial anomalies including microphthalmia, recapitulating the Bosma arhinia microphthalmia syndrome (BAMS – OMIM #603457) phenotype observed in patients with heterozygous missense mutations, confirming a gain-of-function mechanism.[245,246] This phenotype is not recapitulated in mouse models, due to apparent redundancy of *Smchd1* function in rodents.[246]

Morpholino-induced knockdown of co-repressor gene *bcor* in *Xenopus* produces a microphthalmia phenotype, along with systemic features including skeletal and central nervous system abnormalities. These knockdowns phenotypically reflect *BCOR*-associated syndromic microphthalmia 2 (OMIM #300166), also known as oculofaciocardiodental syndrome as hallmarks include cataracts, microphthalmia, facial, cardiac and dental anomalies.[218,243,247,248] Downregulation of transcription factor *Pitx2* in this model highlighted an upstream regulator role for *bcor*, demonstrating a shared pathway in *Xenopus* and humans, as heterozygous *PITX2* variants can cause anterior segment dysgenesis 4 (OMIM #137600) or Axenfeld–Rieger syndrome (OMIM #180500), where patients also exhibit dental hypoplasia and skeletal anomalies.[218] Knockdown of *bcor* in zebrafish does not produce a small eye, instead displaying a less severe ocular coloboma phenotype, and no ocular phenotype has been observed in mouse models of *Bcor*.

*Identification of novel variants through* Xenopus *studies.* Rapid ocular development, along with tolerance for genetic manipulation and injection of additional genetic material, means genes suspected to be involved in microphthalmia pathogenesis can be easily assessed in *Xenopus* using morpholino-induced knockdowns or overexpression to evaluate hypermorphic variants. MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression.[249] While not currently associated with microphthalmia, their role in eye development and disease is being revealed.[249,250] Targeted knockdown or overexpression of *miR-199* in *Xenopus* results in small eyes and reduced cell proliferation in the eye field due to disruption of EFTF expression including *rax1*.[251] This phenotype is rescued by blocking the *miR-199* binding site, demonstrating a distinct role of miRNAs in eye development and ocular maldevelopment,

and a novel set of targets for drug treatments. Additional candidates for patient screens originating from *Xenopus* overexpression modelling include *siah-2*,[252,253] *E-NTPDase*,[254] *PNAS-4*[255] and *pparγ*[256] and knockdowns of *sdr16c5*,[257] *frs3*[258] and *psf2*.[259] Although none of the candidates listed have yet been identified in microphthalmic cohorts, as frequency of next-generation sequencing escalates and large databases such as from the 100,000 genomes project can be analysed in more depth, there is increased capability to identify novel genes in patients through screening performed in animal models.[260]

*Modelling environmental causes of microphthalmia in* Xenopus. External development of *Xenopus* embryos allows for evaluation of adverse effects of environmental changes on ocular development. Alcohol consumption during pregnancy can cause Foetal Alcohol Spectrum Disorder (FASD), leading to microphthalmia, short stature, microcephaly and facial anomalies. Exposure of *Xenopus* embryos to ethanol between the late blastula and early/mid gastrula stages (stage 8.5–18) recapitulates phenotypic aspects of FASD, including shortened rostro–caudal axis, microcephaly and microphthalmia, due to antagonism of vital retinoic acid signalling pathways through competitive inhibition.[261] This knowledge could be beneficial for understanding how genetic and environmental interaction impact eye development and help explain clinical heterogeneity in microphthalmic cohorts.

## Human cellular models of microphthalmia

As discussed, differences exist in genetic regulation and disease manifestation between humans and animals. Hence, *in vitro* human cellular disease models can overcome species-dependent variation for studying molecular mechanisms and therapeutic compound testing, while also reducing the use of animal experimentation.

### *Generation and advantages/drawbacks of different types of cellular models*

*Primary cell lines.* Cells derived directly from patients with molecularly confirmed cause allow researchers to study how specific variants disrupt human cell function, and thereby investigate genotype–phenotype correlations from a molecular and cellular perspective. Furthermore, developing and testing the effects of drugs on patient-derived cells increases capacity to determine drug efficacy, creating more reliable data for which treatments might be successful in clinical trials as well as potential for more personalised medicine options.[262–265] However, a drawback of primary cell lines is as they senesce, they display changes in function and morphology, and eventually stop replicating; for example, primary RPE cells cannot be passaged more than 4–6 times.[266] Additionally, developmental cell types relevant to the onset of microphthalmia such as retinal progenitor cells cannot be derived from adult tissue, and consequently must be isolated from embryonic tissues, which are in short supply and have ethical implications surrounding their usage.[267,268]

*Immortalised cell lines.* Immortalised cell lines provide an unlimited supply of cells at a relatively low cost and are easy to maintain.[269] They are useful for studying various molecular functions in cellular processes, as they are generally tolerant of transfection with exogenous genetic material, and so can be induced to express genes of interest, enabling investigation into their mechanism of action in health and disease. However, misidentification and contamination remain widespread problems in producing reliable data from cell lines.[270,271] Moreover, due to genetic manipulation required to produce the immortalised line, cells may no longer represent their cell type of origin, such as the epithelial phenotype of ARPE-19 cells which diminishes within 3–4 passages, partially due to loss of key claudin tight junctions resulting in reduced functionality.[272,273]

*Embryonic stem cells (ESCs)/Human induced pluripotent stem cells (hiPSCs).* Embryonic stem cells (ESCs) and human induced pluripotent stem cells (hiPSCs) have the capacity to differentiate into any lineage, and therefore can model cellular functions and molecular regulation in any cell type, at different stages of development.[262,274–276] By providing an unlimited source of cells for disease modelling, ESCs/hiPSCs are an excellent resource for research and developing therapies, although can be expensive and more difficult to culture than other cells.[277] HiPSCs are also a promising source of cells to treat disease by transplanting into patients, either as differentiated cells, or in a pluripotent/multipotent state.[278,279] Cell-based therapies are being developed for multiple ocular diseases, such as age-related macular degeneration and retinitis pigmentosa, and show initial success with many ongoing clinical trials.[278–280] The

majority of ocular cell therapies currently focus on degenerative diseases, but transplantation of stem/progenitor cells may yet prove valuable for treating developmental disorders such as microphthalmia, by boosting eye growth postnatally.[281,282]

*3D cellular models.* Traditionally, cells are grown as a monolayer of a specific cell type on a flat surface. However, 2D cell culture has been shown to alter cell morphology, gene expression and function.[283–285] Furthermore, monoculture of a single cell type lacks the cross-cell-type signalling necessary to recapitulate the *in vivo* complexity.[286,287] Recreating the natural environment experienced in the developing eye using 3D culture techniques with multiple interacting cell types facilitates collection of more clinically relevant data.[264,288] Organoids mimic development through restricted division of progenitor cells and expression of distinct cellular adhesion molecules which allow temporal and spatial organisation of multiple cell types, in a manner similar to that of organs.[288] As such, organoids allow study of human organogenesis at developmental stages which would be otherwise inaccessible, such as within the first weeks of pregnancy.

Optic cup-like organoids were first generated by the Sasai group, using mESCs in 2011, then human ESCs in 2012.[289–292] Their work showed self-organisation of cells into distinct layers reflecting the NR and RPE of the developing optic cup, although with inconsistent efficiency in forming stratified retina, which may have been the result of missing surface ectodermal signalling molecules from the presumptive lens.[35,291,293,294] Modifications (such as addition of retinoic acid receptor antagonist AGN193109 at early stages to improve yield of cells expressing *Rax*[295]) have led to numerous protocols shown to recapitulate stages of early embryonic eye development using transcriptomic analysis.[296–308]

One major criticism of organoids is the heterogeneity in differentiation efficiency observed within and between cell lines, partially due to differences in endogenous genetics and epigenetics.[293,309–317] Attempts to combat background genetic/epigenetic variability include creation of isogenic lines through CRISPR/Cas9 gene editing to induce/correct patient mutations, to reduce noise and generate more reliable data.[318,319] It should also be noted that lack of additional signals such as the embryonic axis means organoid structures are often highly heterogeneous, with random relative positioning of tissue regions, such as RPE and NR in retinal organoids.[288] Additionally, current constraints of *in vitro* organoid modelling include lack of vasculature thus poor nutrient diffusion, and absent surrounding tissues, which may result in loss of vital external developmental cues.[35,288,294] However, advances in co-culturing techniques and organ-on-a-chip technologies may provide a solution for more complex cellular modelling, by facilitating signalling between different cell types, and a more vasculature-like perfusion of nutrients across organoids.[286,320,321] The potential of these more advanced culturing systems for drug toxicity screening has been demonstrated through chloroquine and gentamicin treatment to induce retinopathies, although successful use of retinal organoids in drug discovery screens has yet to be reported.[320,322]

*Understanding molecular pathways in microphthalmia.* Studying the genetic basis of microphthalmia directly in human cells has a clear advantage over animal models, as genetic pathways and potential therapies can be studied without possibility for divergence or functional redundancy. By modelling gene function at a cellular level in human tissue, a more translational understanding of microphthalmia pathogenesis can be established. Homozygous frameshift mutations in *FAT1* have been associated with colobomatous microphthalmia, ptosis, syndactyly and facial dysmorphism in patients.[323] Study of RPE cells showed FAT1 localised to cell–cell junctions required for optic fissure fusion in eye development, and knockdown of *FAT1* using short hairpin RNA (shRNA) resulted in disruption of β-Catenin, ZO-1 and F-actin fibres at junction sites, and a failure of RPE cells to form an organised epithelial monolayer.[323] These disruptions were not observed in differentiating RPE tissue from *in vivo* $Fat^{-/-}$ mouse models, although mice did display a microphthalmia and coloboma phenotype. The ability to study molecular pathways in human cells allows for clarity in where molecular mechanisms are conserved and where they deviate from animal models.

In 2014, Phillips *et al.* generated optic vesicle-like models of early eye development with iPSCs derived from a microphthalmic patient with a homozygous $VSX2^{R200Q}$ mutation.[324] Molecular techniques including RNAseq and ChIPseq identified that WNT pathway components were direct targets for

*VSX2* DNA binding and transcriptional downregulation in retina development. Upregulation of the WNT pathway in the *VSX2*-disrupted models resulted in erroneous RPE differentiation, partially rescued by pharmacological inhibition of the WNT pathway.[29,324] Furthermore, supplementation with growth factors including FGF9 partially rescued the phenotype in mutant organoids, although suppression of FGF9 alone in wildtype organoids did not produce a phenotype, indicating redundancy of pathways in retinal development.[325] The valuable insights gained from this study demonstrate the ability of these 3D cellular models to advance our understanding of how individual genes function in human eye development and which pathways are disrupted in microphthalmic patients with the corresponding variant.

