Molecular diagnostic challenges for non-retinal developmental eye disorders in the United Kingdom

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Abstract
Overall, approximately one-quarter of patients with genetic eye diseases will receive a molecular diagnosis. Patients with developmental eye disorders face a number of diagnostic challenges including phenotypic heterogeneity with significant asymmetry, coexisting ocular and systemic disease, limited understanding of human eye development and the associated genetic repertoire, and lack of access to next generation sequencing as regarded not to impact on patient outcomes/management with cost implications. Herein, we report our real world experience from a pediatric ocular genetics service over a 12 month period with 72 consecutive patients from 62 families, and that from a cohort of 322 patients undergoing whole genome sequencing (WGS) through the Genomics England 100,000 Genomes Project; encompassing microphthalmia, anophthalmia, ocular coloboma (MAC), anterior segment dysgenesis anomalies (ASDA), primary congenital glaucoma, congenital cataract, infantile nystagmus, and albinism. Overall molecular diagnostic rates reached 24.9% for those recruited to the 100,000 Genomes Project (73/293 families were solved), but up to 33.9% in the clinic setting (20/59 families). WGS was able to improve genetic diagnosis for MAC patients (15.7%), but not for ASDA (15.0%) and congenital cataracts (44.7%). Increased sample sizes and accurate human phenotype ontology (HPO) terms are required to improve diagnostic accuracy. The significant mixed complex ocular phenotypes distort these rates and lead to missed variants if the correct gene panel is not applied. Increased molecular diagnoses will help to explain the genotype-phenotype relationships of these developmental eye disorders. In turn, this will lead to improved integrated care pathways, understanding of disease, and future therapeutic development.

KEYWORDS
developmental eye disorders, genetic eye disease, next generation sequencing, targeted gene panels, whole genome sequencing
1  |  INTRODUCTION

Childhood visual impairment has a significant emotional, social and economic impact on the individual, their family, and society as a whole. An estimated 1.4 million children are blind worldwide (Gilbert, 2001), and in the United Kingdom, 1 in 2500 children under the age of 1 year are diagnosed as severely sight impaired with an estimated one-third having a genetic basis (Rahi and Cable, 2003). The spectrum of developmental eye disorders is vast, and includes microphthalmia, anophthalmia and ocular coloboma (collectively grouped as MAC), anterior segment dysgenesis anomalies (ASDA), congenital cataracts, primary congenital glaucoma (PCG), Leber congenital amaurosis (LCA) and vitreoretinal dysplasia, optic nerve disorders, infantile nystagmus, and albinism (ocular and syndromic). Environmental factors, such as maternal alcohol intake or in utero infections, may cause some of these conditions (Busby, Dolk, & Armstrong, 2005; Chassaing et al., 2014; Givens, Lee, Jones, & Ilstrup, 1993), therefore a detailed prenatal history should be obtained. If an unremarkable pregnancy is reported a genetic basis should be considered.

MAC contributes up to 15% of childhood blindness and severe visual impairment worldwide (Horbury et al., 2000), with a cumulative incidence of 11.9 per 100,000 children (<16 years of age) in the United Kingdom (Shah et al., 2011). A prospective incidence study found that 2% of cases were due to environmental causes, and despite the assumption that the rest were genetic, only 6% of patients received a molecular diagnosis (Shah et al., 2011). MAC patients display significant phenotypic heterogeneity, forming part of a clinical spectrum and mixed phenotypes can often be seen in individuals, for example right microphthalmia with chorioretinal coloboma and left anophthalmia. Other ocular abnormalities, such as ASDA and cataract, can also be found in MAC patients causing a more complex presentation, with 60% having systemic associations (Richardson, Sowden, Gerth-Kahlert, Moore, & Moosaiee, 2017). Over 90 genes linked to MAC have been identified with all forms of inheritance (de novo sporadic, autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive), demonstrating genetic heterogeneity (Harding & Moosaiee, 2019). In severe bilateral cases of microphthalmia and anophthalmia, a genetic cause can be found in up to 80% (Plaisancie, Calvas, & Chassaing, 2016), with heterozygous loss of function variants involving SOX2 and OTX2, and recessive biallelic changes in STRA6 being the most common (Williamson & FitzPatrick, 2014). However, this represents a small subset of patients with a prevalence of 2–3 per 100,000. For the majority, where asymmetry exists, particularly isolated unilateral cases, the diagnostic rates fall below 10%. Germline mosaicism, nonpenetration and variable expressivity may be contributing factors (Chassaing et al., 2007; Faivre et al., 2006; Morrison et al., 2002).