*Modelling variable ocular and syndromic phenotypes.* Studying the impact of disease-causing proteins on cell function can elucidate the effect of different alleles and genetic/epigenetic background on phenotypic variability. *FZD5* is a transmembrane receptor which regulates WNT signalling in the early optic vesicle.[326] Investigation of *FZD5* in HEK (human embryonic kidney) cells revealed that transfection of microphthalmic patient-originated cDNA produced truncated protein which did not localise to the outer cell membrane or mediate WNT signalling like wildtype protein, instead inhibiting the pathway due to antagonistic competition, resulting in a dominant-negative effect.[326] Heterozygous pathogenic mutations in *FZD5* have predominantly been identified in coloboma cohorts, but in one large family with the frameshift variant c.656del CinsAG, p.Ala219Glufs*49, two members were non-penetrant.[326,327] Animal models also display variable MAC phenotypes, such as zebrafish with *fzd5* knockdown or overexpression of mutant protein,[326] and $Fzd5^{-/-}$ mice which exhibit 50% penetrance of mild microphthalmia/coloboma.[328] This may be the result of overlapping function with *Fzd8*, as triallelic $Fzd5^{-/-}$;$Fzd8^{+/-}$ mutants develop severe retinal coloboma and microphthalmia with full penetrance. No *FZD8* protective alleles were identified in non-penetrant individuals from whole exome sequencing; however, further analysis of human cellular models could help identify other effects of genetic background or compensatory gene mechanisms on *FZD5* function.

Cell models are not representative of the whole organism and hence it is more difficult to explore systemic manifestations. However, they can be used to extrapolate tissue involvement through investigating the transcriptome and molecular pathways, as gene ontology tools can link developmental pathways in other parts of the body to shed light on syndromic features. For example, transcriptomic analysis of zebrafish optic fissure tissue identified differentially expressed genes between optic fissure and dorsal retina which are known to be involved in heart development (*tbx2a*/*3a*), providing new pathways to explore through cellular research.[329] In addition, patient-derived fibroblasts with a heterozygous splice-site *NAA10* variant show reduced cell proliferation and disrupted retinoic acid signalling, which may explain both microphthalmia and extraocular growth defects observed in patients with syndromic microphthalmia 1 (Lenz microphthalmia syndrome – OMIM #309800).[330]

*Identification of novel variants with cellular models.* Converting genomic annotations from animal models to humans can be misleading, due to divergence in genetic regulation of eye development. Evidence from human cellular studies can therefore be more practical for identifying and validating novel candidates. Generation of transcriptomic and epigenomic data from human-derived 3D microphthalmic models could provide datasets from which pathway components and disease mechanisms can be identified, providing both validation for putative genetic causes found in patients as well as resources to discover new genes to screen in microphthalmic cohorts by next-generation sequencing. To date, few 3D cellular disease models have been generated, but as protocols grow more efficient, and multi-omic technologies become more affordable, cellular modelling could become an effective strategy for detecting molecular causes of microphthalmia.

*Modelling environmental causes of microphthalmia in cells.* The effect of exogenous chemicals on cellular function can be quickly investigated in 2D cell culture, due to efficient diffusion of compounds. Retinoic acid treatment of ARPE-19 cells induced dose-dependent increase in *RAR*β mRNA and protein within 24 h, which was inhibited by treatment with antagonist LE135.[331] Utilising more complex 3D models, toxins and potential treatments can be applied directly to mature human ocular tissues without bioavailability and drug metabolism issues, allowing greater understanding of effect on ocular development and its regulation. Importantly, using

patient-derived cells can shed light on the effects of environmental factors on different genetic backgrounds and particular modifiers, leading to more precise clinical advice and care.

## Conclusion

Through work studying patients, animals and cellular models, considerable progress has been made in understanding the genetic basis of eye development, and how dysregulation of molecular pathways can result in microphthalmia. Over 90 monogenic causes of microphthalmia have been identified, and yet molecular diagnosis can only be made in less than 10% of unilateral patients and few genotype–phenotype correlations have been established. Numerous animal models for microphthalmia have been generated; however, many still have not been genetically characterised (including 25% of mouse lines) and several causative microphthalmia genes have not been disrupted in animals.[25] Many known human genetic variants have not been studied in detail due to a lack of a corresponding model. Screening animal lines for novel candidate genes/genetic modifiers and functionally validating variants identified in patients could increase understanding of the roles of disease-causing genes, improve molecular diagnostic rates and provide patients with appropriate multidisciplinary care and genetic counselling by clarifying genotype–phenotype relationships.

Cutting-edge developments in 3D cellular modelling techniques show potential as an animal-free approach for deepening understanding of human eye development and molecular disease mechanisms at early stages of oculogenesis, which would otherwise be inaccessible to study, as well as providing promising results in understanding patient-specific mutations and developing novel therapeutics.[29,264] Nevertheless, whole-organism modelling in animals is necessary for understanding the systemic effect of gene disruption and screening drugs, particularly when studying syndromic microphthalmia.[155] Research on a combination of animal and cellular models is essential to gaining a clear picture of the molecular basis of microphthalmia and developing life-changing treatments.

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## Author contributions

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Mariya Moosajee – Conceptualisation; Funding acquisition; Supervision; Writing-review & editing

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## Supplemental material

Supplemental material for this article is available online.

## References

1. Richardson R, Sowden J, Gerth-Kahlert C, *et al.* Clinical utility gene card for: non-syndromic microphthalmia including next-generation sequencing-based approaches. *Eur J Hum Genet* 2017; 25: 512.
2. Williamson KA and FitzPatrick DR. The genetic architecture of microphthalmia, anophthalmia and coloboma. *Eur J Med Genet* 2014; 57: 369–380.
3. Harding P and Moosajee M. The molecular basis of human anophthalmia and microphthalmia. *J Dev Biol* 2019; 7: 16.

4. Verma AS and FitzPatrick DR. Anophthalmia and microphthalmia. *Orphanet J Rare Dis* 2007; 2: 47.
5. Ragge NK, Subak-Sharpe ID and Collin JRO. A practical guide to the management of anophthalmia and microphthalmia. *Eye* 2007; 21: 1290–1300.
6. Shah SP, Taylor AE, Sowden JC, *et al.* Anophthalmos, microphthalmos, and coloboma in the United Kingdom: clinical features, results of investigations, and early management. *Ophthalmology* 2012; 119: 362–368.
7. Chassaing N, Causse A, Vigouroux A, *et al.* Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. *Clin Genet* 2014; 86: 326–334.
8. Busby A, Dolk H and Armstrong B. Eye anomalies: seasonal variation and maternal viral infections. *Epidemiology* 2005; 16: 317–322.
9. Gerth-Kahlert C, Williamson K, Ansari M, *et al.* Clinical and mutation analysis of 51 probands with anophthalmia and/or severe microphthalmia from a single center. *Mol Genet Genomic Med* 2013; 1: 15–31.
10. Schneider A, Bardakjian T, Reis LM, *et al.* Novel SOX2 mutations and genotype-phenotype correlation in anophthalmia and microphthalmia. *Am J Med Genet A* 2009; 149A: 2706–2715.
11. Biesecker LG and Spinner NB. A genomic view of mosaicism and human disease. *Nat Rev Genet* 2013; 14: 307–320.
12. Ragge NK, Brown AG, Poloschek CM, *et al.* Heterozygous mutations of OTX2 cause severe ocular malformations. *Am J Hum Genet* 2005; 76: 1008–1022.
13. Faivre L, Williamson KA, Faber V, *et al.* Recurrence of SOX2 anophthalmia syndrome with gonosomal mosaicism in a phenotypically normal mother. *Am J Med Genet A* 2006; 140A: 636–639.
14. Chassaing N, Gilbert-Dussardier B, Nicot F, *et al.* Germinal mosaicism and familial recurrence of a SOX2 mutation with highly variable phenotypic expression extending from AEG syndrome to absence of ocular involvement. *Am J Med Genet A* 2007; 143A: 289–291.
15. Burkitt Wright EMM, Perveen R, Bowers N, *et al.* VSX2 in microphthalmia: a novel splice site mutation producing a severe microphthalmia phenotype. *Br J Ophthalmol* 2010; 94: 386–388.
16. Plaisancié J, Ceroni F, Holt R, *et al.* Genetics of anophthalmia and microphthalmia. Part 1: non-syndromic anophthalmia/microphthalmia. *Hum Genet* 2019; 138: 799–830.
17. Chao R, Nevin L, Agarwal P, *et al.* A male with unilateral microphthalmia reveals a role for TMX3 in eye development. *PLoS One* 2010; 5: e10565.
18. Plaisancie J, Calvas P and Chassaing N. Genetic advances in microphthalmia. *J Pediatr Genet* 2016; 5: 184–188.
19. Acuna-Hidalgo R, Veltman JA and Hoischen A. New insights into the generation and role of de novo mutations in health and disease. *Genome Biol* 2016; 17: 1–19.
20. Jimenez NL, Flannick J, Yahyavi M, *et al.* Targeted 'next-generation' sequencing in anophthalmia and microphthalmia patients confirms SOX2, OTX2 and FOXE3 mutations. *BMC Med Genet* 2011; 12: 172.
21. Kallen B, Robert E and Harris J. The descriptive epidemiology of anophthalmia and microphthalmia. *Int J Epidemiol* 1996; 25: 1009–1016.
22. Forrester MB and Merz RD. Descriptive epidemiology of anophthalmia and microphthalmia, Hawaii, 1986–2001. *Birth Defects Res A Clin Mol Teratol* 2006; 76: 187–192.
23. Riera M, Wert A, Nieto I, *et al.* Panel-based whole exome sequencing identifies novel mutations in microphthalmia and anophthalmia patients showing complex Mendelian inheritance patterns. *Mol Genet Genomic Med* 2017; 5: 709–719.
24. Cavodeassi F and Wilson SW. Looking to the future of zebrafish as a model to understand the genetic basis of eye disease. *Hum Genet* 2019; 138: 993–1000.
25. Graw J. Mouse models for microphthalmia, anophthalmia and cataracts. *Hum Genet* 2019; 138: 1007–1018.
26. Richardson R, Tracey-White D, Webster A, *et al.* The zebrafish eye-a paradigm for investigating human ocular genetics. *Eye* 2017; 31: 68–86.
27. Zuber ME. Eye field specification in Xenopus laevis. *Curr Top Dev Biol* 2010; 93: 29–60.
28. Slijkerman RWN, Song F, Astuti GDN, *et al.* The pros and cons of vertebrate animal models for functional and therapeutic research on

inherited retinal dystrophies. *Prog Retin Eye Res* 2015; 48: 137–159.

29. Capowski EE, Wright LS, Liang K, *et al.* Regulation of WNT signaling by VSX2 during optic vesicle patterning in human induced pluripotent stem cells. *Stem Cells* 2016; 34: 2625–2634.
30. Chow RL and Lang RA. Early eye development in vertebrates. *Annu Rev Cell Dev Biol* 2001; 17: 255–296.
31. Sinn R and Wittbrodt J. An eye on eye development. *Mech Dev* 2013; 130: 347–358.
32. Zagozewski J, Zhang Q and Eisenstat D. Genetic regulation of vertebrate eye development. *Clin Genet* 2014; 86: 453–460.
33. Zuber ME, Gestri G, Viczian AS, *et al.* Specification of the vertebrate eye by a network of eye field transcription factors. *Development* 2003; 130: 5155–5167.
34. Cvekl A and Ashery-Padan R. The cellular and molecular mechanisms of vertebrate lens development. *Development* 2014; 141: 4432–4447.
35. Fuhrmann S. Eye morphogenesis and patterning of the optic vesicle. *Curr Top Dev Biol* 2010; 93: 61–84.
36. Patel A and Sowden JC. Genes and pathways in optic fissure closure. *Semin Cell Dev Biol.* Epub ahead of print 6 December 2017. DOI: 10.1016/j.semcdb.2017.10.010.
37. Cvekl A, McGreal R and Liu W. Lens development and crystallin gene expression. *Prog Mol Biol Transl Sci* 2015; 134: 129–167.
38. Graw J. Eye development. *Curr Top Dev Biol* 2010; 90: 343–386.
39. Reis LM and Semina EV. Conserved genetic pathways associated with microphthalmia, anophthalmia, and coloboma. *Birth Defects Res C Embryo Today* 2015; 105: 96–113.
40. Deml B, Kariminejad A, Borujerdi RHR, *et al.* Mutations in MAB21L2 result in ocular coloboma, microcornea and cataracts. *PLoS Genet* 2015; 11: 1–26.
41. Chhetri J, Jacobson G and Gueven N. Zebrafish—on the move towards ophthalmological research. *Eye* 2014; 28: 367–380.
42. Kimmel CB, Ballard WW, Kimmel SR, *et al.* Stages of embryonic development of the zebrafish. *Dev Dyn* 1995; 203: 253–310.
43. Ledford KL, Martinez-De Luna RI, Theisen MA, *et al.* Distinct cis-acting regions control six6 expression during eye field and optic cup stages of eye formation. *Dev Biol* 2017; 426: 418–428.
44. Holt C. Cell movements in Xenopus eye development. *Nature* 1980; 287: 850–852.
45. Feldman JD. Retino-tectal projections from half-ventral and half-dorsal eye rudiments in Xenopus. *J Embryol Exp Morphol* 1978; 46: 89–97.
46. Henry JJ, Wever JM, Natalia Vergara M, *et al.* Xenopus, an ideal vertebrate system for studies of eye development and regeneration. In: Tsonis PA (ed.) *Animal models in eye research*. Elsevier Ltd, 2008, pp.57–92.
47. Bovolenta P and Martinez-Morales J-R. Genetics of congenital eye malformations: insights from chick experimental embryology. *Hum Genet*. Epub ahead of print 6 July 2018. DOI: 10.1007/s00439-018-1900-5.
48. Sghari S and Gunhaga L. Temporal requirement of mab21l2 during eye development in chick reveals stage-dependent functions for retinogenesis. *Investig Ophthalmol Vis Sci* 2018; 59: 3869–3878.
49. Kennelly K, Brennan D, Chummun K, *et al.* Histological characterisation of the ethanol-induced microphthalmia phenotype in a chick embryo model system. *Reprod Toxicol* 2011; 32: 227–234.
50. Hallsson JH, Haflidadóttir BS, Stivers C, *et al.* The basic helix-loop-helix leucine zipper transcription factor Mitf is conserved in Drosophila and functions in eye development. *Genetics* 2004; 167: 233–241.
51. Taranova OV, Magness ST, Fagan BM, *et al.* SOX2 is a dose-dependent regulator of retinal neural progenitor competence. *Genes Dev* 2006; 20: 1187–1202.
52. Bernard C, Kim H-T, Torero Ibad R, *et al.* Graded Otx2 activities demonstrate dose-sensitive eye and retina phenotypes. *Hum Mol Genet* 2014; 23: 1742–1753.
53. Matsuo I, Kuratani S, Kimura C, *et al.* Mouse Otx2 functions in the formation and patterning of rostral head. *Genes Dev* 1995; 9: 2646–2658.
54. Bando H, Gergics P, Bohnsack BL, *et al.* Otx2b mutant zebrafish have pituitary, eye and mandible defects that model mammalian disease. *Hum Mol Genet* 2020; 29: 1648–1657.
55. Plageman TF Jr and Lang RA. Generation of an Rx-tTA: TetOp-Cre knock-in mouse line for doxycycline regulated cre activity in the Rx expression domain. *PLoS One* 2012; 7: e50426.

56. Chase HB. Studies on an anophthalmic strain of mice. IV. A second major gene for anophthalmia. *Genetics* 1944; 29: 264–269.

57. Yin J, Morrissey ME, Shine L, *et al.* Genes and signaling networks regulated during zebrafish optic vesicle morphogenesis. *BMC Genomics* 2014; 15: 825.

58. Loosli F, Staub W, Finger-Baier KC, *et al.* Loss of eyes in zebrafish caused by mutation of chokh/rx3. *EMBO Rep* 2003; 4: 894–899.

59. Prochazka M, Leiter EH, Cook S, *et al.* Or-2J; a new remutation at ocular retardation (or) associated with sterility. *Mouse Genome* 1990; 87–93.

60. Zou C and Levine EM. Vsx2 controls eye organogenesis and retinal progenitor identity via homeodomain and non-homeodomain residues required for high affinity DNA binding. *PLoS Genet* 2012; 8: e1002924.

61. Truslove GM. A gene causing ocular retardation in the mouse. *J Embryol Exp Morphol* 1962; 10: 652–660.

62. Rossant J. A new allele at the Pax6 locus from the center of modeling human disease. *MGI Direct Data Submission* 2003.

63. Favor J, Peters H, Hermann T, *et al.* Molecular characterization of Pax6 2Neu through Pax6 10Neu : an extension of the Pax6 allelic series and the identification of two possible hypomorph alleles in the mouse Mus musculus. *Genet Soc Am* 2001; 159: 1689–1700.

64. Favor J and Neuhäuser-Klaus A. Saturation mutagenesis for dominant eye morphological defects in the mouse Mus musculus. *Mamm Genome* 2000; 11: 520–525.

65. Favor J, Bradley A, Conte N, *et al.* Analysis of Pax6 contiguous gene deletions in the mouse, Mus musculus, identifies regions distinct from Pax6 responsible for extreme small-eye and belly-spotting phenotypes. *Genetics* 2009; 182: 1077–1088.

66. Favor J, Grimes P, Neuhäuser-Klaus A, *et al.* The mouse Cat4 locus maps to chromosome 8 and mutants express lens-corneal adhesion. *Mamm Genome* 1997; 8: 403–406.

67. Graw J, Löster J, Puk O, *et al.* Three novel Pax6 alleles in the mouse leading to the same small-eye phenotype caused by different consequences at target promoters. *Investig Ophthalmol Vis Sci* 2005; 46: 4671–4683.

68. Puk O, Yan X, Sabrautzki S, *et al.* Novel small-eye allele in paired box gene 6 (Pax6) is caused by a point mutation in intron 7 and creates a new exon. *Mol Vis* 2013; 19: 877–884.

69. Lyon MF, Bogani D, Boyd Y, *et al.* Further genetic analysis of two autosomal dominant mouse eye defects, Ccw and Pax6coop. *Mol Vis* 2000; 6: 199–203.

70. Thaung C, West K, Clark BJ, *et al.* Novel ENU-induced eye mutations in the mouse: models for human eye disease. *Hum Mol Genet* 2002; 11: 755–767.

71. European Mouse Mutant Archive. Information obtained from the European Mouse Mutant Archive (EMMA). (Unpublished). 2003-2013.

72. RIKEN BioResource Center/RIKEN Genomic Sciences Center. A large scale mutagenesis PROGRAM in RIKEN GSC. PhenoSITE, http://www.brc.riken.jp/lab/gsc/mouse/ (2008)

73. Theiler K, Varnum DS and Stevens LC. Development of Dickie's small eye, a mutation in the house mouse. *Anat Embryol (Berl)* 1979; 155: 81–86.

74. Hogan BLM, Horsburgh G, Cohen J, *et al.* Small eyes (Sey): a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse. *J Exp Morphol* 1986; 97: 95–110.

75. Ramaesh T, Collinson JM, Ramaesh K, *et al.* Corneal abnormalities in Pax6+/- small eye mice mimic human aniridia-related keratopathy. *Investig Ophthalmol Vis Sci* 2003; 44: 1871–1878.

76. Hill RE, Favor J, Hogan BLMM, *et al.* Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature* 1991; 354: 522–525.

77. Klimova L and Kozmik Z. Stage-dependent requirement of neuroretinal Pax6 for lens and retina development. *Development* 2014; 141: 1292–1302.

78. Carbe C, Hertzler-Schaefer K and Zhang X. The functional role of the Meis/Prep-binding elements in Pax6 locus during pancreas and eye development. *Dev Biol* 2012; 363: 320–329.