The involvement of multiple ocular structures in patients with developmental eye disorders is due to the genes (a significant number being transcription factors) involved in early eye development having a spatiotemporal role in various ocular tissues, thus having a pleiotropic effect if defective. Current genetic testing practice in the United Kingdom for genetically heterogeneous eye conditions utilizes targeted gene panels (e.g., the Oculome; http://www.labs.gosh.nhs.uk/media/764794/oculome_v8.pdf) which encompass known disease-causing genes that cause both nonsyndromic and syndromic forms of disease. There are exceptions, for example, for children born with aniridia, an array-CGH is commonly used to detect a deletion involving the WT1 and PAX6 genes, if negative then Wilms tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome, can be ruled out and single gene PCR-based sequencing of PAX6 is undertaken to identify pathogenic variants causing isolated aniridia. It is important to consider that although targeted gene panels, such as the Oculome "Anterior Segment Dysgenesis" panel encompasses related conditions such as ASDA, corneal dystrophies and glaucoma related genes, if a patient also had cataract or coloboma, then the relevant panel may not be selected and the molecular cause missed. Hence accurate phenotypic descriptions using Human Phenotype Ontology (HPO) terms must be given so the clinical scientists can consider the differential genes that may be involved and apply multiple gene panels if necessary. Whole genome sequencing (WGS) remains a research-based test in the United Kingdom but is transitioning to clinical accreditation, however, similar principles will apply to selecting the correct panel of genes to be screened based on phenotype.

In contrast, inherited retinal disorders (IRDs), although considered phenotypically heterogeneous, commonly have a symmetrical appearance with an onset after birth through to late adulthood which can be monitored closely with state-of-the-art retinal imaging. Over 250 disease-causing genes have been identified, mainly over the past two decades, and this had led to the first approved retinal gene therapy, voretigene neparovec, for autosomal recessive biallelic RPE65-retinopathy, with a multitude of gene/mutation-based clinical trials underway (Maguire et al., 2019; Miraldi Utz, Coussa, Antaki, & Traboulsi, 2018; Russell et al., 2017). Genetic diagnostic rates in IRDs vary according to the population being tested, but range between 50 and 70% (Audo et al., 2012; Bernardis et al., 2016; Consugar et al., 2015; Ellingford et al., 2016; Jiman et al., 2020; Tayebi et al., 2019). The progress seen in the IRD field is likely due to the consistent scientific investment made internationally. A PubMed search on 16th May 2020 of papers relating to IRDs with search terms "inheritance" came to 12,953, whereas those for "developmental eye disorders" was 7,689; "anterior segment dysgenesis" 2,402; "microphthalmia" 4,837; "anophthalmia" 1,612; coloboma 5,152; and "congenital cataract" 5,793.

In this study, we report our real-world clinical experience of genetic testing of pediatric patients with developmental eye disorders, excluding IRDs, vitreoretinopathies and optic nerve disorders (including hereditary optic neuropathies). We compare our rates of diagnosis to published studies, using current clinically accredited targeted gene panels and research-based whole genome sequencing (WGS) tests, for patients that presented to the oculars genetics service at Moorfields Eye Hospital NHS Foundation Trust (MEH), which oversees the care of the largest number of genetic eye disease patients of any one site in the United Kingdom.
2 | METHODS

2.1 | Editorial policies and ethical considerations

This study had relevant local and national research ethics committee approvals (MEH and the Northwest London Research Ethics Committee), and adhered to the tenets of the Declaration of Helsinki. Patients and relatives gave written informed consent for genetic testing through either the Genetic Study of Inherited Eye Disease (REC reference 12/LO/0141) or Genomics England 100,000 Genomes project (REC reference 14/EE/1112).

2.2 | Genetic screening methods

Only families with nonsyndromic and syndromic microphthalmia, anophthalmia, ocular coloboma (MAC), anterior segment dysgenesis anomalies (ASDA) including primary congenital glaucoma, corneal dystrophies, and anirida, congenital cataract, infantile nystagmus and albinism were included in this analysis. Consecutive patients presenting to the pediatric ocular genetics service at MEH between 1st October 2017 and 30th September 2018 were investigated. In addition, the cohort of patients with corresponding diagnoses recruited into the UK Genomics England 100,000 Genomes Project (Turnbull et al., 2018) from 2015 to 2018 at MEH were also scrutinized.

Molecular testing was performed in the clinical and research setting, using targeted gene panel testing (Oculome; http://www.labs.gosh.nhs.uk/media/764794/oculome_v8.pdf) through the Rare & Inherited Disease Genomic Laboratory at Great Ormond Street Hospital (London, UK) and whole genome sequencing (WGS) as part of the UK Genomics England 100,000 Genomes Project, for which results were reviewed by a multidisciplinary team (including molecular biologists, clinical geneticists, as well as the ophthalmology specialist managing the family), to confirm variant pathogenicity, prevalence in publicly available genome databases, the clinical phenotype and mode of inheritance, before the molecular diagnosis was established. The datasets (variants) generated for this study were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) through the Rare & Inherited Disease Genomic Laboratory at Great Ormond Street Hospital (London, UK).