79. Kroeber M, Davis N, Holzmann S, *et al.* Reduced expression of Pax6 in lens and cornea of mutant mice leads to failure of chamber angle development and juvenile glaucoma. *Hum Mol Genet* 2010; 19: 3332–3342.

80. Shaham O, Smith AN, Robinson ML, *et al.* Pax6 is essential for lens fiber cell differentiation. *Development* 2009; 136: 2567–2578.

81. Davis N, Yoffe C, Raviv S, *et al.* Pax6 dosage requirements in iris and ciliary body differentiation. *Dev Biol* 2009; 333: 132–142.

82. Kleinjan DA, Bancewicz RM, Gautier P, *et al.* Subfunctionalization of duplicated zebrafish pax6 genes by cis-regulatory divergence. *PLoS Genet* 2008; 4: e29.

83. Nakayama T, Fisher M, Nakajima K, *et al.* Xenopus pax6 mutants affect eye development and other organ systems, and have phenotypic similarities to human aniridia patients. *Dev Biol* 2015; 408: 328–344.

84. Ruiz A, Mark M, Jacobs H, *et al.* Retinoid content, visual responses, and ocular morphology are compromised in the retinas of mice lacking the retinol-binding protein receptor, STRA6. *Invest Ophthalmol Vis Sci* 2012; 53: 3027–3039.

85. Shi Y, Obert E, Rahman B, *et al.* The retinol binding protein receptor 2 (Rbpr2) is required for photoreceptor outer segment morphogenesis and visual function in zebrafish. *Sci Rep* 2017; 7: 16207.

86. Sanyal S and Hawkins RK. Dysgenetic lens (dyl) - a new gene in the mouse. *Investig Ophthalmol Vis Sci* 1979; 18: 642–645.

87. Medina-Martinez O, Brownell I, Amaya-Manzanares F, *et al.* Severe defects in proliferation and differentiation of lens cells in Foxe3 null mice. *Mol Cell Biol* 2005; 25: 8854–8863.

88. Krall M, Htun S, Anand D, *et al.* A zebrafish model of foxe3 deficiency demonstrates lens and eye defects with dysregulation of key genes involved in cataract formation in humans. *Hum Genet* 2018; 137: 315–328.

89. Dunn NR, Winnier GE, Hargett LK, *et al.* Haploinsufficient phenotypes in Bmp4 heterozygous null mice and modification by mutations in Gli3 and Alx4. *Dev Biol* 1997; 188: 235–247.

90. Zouvelou V, Passa O, Segklia K, *et al.* Generation and functional characterization of mice with a conditional BMP7 allele. *Int J Dev Biol* 2009; 53: 597–603.

91. Dudley AT, Lyons KM and Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev* 1995; 9: 2795–2807.

92. Oxburgh L, Dudley AT, Godin RE, *et al.* BMP4 substitutes for loss of BMP7 during kidney development. *Dev Biol* 2005; 286: 637–646.

93. French CR, Stach TR, March LD, *et al.* Apoptotic and proliferative defects characterize ocular development in a microphthalmic BMP model. *Investig Ophthalmol Vis Sci* 2013; 54: 4636–4647.

94. Pant SD, March LD, Famulski JK, *et al.* Molecular mechanisms regulating ocular apoptosis in zebrafish gdf6a mutants. *Investig Ophthalmol Vis Sci* 2013; 54: 5871–5879.

95. den Hollander AI, Biyanwila J, Kovach P, *et al.* Genetic defects of GDF6 in the zebrafish out of sight mutant and in human eye developmental anomalies. *BMC Genet* 2010; 11: 102.

96. Okada I, Hamanoue H, Terada K, *et al.* SMOC1 is essential for ocular and limb development in humans and mice. *Am J Hum Genet* 2011; 88: 30–41.

97. Wang YP, Dakubo G, Howley P, *et al.* Development of normal retinal organization depends on sonic Hedgehog signaling from ganglion cells. *Nat Neurosci* 2002; 5: 831–832.

98. Dakubo GD, Wang YP, Mazerolle C, *et al.* Retinal ganglion cell-derived sonic Hedgehog signaling is required for optic disc and stalk neuroepithelial cell development. *Development* 2003; 130: 2967–2980.

99. Ratzka A, Kalus I, Moser M, *et al.* Redundant function of the heparan sulfate 6-O-endosulfatases Sulf1 and Sulf2 during skeletal development. *Dev Dyn* 2008; 237: 339–353.

100. Grobe K, Inatani M, Pallerla SR, *et al.* Cerebral hypoplasia and craniofacial defects in mice lacking heparan sulfate Ndst1 gene function. *Development* 2005; 132: 3777–3786.

101. Bulgakov OV, Eggenschwiler JT, Hong DH, *et al.* FKBP8 is a negative regulator of mouse sonic Hedgehog signaling in neural tissues. *Development* 2004; 131: 2149–2159.

102. Chan JA, Balasubramanian S, Witt RM, *et al.* Proteoglycan interactions with Sonic Hedgehog specify mitogenic responses. *Nat Neurosci* 2009; 12: 409–417.

103. Brand M, Heisenberg CP, Warga RM, *et al.* Mutations affecting development of the midline and general body shape during zebrafish embryogenesis. *Development* 1996; 123: 129–142.

104. Stenkamp DL, Frey RA, Mallory DE, *et al.* Embryonic retinal gene expression insonic-you mutant zebrafish. *Dev Dyn* 2002; 225: 344–350.

105. Gath N and Gross JM. Zebrafish mab21l2 mutants possess severe defects in optic cup morphogenesis, lens and cornea development. *Dev Dyn* 2019; 248: 514–529.

106. Bankhead EJ, Colasanto MP, Dyorich KM, *et al.* Multiple requirements of the focal dermal hypoplasia gene porcupine during ocular morphogenesis. *Am J Pathol* 2015; 185: 197–213.

107. Phillips RJS. Blebbed, bl. *Mouse News Lett* 1970; 42: 26.

108. Kile BT, Hentges KE, Clark AT, *et al.* Functional genetic analysis of mouse chromosome 11. *Nature* 2003; 425: 81–86.

109. Beck TF, Shchelochkov OA, Yu Z, *et al.* Novel frem1-related mouse phenotypes and evidence of genetic interactions with gata4 and slit3. *PLoS One* 2013; 8: e58830.

110. Beck TF, Veenma D, Shchelochkov OA, *et al.* Deficiency of FRAS1-related extracellular matrix 1 (FREM1) causes congenital diaphragmatic hernia in humans and mice. *Hum Mol Genet* 2013; 22: 1026–1038.

111. Sang L, Miller JJ, Corbit KC, *et al.* Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. *Cell* 2011; 145: 513–528.

112. Favor J, Gloeckner CJ, Janik D, *et al.* Type IV procollagen missense mutations associated with defects of the eye, vascular stability, the brain, kidney function and embryonic or postnatal viability in the mouse, Mus musculus: an extension of the Col4a1 allelic series and the identification of the first two Col4a2 mutant alleles. *Genetics* 2007; 175: 725–736.

113. Labelle-Dumais C, Dilworth DJ, Harrington EP, *et al.* COL4A1 mutations cause ocular dysgenesis, neuronal localization defects, and myopathy in mice and Walker-Warburg syndrome in humans. *PLoS Genet* 2011; 7: e1002062.

114. Lee J, Cox BD, Daly CMS, *et al.* An ENU mutagenesis screen in zebrafish for visual system mutants identifies a novel splice-acceptor site mutation in patched2 that results in colobomas. *Investig Ophthalmol Vis Sci* 2012; 53: 8214–8221.

115. Lo C. Information submitted by the NHLBI Cardiovascular Development Consortium (CvDC), Bench to Bassinet Program (B2B/CvDC). *MGI Direct Data Submiss (B2B/CvDC)* 2011-2015.

116. Collery RF, Volberding PJ, Bostrom JR, *et al.* Loss of zebrafish Mfrp causes nanophthalmia, hyperopia, and accumulation of subretinal macrophages. *Investig Ophthalmol Vis Sci* 2016; 57: 6805–6814.

117. Nair KS, Hmani-Aifa M, Ali Z, *et al.* Alteration of the serine protease PRSS56 causes angle-closure glaucoma in mice and posterior microphthalmia in humans and mice. *Nat Genet* 2011; 43: 579–584.

118. Yan X, Sabrautzki S, Horsch M, *et al.* Peroxidasin is essential for eye development in the mouse. *Hum Mol Genet* 2014; 23: 5597–5614.

119. Gage PJ, Suh H and Camper SA. Dosage requirement of Pitx2 for multiple organs. *Development* 1999; 126: 4643–4651.

120. Liu P and Johnson RL. Lmx1b is required for murine trabecular meshwork formation and for maintenance of corneal transparency. *Dev Dyn* 2010; 239: 2161–2171.

121. Hendee KE, Sorokina EA, Muheisen SS, *et al.* PITX2 deficiency and associated human disease: insights from the zebrafish model. *Hum Mol Genet* 2018; 27: 1675–1695.

122. Rosemann M, Ivashkevich A, Favor J, *et al.* Microphthalmia, parkinsonism, and enhanced nociception in Pitx3 416insG mice. *Mamm Genome* 2010; 21: 13–27.

123. Varnum DS and Stevens LC. Aphakia, a new mutation in the mouse. *J Hered* 1968; 59: 147–150.

124. Hetherington C. Microphthalmic mutant in CBA strain. *Mouse News Lett* 1976; 54: 34.

125. Zimring DC, Lamoreux ML, Millichamp NJ, *et al.* Microphthalmia cloudy-eye ($mi^{ce}$): a new murine allele. *J Hered* 1996; 87: 334–338.

126. Steingrímsson E, Tessarollo L, Pathak B, *et al.* Mitf and Tfe3, two members of the Mitf-Tfe family of bHLH-Zip transcription factors, have important but functionally redundant roles in osteoclast development. *Proc Natl Acad Sci U S A* 2002; 99: 4477–4482.

127. Munford RE. Mutation at mi locus. *Mouse News Lett* 1965; 33: 52.

128. Hallsson JH, Favor J, Hodgkinson C, *et al.* Genomic, transcriptional and mutational analysis of the mouse microphthalmia locus. *Genetics* 2000; 155: 291–300.

129. Hollander WF. mi. *Mouse News Lett* 1964; 30: 29.

130. Packer SO. The eye and skeletal effects of two mutant alleles at the microphthalmia locus of Mus musculus. *J Exp Zool* 1967; 165: 21–45.

131. Krakowsky JM, Boissy RE, Neumann JC, *et al.* A DNA insertional mutation results in microphthalmia in transgenic mice. *Transgenic Res* 1993; 2: 14–20.

132. Southard JL. Red-eyed white (mi). *Mouse News Lett* 1974; 51: 23.

133. Stelzner KF. Dominant mutation resembling Mi. *Mouse News Lett* 1964; 31: 40–41.

134. Hansdottir AG, Pálsdóttir K, Favor J, *et al.* The novel mouse microphthalmia mutations Mitfmi-enu5 and Mitfmi-bcc2 produce dominant negative Mitf proteins. *Genomics* 2004; 83: 932–935.

135. Silvers K. *The coat colors of mice*. Springer-Verlag, http://www.informatics.jax.org/wksilvers/ (1979, accessed 15 May 2020).

136. West JD, Fisher G, Loutit JF, *et al.* A new allele of microphthalmia induced in the mouse: microphthalmia - defective iris (midi). *Genet Res* 1985; 46: 309–324.

137. Zarbalis K, Siegenthaler JA, Choe Y, *et al.* Cortical dysplasia and skull defects in mice with a Foxc1 allele reveal the role of meningeal differentiation in regulating cortical development. *Proc Natl Acad Sci U S A* 2007; 104: 14002–14007.

138. Xia CH, Liu H, Chang B, *et al.* Arginine 54 and tyrosine 118 residues of αA-crystallin are crucial for lens formation and transparency. *Investig Ophthalmol Vis Sci* 2006; 47: 3004–3010.

139. Graw J, Jana Löster Soewarto D, *et al.* Characterization of a new, dominant V124E mutation in the mouse αA-crystallin–encoding gene | IOVS | ARVO journals. *Invest Ophthalmol Vis Sci* 2001; 42: 2909–2915.

140. Xi JH, Bai F, Gross J, *et al.* Mechanism of small heat shock protein function in vivo: a knock-in mouse model demonstrates that the R49C mutation in αA-crystallin enhances protein insolubility and cell death. *J Biol Chem* 2008; 283: 5801–5814.

141. Brady JP, Garland D, Duglas-Tabor Y, *et al.* Targeted disruption of the mouse αA-crystallin gene induces cataract and cytoplasmic inclusion bodies containing the small heat shock protein αB-crystallin. *Proc Natl Acad Sci U S A* 1997; 94: 884–889.

142. Timmer JR, Mak TW, Manova K, *et al.* Tissue morphogenesis and vascular stability require the Frem2 protein, product of the mouse myelencephalic blebs gene. *Proc Natl Acad Sci U S A* 2005; 102: 11746–11750.

143. Curtain MM and Donahue LR. *A spontaneous mouse strain with cryptophthalmos*. The Jackson Laboratory, Craniofacial Mutant Resource, 2008.

144. Vierkotten J, Dildrop R, Peters T, *et al.* Ftm is a novel basal body protein of cilia involved in Shh signalling. *Development* 2007; 134: 2569–2577.

145. Delous M, Baala L, Salomon R, *et al.* The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet* 2007; 39: 875–881.

146. Shaheen R, Anazi S, Ben-Omran T, *et al.* Mutations in SMG9, encoding an essential component of nonsense-mediated decay machinery, cause a multiple congenital anomaly syndrome in humans and mice. *Am J Hum Genet* 2016; 98: 643–652.

147. Samuel A, Rubinstein AM, Azar TT, *et al.* Six3 regulates optic nerve development via multiple mechanisms. *Sci Rep* 2016; 6: 1–14.

148. Mouse Genome Informatics and the International Mouse Phenotyping Consortium (IMPC). Obtaining and loading phenotype annotations from the International Mouse Phenotyping Consortium (IMPC) Database. *Database Release* 2014.

149. Satz JS, Philp AR, Nguyen H, *et al.* Visual impairment in the absence of dystroglycan. *J Neurosci* 2009; 29: 13136–13146.

150. Munroe RJ, Prabhu V, Acland GM, *et al.* Mouse H6 Homeobox 1 (Hmx1) mutations cause cranial abnormalities and reduced body mass. *BMC Dev Biol* 2009; 9: 1–9.

151. Kim BJ, Zaveri HP, Shchelochkov OA, *et al.* An allelic series of mice reveals a role for RERE in the development of multiple organs affected in chromosome 1p36 deletions. *PLoS One* 2013; 8: e57460.

152. Plaster N, Sonntag C, Schilling TF, *et al.* REREa/Atrophin-2 interacts with histone deacetylase and Fgf8 signaling to regulate multiple processes of zebrafish development. *Dev Dyn* 2007; 236: 1891–1904.

153. Schilling TF, Piotrowski T, Grandel H, *et al.* Jaw and branchial arch mutants in zebrafish I: branchial arches. *Development* 1996; 123: 329–344.

154. Cheng CY, Wu JC, Tsai JW, *et al.* ENU mutagenesis identifies mice modeling Warburg micro syndrome with sensory axon degeneration caused by a deletion in Rab18. *Exp Neurol* 2015; 267: 143–151.

155. Krebs MP, Collin GB, Hicks WL, *et al.* Mouse models of human ocular disease for translational research. *PLoS One* 2017; 12: e0183837.

156. Zeiss CJ. Translational models of ocular disease. *Vet Ophthalmol* 2013; 16(Suppl. 1): 15–33.

157. Veleri S, Lazar CH, Chang B, *et al.* Biology and therapy of inherited retinal degenerative disease: insights from mouse models. *Dis Model Mech* 2015; 8: 109–129.

158. Vandamme TF. Use of rodents as models of human diseases. *J Pharm Bioallied Sci* 2014; 6: 2–9.

159. Samardzija M and Grimm C. Mouse models for cone degeneration. *Adv Exp Med Biol* 2014; 801: 567–573.

160. Yahyavi M, Abouzeid H, Gawdat G, *et al.* ALDH1A3 loss of function causes bilateral anophthalmia/microphthalmia and hypoplasia of the optic nerve and optic chiasm. *Hum Mol Genet* 2013; 22: 3250–3258.

161. Srour M, Caron V, Pearson T, *et al.* Gain-of-function mutations in RARB cause intellectual disability with progressive motor impairment. *Hum Mutat* 2016; 37: 786–793.

162. Medina-Martinez O, Shah R and Jamrich M. Pitx3 controls multiple aspects of lens development. *Dev Dyn* 2009; 238: 2193–2201.

163. Semina EV, Murray JC, Reiter R, *et al.* Deletion in the promoter region and altered expression of Pitx3 homeobox gene in aphakia mice. *Hum Mol Genet* 2000; 9: 1575–1585.

164. Wada K, Matsushima Y, Tada T, *et al.* Expression of truncated PITX3 in the developing lens leads to microphthalmia and aphakia in mice. *PLoS One* 2014; 9: e111432.

165. Ahmad N, Aslam M, Muenster D, *et al.* Pitx3 directly regulates Foxe3 during early lens development. *Int J Dev Biol* 2013; 57: 741–751.

166. Anand D, Agrawal SA, Slavotinek A, *et al.* Mutation update of transcription factor genes FOXE3, HSF4, MAF, and PITX3 causing cataracts and other developmental ocular defects. *Hum Mutat* 2018; 39: 471–494.

167. Steingrímsson E, Copeland NG and Jenkins NA. Melanocytes and the microphthalmia transcription factor network. *Annu Rev Genet* 2004; 38: 365–411.

168. Tajima T, Ohtake A, Hoshino M, *et al.* OTX2 loss of function mutation causes anophthalmia and combined pituitary hormone deficiency with a small anterior and ectopic posterior pituitary. *J Clin Endocrinol Metab* 2009; 94: 314–319.

169. Larder R, Kimura I, Meadows J, *et al.* Gene dosage of Otx2 is important for fertility in male mice. *Mol Cell Endocrinol* 2013; 377: 16–22.

170. Patat O, van Ravenswaaij-Arts CMA, Tantau J, *et al.* Otocephaly-dysgnathia complex: description of four cases and confirmation of the role of OTX2. *Mol Syndromol* 2013; 4: 302–305.

171. Hide T, Hatakeyama J, Kimura-Yoshida C, *et al.* Genetic modifiers of otocephalic phenotypes in Otx2 heterozygous mutant mice. *Development* 2002; 129: 4347–4357.

172. Chassaing N, Sorrentino S, Davis EE, *et al.* OTX2 mutations contribute to the otocephaly-dysgnathia complex. *J Med Genet* 2012; 49: 373–379.

173. Tajima T, Ishizu K and Nakamura A. Molecular and clinical findings in patients with LHX4 and OTX2 mutations. *Clin Pediatr Endocrinol* 2013; 22: 15–23.

174. Matsumoto R, Suga H, Aoi T, *et al.* Congenital pituitary hypoplasia model demonstrates hypothalamic OTX2 regulation of pituitary progenitor cells. *J Clin Invest*. Epub ahead of print 17 December 2019. DOI: 10.1172/JCI127378.

175. Hertwig P. Neue Mutationen und Koppelungsgruppen bei der Hausmaus. *Z Indukt Abstamm Vererbungsl* 1942; 80: 220–246.

176. Arnheiter H. The discovery of the microphthalmia locus and its gene, Mitf. *Pigment Cell Melanoma Res* 2010; 23: 729–735.

177. Acevedo-Arozena A, Wells S, Potter P, *et al.* ENU mutagenesis, a way forward to understand gene function. *Annu Rev Genomics Hum Genet* 2008; 9: 49–69.

178. Hodgkinson CA, Moore KJ, Nakayama A, *et al.* Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell* 1993; 74: 395–404.

179. White TW, Goodenough DA and Paul DL. Targeted ablation of connexin50 in mice results in microphthalmia and zonular pulverulent cataracts. *J Cell Biol* 1998; 143: 815–825.

180. White TW, Sellitto C, Paul DL, *et al.* Prenatal lens development in connexin43 and connexin50 double knockout mice. *Investig Ophthalmol Vis Sci* 2001; 42: 2916–2923.

181. Gerido DA, Sellitto C, Li L, *et al.* Genetic background influences cataractogenesis, but not lens growth deficiency, in Cx50-knockout mice. *Investig Ophthalmol Vis Sci* 2003; 44: 2669–2674.

182. Källén B and Tornqvist K. The epidemiology of anophthalmia and microphthalmia in Sweden. *Eur J Epidemiol* 2005; 20: 345–350.

183. Chambers TM, Agopian AJ, Lewis RA, *et al.* Epidemiology of anophthalmia and microphthalmia: prevalence and patterns in Texas, 1999-2009. *Am J Med Genet A* 2018; 176: 1810–1818.

184. Crider KS, Cleves MA, Reefhuis J, *et al.* Antibacterial medication use during pregnancy and risk of birth defects. *Arch Pediatr Adolesc Med* 2009; 163: 978–985.

185. Givens KT, Lee DA, Jones T, *et al.* Congenital rubella syndrome: ophthalmic manifestations and associated systemic disorders. *Br J Ophthalmol* 1993; 77: 358–363.

186. Frenkel LD, Keys MP, Hefteren SJ, *et al.* Unusual eye abnormalities associated with congenital cytomegalovirus infection. *Pediatrics* 1980; 66: 763–766.

187. Hoshino K, Nakane K and Kameyama Y. Influences of teratogenic agents on the manifestation of genetic malformations-effects of the maternal administration of trypan blue during pregnancy on the manifestation of genetic microphthalmia (mc) in mice. *Annu Rep Res Inst Environ Med Nagoya Univ* 1972; 19: 85–87.

188. Hoshino K, Nakane K and Kameyama Y. Influence of trypan blue on the manifestation of genetic microphthalmia (mic) in mouse fetuses. *Congenit Anom* 1976; 16: 105–110.

189. López-Escobar B, Cano DA, Rojas A, *et al.* The effect of maternal diabetes on the Wnt-PCP pathway during embryogenesis as reflected in the developing mouse eye. *DMM Dis Model Mech* 2015; 8: 157–168.

190. Maestro-de-las-Casas C, Pérez-Miguelsanz J, López-Gordillo Y, *et al.* Maternal folic acid-deficient diet causes congenital malformations in the mouse eye. *Birth Defects Res A Clin Mol Teratol* 2013; 97: 587–596.

191. Parnell SE, Dehart DB, Wills TA, *et al.* Maternal oral intake mouse model for fetal alcohol spectrum disorders: ocular defects as a measure of effect. *Alcohol Clin Exp Res* 2006; 30: 1791–1798.

192. Sulik KK and Johnston MC. Sequence of developmental alterations following acute ethanol exposure in mice: craniofacial features of the fetal alcohol syndrome. *Am J Anat* 1983; 166: 257–269.

193. Rutland CS, Jiang K, Soff GA, *et al.* Maternal administration of anti-angiogenic agents, TNP-470 and Angiostatin 4.5, induces fetal microphthalmia. *Mol Vis* 2009; 15: 1260–1269.

194. Tsutsui Y. Developmental disorders of the mouse brain induced by murine cytomegalovirus: animal models for congenital cytomegalovirus infection. *Pathol Int* 1995; 45: 91–102.

195. Chen B-Y, Chang H-H, Chiou H-L, *et al.* Influenza-B-virus-induced eye and brain malformations during early chick embryogenesis and localization of the viral RNA in specific areas. *J Biomed Sci* 2004; 11: 266–274.

196. Fadool JM and Dowling JE. Zebrafish: a model system for the study of eye genetics. *Prog Retin Eye Res* 2008; 27: 89–110.

197. Adler R and Canto-Soler MV. Molecular mechanisms of optic vesicle development: complexities, ambiguities and controversies. *Dev Biol* 2007; 305: 1–13.

198. Soules KA and Link BA. Morphogenesis of the anterior segment in the zebrafish eye. *BMC Dev Biol* 2005; 5: 12.

199. Moreno-Marmol T, Cavodeassi F and Bovolenta P. Setting eyes on the retinal pigment epithelium. *Front Cell Dev Biol* 2018; 6: 145.

200. Howe K, Clark MD, Torroja CF, *et al.* The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 2013; 496: 498–503.

201. Wu RS, Lam II, Clay H, *et al.* A rapid method for directed gene knockout for screening in G0 zebrafish. *Dev Cell* 2018; 46: 112–125.e4.

202. Williamson KA, Hall HN, Owen LJ, *et al.* Recurrent heterozygous PAX6 missense variants cause severe bilateral microphthalmia via predictable effects on DNA–protein interaction. *Genet Med*. Epub ahead of print 8 November 2019. DOI: 10.1038/s41436-019-0685-9.

203. Coutinho P, Pavlou S, Bhatia S, *et al.* Discovery and assessment of conserved Pax6 target genes and enhancers. *Genome Res* 2011; 21: 1349–1359.

204. Bill BR, Petzold AM, Clark KJ, *et al.* A primer for morpholino use in zebrafish. *Zebrafish* 2009; 6: 69–77.

205. Wang L, He F, Bu J, *et al.* ABCB6 mutations cause ocular coloboma. *Am J Hum Genet* 2012; 90: 40–48.

206. Huang X-F, Xiang L, Cheng W, *et al.* Mutation of IPO13 causes recessive ocular coloboma, microphthalmia, and cataract. *Exp Mol Med* 2018; 50: 53.

207. Bedell VM, Westcot SE and Ekker SC. Lessons from morpholino-based screening in zebrafish. *Brief Funct Genomics* 2011; 10: 181–188.

208. Kennedy BN, Stearns GW, Smyth VA, *et al.* Zebrafish rx3 and mab21l2 are required during eye morphogenesis. *Dev Biol* 2004; 270: 336–349.

209. Nelson SM, Park L and Stenkamp DL. Retinal homeobox 1 is required for retinal neurogenesis and photoreceptor differentiation in embryonic zebrafish. *Dev Biol* 2009; 328: 24–39.

210. Carnes MU, Liu YP, Allingham RR, *et al.* Discovery and functional annotation of SIX6 variants in primary open-angle glaucoma. *PLoS Genet* 2014; 10: e1004372.

211. Iglesias AI, Springelkamp H, van der Linde H, *et al.* Exome sequencing and functional analyses suggest that SIX6 is a gene involved in an altered proliferation-differentiation balance early in life and optic nerve degeneration at old age. *Hum Mol Genet* 2014; 23: 1320–1332.

212. Dee CT, Szymoniuk CR, Mills PED, *et al.* Defective neural crest migration revealed by a zebrafish model of Alx1-related frontonasal dysplasia. *Hum Mol Genet* 2013; 22: 239–251.

213. Kalaskar VK, Alur RP, Li LAK, *et al.* High-throughput custom capture sequencing identifies novel mutations in coloboma-associated genes: mutation in DNA-binding domain of retinoic acid receptor beta affects nuclear localization causing ocular coloboma. *Hum Mutat* 2020; 41: 678–695.

214. Asai-Coakwell M, French CR, Berry KM, *et al.* GDF6, a novel locus for a spectrum of ocular developmental anomalies. *Am J Hum Genet* 2007; 80: 306–315.

215. Chassaing N, Davis EE, McKnight KL, *et al.* Targeted resequencing identifies PTCH1 as a major contributor to ocular developmental anomalies and extends the SOX2 regulatory network. *Genome Res* 2016; 26: 474–485.

216. Barabino SM, Spada F, Cotelli F, *et al.* Inactivation of the zebrafish homologue of Chx10 by antisense oligonucleotides causes eye malformations similar to the ocular retardation phenotype. *Mech Dev* 1997; 63: 133–143.

217. Casey J, Kawaguchi R, Morrissey M, *et al.* First implication of STRA6 mutations in isolated anophthalmia, microphthalmia, and coloboma: a new dimension to the STRA6 phenotype. *Hum Mutat* 2011; 32: 1417–1426.

218. Slavotinek AM. Eye development genes and known syndromes. *Mol Genet Metab* 2011; 104: 448–456.

219. Chassaing N, Ragge N, Kariminejad A, *et al.* Mutation analysis of the STRA6 gene in isolated and non-isolated anophthalmia/microphthalmia. *Clin Genet* 2013; 83: 244–250.

220. Isken A, Golczak M, Oberhauser V, *et al.* RBP4 disrupts vitamin A uptake homeostasis in a STRA6-deficient animal model for Matthew-Wood syndrome. *Cell Metab* 2008; 7: 258–268.

221. Le HGT, Dowling JE and Cameron DJ. Early retinoic acid deprivation in developing zebrafish results in microphthalmia. *Vis Neurosci* 2012; 29: 219–228.

222. Tingaud-Sequeira A, Forgue J, André M, *et al.* Epidermal transient down-regulation ofretinol-binding protein 4 and mirror expression ofapolipoprotein Eb andestrogen receptor 2a during zebrafish fin and scale development. *Dev Dyn* 2006; 235: 3071–3079.

223. Biehlmaier O, Lampert JM, von Lintig J, *et al.* Photoreceptor morphology is severely affected in the β,β-carotene-15,15'-oxygenase (bcox) zebrafish morphant. *Eur J Neurosci* 2005; 21: 59–68.

224. Brastrom L, Dash S, Scott CA, *et al.* RNA binding proteins in eye development: RBM24a regulates sox2 and leads to microphthalmia and visual processing defects in zebrafish | IOVS | ARVO journals. *Invest Ophthalmol Vis Sci* 2019; 60: 4952.

225. Dash S, Brastrom LK, Patel SD, *et al.* The master transcription factor SOX2, mutated in anophthalmia/microphthalmia, is post-transcriptionally regulated by the conserved RNA-binding protein RBM24 in vertebrate eye development. *Hum Mol Genet* 2020; 29: 591–604.

226. Beqqali A, Bollen IAE, Rasmussen TB, *et al.* A mutation in the glutamate-rich region of RNA-binding motif protein 20 causes dilated cardiomyopathy through missplicing of titin and impaired Frank-Starling mechanism. *Cardiovasc Res* 2016; 112: 452–463.

227. Brauch KM, Karst ML, Herron KJ, *et al.* Mutations in ribonucleic acid binding protein gene cause familial dilated cardiomyopathy. *J Am Coll Cardiol* 2009; 54: 930–941.

228. Jackson D, Malka S, Harding P, *et al.* Molecular diagnostic challenges for non-retinal developmental eye disorders in the United Kingdom. *Am J Med Genet C Semin Med Genet* 2020; 184: 578–589.

229. Gestri G, Osborne RJ, Wyatt AW, *et al.* Reduced TFAP2A function causes variable optic fissure closure and retinal defects and sensitizes eye development to mutations in other morphogenetic regulators. *Hum Genet* 2009; 126: 791–803.

230. Knight RD, Nair S, Nelson SS, *et al.* Lockjaw encodes a zebrafish tfap2a required for early neural crest development. *Development* 2003; 130: 5755–5768.

231. Young RM, Hawkins TA, Cavodeassi F, *et al.* Compensatory growth renders Tcf7l1a dispensable for eye formation despite its requirement in eye field specification. *Elife* 2019; 8: e40093.

232. Viet J, Reboutier D, Hardy S, *et al.* Modeling ocular lens disease in Xenopus. *Dev Dyn* 2020; 249: 610–621.

233. Tseng A-S. Seeing the future: using *Xenopus* to understand eye regeneration. *Genesis* 2017; 55: e23003.

234. Harland RM and Grainger RM. Xenopus research: metamorphosed by genetics and genomics. *Trends Genet* 2011; 27: 507–515.

235. Tandon P, Conlon F, Furlow JD, *et al.* Expanding the genetic toolkit in Xenopus: approaches and opportunities for human disease modeling. *Dev Biol* 2017; 426: 325–335.

236. Hellsten U, Harland RM, Gilchrist MJ, *et al.* The genome of the western clawed frog Xenopus tropicalis. *Science* 2010; 328: 633–636.

237. Khokha MK. Xenopus white papers and resources: folding functional genomics and genetics into the frog. *Genesis* 2012; 50: 133–142.

238. Ochi H, Kawaguchi A, Tanouchi M, *et al.* Co-accumulation of cis-regulatory and coding mutations during the pseudogenization of the Xenopus laevis homoeologs six6.L and six6.S. *Dev Biol* 2017; 427: 84–92.

239. Danno H, Michiue T, Hitachi K, *et al.* Molecular links among the causative genes for ocular malformation: Otx2 and Sox2 coregulate Rax expression. *Proc Natl Acad Sci U S A* 2008; 105: 5408–5413.

240. Hu W, Haamedi N, Lee J, *et al.* The structure and development of Xenopus laevis cornea. *Exp Eye Res* 2013; 116: 109–128.

241. Wheeler GN and Brändli AW. Simple vertebrate models for chemical genetics and drug discovery screens: lessons from zebrafish and *Xenopus*. *Dev Dyn* 2009; 238: 1287–1308.

242. Suzuki KIT, Isoyama Y, Kashiwagi K, *et al.* High efficiency TALENs enable F0 functional analysis by targeted gene disruption in Xenopus laevis embryos. *Biol Open* 2013; 2: 448–452.

243. Hilton EN, Manson FDC, Urquhart JE, *et al.* Left-sided embryonic expression of the BCL-6 corepressor, BCOR, is required for vertebrate laterality determination. *Hum Mol Genet* 2007; 16: 1773–1782.

244. Viczian AS, Solessio EC, Lyou Y, *et al.* Generation of functional eyes from pluripotent cells. *PLoS Biol* 2009; 7: 1000174.

245. Gordon CT, Xue S, Yigit G, *et al.* De novo mutations in SMCHD1 cause Bosma arhinia microphthalmia syndrome and abrogate nasal development. *Nat Genet* 2017; 49: 249–255.

246. Gurzau AD, Chen K, Xue S, *et al.* FSHD2- and BAMS-associated mutations confer opposing effects on SMCHD1 function. *J Biol Chem* 2018; 293: 9841–9853.

247. Ng D, Thakker N, Corcoran CM, *et al.* Oculofaciocardiodental and Lenz microphthalmia syndromes result from distinct classes of mutations in BCOR. *Nat Genet* 2004; 36: 411–416.

248. Ragge N, Isidor B, Bitoun P, *et al.* Expanding the phenotype of the X-linked BCOR microphthalmia syndromes. *Hum Genet* 2018; 138: 1051–1069.

249. Zuzic M, Arias JER, Wohl SG, *et al.* Retinal miRNA functions in health and disease. *Genes (Basel)*. Epub ahead of print 17 May 2019. DOI: 10.3390/genes10050377.

250. Ohana R, Weiman-Kelman B, Raviv S, *et al.* MicroRNAs are essential for differentiation of the retinal pigmented epithelium and maturation of adjacent photoreceptors. *Development* 2015; 142: 2487–2498.

251. Ritter RA, Ulrich CH, Brzezinska BN, *et al.* miR-199 plays both positive and negative regulatory roles in Xenopus eye development. *Genesis*. Epub ahead of print 7 January 2020. DOI: 10.1002/dvg.23354.

252. Bogdan S, Senkel S, Esser F, *et al.* Misexpression of Xsiah-2 induces a small eye phenotype in Xenopus. *Mech Dev* 2001; 103: 61–69.

253. Imaoka S, Muraguchi T and Kinoshita T. Isolation of Xenopus HIF-prolyl 4-hydroxylase and rescue of a small-eye phenotype caused by Siah2 over-expression. *Biochem Biophys Res Commun* 2007; 355: 419–425.

254. Massé K, Bhamra S, Eason R, *et al.* Purine-mediated signalling triggers eye development. *Nature* 2007; 449: 1058–1062.

255. Yan F, Ruan X-Z, Yang H-S, *et al.* Identification, characterization, and effects of Xenopus laevis PNAS-4 gene on embryonic development. *J Biomed Biotechnol* 2010; 2010: 134764.

256. Zhu J, Huang X, Jiang H, *et al.* The role of pparγ in embryonic development of Xenopus tropicalis under triphenyltin-induced teratogenicity. *Sci Total Environ* 2018; 633: 1245–1252.

257. Belyaeva OV, Lee S-A, Adams MK, *et al.* Short chain dehydrogenase/reductase rdhe2 is a novel retinol dehydrogenase essential for frog embryonic development. *J Biol Chem* 2012; 287: 9061–9071.

258. Kim Y-J, Bahn M, Kim YH, *et al.* Xenopus laevis FGF receptor substrate 3 (XFrs3) is important for eye development and mediates Pax6 expression in lens placode through its Shp2-binding sites. *Dev Biol* 2015; 397: 129–139.

259. Walter BE, Perry KJ, Fukui L, *et al.* Psf2 plays important roles in normal eye development in Xenopus laevis. *Mol Vis* 2008; 14: 906–921.

260. Turnbull C, Scott RH, Thomas E, *et al.* The 100 000 genomes project: bringing whole genome sequencing to the NHS. *BMJ*. Epub ahead of print 24 April 2018. DOI: 10.1136/bmj.k1687.

261. Yelin R, Schyr RBH, Kot H, *et al.* Ethanol exposure affects gene expression in the embryonic organizer and reduces retinoic acid levels. *Dev Biol* 2005; 279: 193–204.

262. Hirschi KK, Li S and Roy K. Induced pluripotent stem cells for regenerative medicine. *Annu Rev Biomed Eng* 2014; 16: 277–294.

263. Hung SSC, Khan S, Lo CY, *et al.* Drug discovery using induced pluripotent stem cell models of neurodegenerative and ocular diseases. *Pharmacol Ther* 2017; 177: 32–43.

264. Llonch S, Carido M and Ader M. Organoid technology for retinal repair. *Dev Biol* 2018; 433: 132–143.

265. Rossi G, Manfrin A and Lutolf MP. Progress and potential in organoid research. *Nat Rev Genet* 2018; 19: 671–687.

266. Fronk AH and Vargis E. Methods for culturing retinal pigment epithelial cells: a review of current protocols and future recommendations. *J Tissue Eng* 2016; 7: 1–23.

267. Meyer JS, Shearer RL, Capowski EE, *et al.* Modeling early retinal development with human embryonic and induced pluripotent stem cells. *Proc Natl Acad Sci U S A* 2009; 106: 16698–16703.

268. Ilic D and Ogilvie C. Concise review: human embryonic stem cells—what have we done? What are we doing? Where are we going? *Stem Cells* 2017; 35: 17–25.

269. Kaur G and Dufour JM. Cell lines. *Spermatogenesis* 2012; 2: 1–5.

270. Lorsch JR, Collins FS and Lippincott-Schwartz J. Fixing problems with cell lines. *Science* 2014; 346: 1452–1453.

271. Alston-Roberts C, Barallon R, Bauer SR, *et al.* Cell line misidentification: the beginning of the end. *Nat Rev Cancer* 2010; 10: 441–448.

272. Peng S, Wang S-B, Singh D, *et al.* Claudin-3 and claudin-19 partially restore native phenotype to ARPE-19 cells via effects on tight junctions and gene expression. *Exp Eye Res* 2016; 151: 179–189.

273. Samuel W, Jaworski C, Postnikova OA, *et al.* Appropriately differentiated ARPE-19 cells regain phenotype and gene expression profiles similar to those of native RPE cells. *Mol Vis* 2017; 23: 60–89.

274. Walmsley G, Hyun J, McArdle A, *et al.* Induced pluripotent stem cells in regenerative medicine and disease modeling. *Curr Stem Cell Res Ther* 2014; 9: 73–81.

275. Takahashi K, Tanabe K, Ohnuki M, *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131: 861–872.

276. Singh VK, Kalsan M, Kumar N, *et al.* Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. *Front Cell Dev Biol* 2015; 3: 2.

277. Gao M-L, Lei X-L, Han F, *et al.* Patient-specific retinal organoids recapitulate disease features of late-onset retinitis pigmentosa. *Front Cell Dev Biol* 2020; 8: 128.

278. Higuchi A, Suresh Kumar S, Benelli G, *et al.* Stem cell therapies for reversing vision loss. *Trends Biotechnol* 2017; 35: 1102–1117.

279. Trounson A and McDonald C. Stem cell therapies in clinical trials: progress and challenges. *Cell Stem Cell* 2015; 17: 11–22.

280. Maeda A, Mandai M and Takahashi M. Gene and induced pluripotent stem cell therapy for retinal diseases. *Annu Rev Genomics Hum Genet* 2019; 20: 201–216.

281. Bobba S, Di Girolamo N, Munsie M, *et al.* The current state of stem cell therapy for ocular disease. *Exp Eye Res* 2018; 177: 65–75.

282. Sivan PP, Syed S, Mok P-L, *et al.* Stem cell therapy for treatment of ocular disorders. *Stem Cells Int*. Epub ahead of print 15 May 2016. DOI: 10.1155/2016/8304879.

283. Vergani L, Grattarola M and Nicolini C. Modifications of chromatin structure and gene expression following induced alterations of cellular shape. *Int J Biochem Cell Biol* 2004; 36: 1447–1461.

284. Thomas CH, Collier JH, Sfeir CS, *et al.* Engineering gene expression and protein synthesis by modulation of nuclear shape. *Proc Natl Acad Sci U S A* 2002; 99: 1972–1977.

285. Weaver VM, Petersen OW, Wang F, *et al.* Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J Cell Biol* 1997; 137: 231–245.

286. Akhtar T, Xie H, Khan MI, *et al.* Accelerated photoreceptor differentiation of hiPSC-derived retinal organoids by contact co-culture with retinal pigment epithelium. *Stem Cell Res* 2019; 39: 101491.

287. Lidgerwood GE, Hewitt AW and Pébay A. Human pluripotent stem cells for the modelling of diseases of the retina and optic nerve: toward a retina in a dish. *Curr Opin Pharmacol* 2019; 48: 114–119.

288. Lancaster MA and Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 2014; 345: 1247125.

289. Eiraku M and Sasai Y. Mouse embryonic stem cell culture for generation of three-dimensional retinal and cortical tissues. *Nat Protoc* 2011; 7: 69–79.

290. Eiraku M, Takata N, Ishibashi H, *et al.* Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 2011; 472: 51–56.

291. Nakano T, Ando S, Takata N, *et al.* Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 2012; 10: 771–785.

292. Sasai Y, Eiraku M and Suga H. In vitro organogenesis in three dimensions: self-organising stem cells. *Development* 2012; 139: 4111–4121.

293. Hiler D, Chen X, Hazen J, *et al.* Quantification of retinogenesis in 3D cultures reveals epigenetic memory and higher efficiency in iPSCs derived from rod photoreceptors. *Cell Stem Cell* 2015; 17: 101–115.

294. Hyer J, Kuhlman J, Afif E, *et al.* Optic cup morphogenesis requires pre-lens ectoderm but not lens differentiation. *Dev Biol* 2003; 259: 351–363.

295. Assawachananont J, Mandai M, Okamoto S, *et al.* Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports* 2014; 2: 662–674.

296. Zhong X, Gutierrez C, Xue T, *et al.* Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. *Nat Commun* 2014; 5: 4047.

297. Parfitt DA, Lane A, Ramsden CM, *et al.* Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. *Cell Stem Cell* 2016; 18: 769–781.

298. Chen HY, Kaya KD, Dong L, *et al.* Three-dimensional retinal organoids from mouse pluripotent stem cells mimic in vivo development with enhanced stratification and rod photoreceptor differentiation. *Mol Vis* 2016; 22: 1077–1094.

299. Völkner M, Zschätzsch M, Rostovskaya M, *et al.* Retinal organoids from pluripotent stem cells efficiently recapitulate retinogenesis. *Stem Cell Reports* 2016; 6: 525–538.

300. Ohlemacher SK, Iglesias CL, Sridhar A, *et al.* Generation of highly enriched populations of optic vesicle–like retinal cells from human pluripotent stem cells. In: Bhatia M (ed.) *Current protocols in stem cell biology*. Hoboken, NJ: John Wiley & Sons, Inc., 2015, pp.1H.8.1–1H.8.20.

301. Mellough CB, Collin J, Khazim M, *et al.* IGF-1 signaling plays an important role in the formation of three-dimensional laminated neural retina and other ocular structures from human embryonic stem cells. *Stem Cells* 2015; 33: 2416–2430.

302. Reichman S, Terray A, Slembrouck A, *et al.* From confluent human iPS cells to self-forming neural retina and retinal pigmented epithelium. *Proc Natl Acad Sci U S A* 2014; 111: 8518–8523.

303. Gonzalez-Cordero A, Kruczek K, Naeem A, *et al.* Recapitulation of human retinal

development from human pluripotent stem cells generates transplantable populations of cone photoreceptors. *Stem Cell Reports* 2017; 9: 820–837.

304. Decembrini S, Koch U, Radtke F, *et al.* Derivation of traceable and transplantable photoreceptors from mouse embryonic stem cells. *Stem Cell Reports* 2014; 2: 853–865.
305. Garita-HernÁndez M, Diaz-Corrales F, Lukovic D, *et al.* Hypoxia increases the yield of photoreceptors differentiating from mouse embryonic stem cells and improves the modeling of retinogenesis in vitro. *Stem Cells* 2013; 31: 966–978.
306. Ovando-Roche P, West EL, Branch MJ, *et al.* Use of bioreactors for culturing human retinal organoids improves photoreceptor yields. *Stem Cell Res Ther* 2018; 9: 156.
307. Cui Z, Guo Y, Zhou Y, *et al.* Transcriptomic analysis of the developmental similarities and differences between the native retina and retinal organoids. *Invest Opthalmol Vis Sci* 2020; 61: 6.
308. Sridhar A, Hoshino A, Finkbeiner CR, *et al.* Single-cell transcriptomic comparison of human fetal retina, hPSC-derived retinal organoids, and long-term retinal cultures. *Cell Rep* 2020; 30: 1644–1659.e4.
309. Mellough CB, Collin J, Queen R, *et al.* Systematic comparison of retinal organoid differentiation from human pluripotent stem cells reveals stage specific, cell line, and methodological differences. *Stem Cells Transl Med*. Epub ahead of print 27 March 2019. DOI: 10.1002/sctm.18-0267.
310. Chichagova V, Hilgen G, Ghareeb A, *et al.* Human iPSC differentiation to retinal organoids in response to IGF1 and BMP4 activation is line- and method-dependent. *Stem Cells* 2019; 38: 195–201.
311. Hallam D, Hilgen G, Dorgau B, *et al.* Human-induced pluripotent stem cells generate light responsive retinal organoids with variable and nutrient-dependent efficiency. *Stem Cells* 2018; 36: 1535–1551.
312. Capowski EE, Samimi K, Mayerl SJ, *et al.* Reproducibility and staging of 3D human retinal organoids across multiple pluripotent stem cell lines. *Development* 2019; 146: dev171686.
313. Zhu L, Gomez-Duran A, Saretzki G, *et al.* The mitochondrial protein CHCHD2 primes the differentiation potential of human induced pluripotent stem cells to neuroectodermal lineages. *J Cell Biol* 2016; 215: 187–202.
314. Narsinh KH, Sun N, Sanchez-Freire V, *et al.* Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells. *J Clin Invest* 2011; 121: 1217–1221.
315. Choi J, Lee S, Mallard W, *et al.* A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. *Nat Biotechnol* 2015; 33: 1173–1181.
316. Nazor KL, Altun G, Lynch C, *et al.* Recurrent variations in DNA methylation in human pluripotent stem cells and their differentiated derivatives. *Cell Stem Cell* 2012; 10: 620–634.
317. Wang L, Hiler D, Xu B, *et al.* Retinal cell type DNA methylation and histone modifications predict reprogramming efficiency and retinogenesis in 3D organoid cultures. *Cell Rep* 2018; 22: 2601–2614.
318. Khurana V, Tardiff DF, Chung CY, *et al.* Toward stem cell-based phenotypic screens for neurodegenerative diseases. *Nat Rev Neurol* 2015; 11: 339–350.
319. Jiang F and Doudna JA. CRISPR-Cas9 structures and mechanisms. *Annu Rev Biophys* 2017; 46: 505–529.
320. Achberger K, Probst C, Haderspeck J, *et al.* Merging organoid and organ-on-a-chip technology to generate complex multi-layer tissue models in a human retina-on-a-chip platform. *Elife* 2019; 8: e46188.
321. Bai J and Wang C. Organoids and microphysiological systems: new tools for ophthalmic drug discovery. *Front Pharmacol* 2020; 11: 407.
322. Bell CM, Zack DJ and Berlinicke CA. Human organoids for the study of retinal development and disease. *Annu Rev Vis Sci* 2020; 6: 91–114.
323. Lahrouchi N, George A, Ratbi I, *et al.* Homozygous frameshift mutations in FAT1 cause a syndrome characterized by colobomatous-microphthalmia, ptosis, nephropathy and syndactyly. *Nat Commun*. Epub ahead of print 12 March 2019. DOI: 10.1038/s41467-019-08547-w.
324. Phillips MJ, Perez ET, Martin JM, *et al.* Modeling human retinal development with patient-specific induced pluripotent stem cells reveals multiple roles for visual system homeobox 2. *Stem Cells* 2014; 32: 1480–1492.
325. Gamm DM, Clark E, Capowski EE, *et al.* The role of FGF9 in the production of neural retina and RPE in a pluripotent stem cell model of early human retinal development. *Am J Ophthalmol* 2019; 206: 113–131.

326. Liu C, Widen SA, Williamson KA, *et al.* A secreted WNT-ligand-binding domain of FZD5 generated by a frameshift mutation causes autosomal dominant coloboma. *Hum Mol Genet* 2016; 25: 1382–1391.

327. Aubert-Mucca M, Pernin-Grandjean J, Marchasson S, *et al.* Confirmation of FZD5 implication in a cohort of 50 patients with ocular coloboma. *Eur J Hum Genet*. Epub ahead of print 31 July 2020. DOI: 10.1038/s41431-020-0695-8.

328. Liu C, Bakeri H, Li T, *et al.* Regulation of retinal progenitor expansion by Frizzled receptors: implications for microphthalmia and retinal coloboma. *Hum Mol Genet* 2012; 21: 1848–1860.

329. Richardson R, Owen N, Toms M, *et al.* Transcriptome profiling of zebrafish optic fissure fusion. *Sci Rep* 2019; 9: 1541.

330. Esmailpour T, Riazifar H, Liu L, *et al.* A splice donor mutation in NAA10 results in the dysregulation of the retinoic acid signalling pathway and causes Lenz microphthalmia syndrome. *J Med Genet* 2014; 51: 185–196.

331. Gao Z, Huo L, Cui D, et al. The expression of bone morphogenetic protein 2 and matrix metalloproteinase 2 through retinoic acid receptor beta induced by all-trans retinoic acid in cultured ARPE-19 cells. *PLoS One*. Epub ahead of print 1 March 2016. DOI: 10.1371/journal.pone.0150831.