3 | RESULTS

3.1 | Real world genetic outcomes for patients with developmental eye disorders

A retrospective observational study identified 72 consecutive patients from 62 families with developmental eye disorders, who attended the pediatric ocular genetics service at MEH over a 12-month period. The families were divided into 15 MAC (24.2%), 11 ASDA (17.7%, including one anirida, three corneal dystrophies and two glaucoma [congenital and juvenile-onset]), 10 congenital cataracts (16.1%), 13 infantile nystagmus (21.0%), nine albinism (14.5%) and four complex strabismus patients (6.5%, including two congenital fibrosis of extraocular muscles, one Duane syndrome, and one with query blepharophimosis, ptosis and epicanthus inversus syndrome) (Figure 1a). The mean ± SD age of children was 4.9 ± 4.2 years (range 1 month-15 years) with 41.7% (n = 30) being female. The ethnicity of patients was divided into 18 White British (25%), 11 White other (15.3%), five Asian Pakistani (6.9%), three Asian Bangladeshi (4.2%), three Asian Indian (4.2%), six Asian other (8.3%), two Black African (2.8%), one Black Caribbean (1.4%), two Black other (2.8%), two mixed White and Black African (2.8%), 11 other ethnicities (15.3%), and eight were not stated (11.1%). All patient demographics including clinical and genetic details are listed in Supplementary Table S1.

In total, 69 patients from 59 families (95.2%) proceeded with genetic testing following informed consent, two patients declined, and it was not possible to obtain a sample from one patient. Of the 59 families who consented, 13 had a targeted gene panel (22.0%), 45 had WGS through the 100,000 Genomes Project (76.3%) and one family had single gene test for PAX6. Most families opted for WGS due to the superiority of the test and its coverage despite it being on a research basis. Those that underwent a targeted gene panel did so as they either did not meet the 100,000 Genomes Project study eligibility criteria, did not want to partake in a research study, or were concerned about the length of time to get results for family planning purposes.

Overall, 29 patients from 20 families (42.0% of tested patients; 33.9% of tested families) received a molecular diagnosis (Table 1). For the disease subgroups, the following molecular diagnostic rates were achieved (based on number of families undergoing genetic testing): 21.4% MAC (3/14), 60% ASDA (6/10), 44.4% congenital cataracts (4/9), 15.4% infantile nystagmus (2/13), 55.6% albinism (5/9) and nil for the complex strabismus disorders (0/4). If the ASDA group is subdivided: 60% ASDA (3/5), 66.7% corneal dystrophies (2/3) and 50% glaucoma (1/2). Of the solved families, 5/20 had targeted gene panel testing (25%) and 14/20 had WGS (70%), and one had single gene testing (5%). Of the 36 families that received a no primary finding result, seven were from panel test and 29 from WGS, suggesting that overall, those that had a panel test had a 46.2% (6/13) diagnostic rate and for those with WGS was 35.6% (16/45), although this is not a valid comparison due to differing indications and sample size.

Pathogenic variants were found in 19 distinct genes. The genes identified for MAC were KMT2D, MAB2IL2, ALDH1A3; ASDA were KERA, PAX6, MYOC, TGBI, and SLC4A11; congenital cataract were CRYBB2, EPHA2, HSF4 and BCOR; infantile nystagmus were CACNA1A and FRMD7; and albinism were OCA2, GPR143, HPS6 and SLC38A8 (Table 1).

3.2 | Outcomes for developmental eye disorders through whole genome sequencing

As the aforementioned consecutive cohort of patients had a mixed range of genetic testing, we looked at all developmental eye disorder
patients with the same disease criteria who had been recruited into the UK 100,000 Genomes Project with corresponding diagnostic rates. A total of 322 families were recruited, divided into 75 MAC (23.3%), 49 ASDA (15.2%, including three aniridia, two corneal dystrophies, and 42 primary congenital glaucoma), 43 congenital cataracts (13.4%), 25 infantile nystagmus (7.8%), 130 albinism (40.4%) (Figure 1b).

Two hundred and ninety-three families have received their results, with 29 still pending. Of this 73 families (24.9%) received a molecular diagnosis but 220 (75.1%) had no primary findings (Table 2). For the disease subgroups the following molecular diagnostic rates were achieved; 15.7% MAC (11/70), 13.0% ASDA (6/46, but 12.8% for primary congenital glaucoma [5/39]), 44.7% congenital cataracts (17/38), 28.0% infantile nystagmus (7/25), and 28.1% albinism (32/114) (Figure 1c).

Pathogenic variants were found in 38 distinct genes. Those identified for MAC were PRSS56 (two families), ALDH1A3, CREBBP, GJA8, KERA, MAB21L2, MFRP, PAX6, PTPN11, and TAF2PA; ASDA were CYP1B1 (four families), FOXC1 and PAX6; congenital cataract were CRYBB1, CRYBB2 (three families), CRYGD (two families), BCOR, CRYBB1, CRYBB3, CHMP4B, EPHA2, GJA8, HSF4, MAF, MIP, NHS, PITX3, and TDRD7; infantile nystagmus were FRMD7 (four families), OCA2, SLC38A8, CACNA1A; and albinism were TYR (12 families), GPR143 (eight families), OCA2 (seven families), SLC38A8 (three families), HPS6, and SLC24A5 (Table 2). WGS seeks to screen both coding and noncoding regions of the gene, but only 8 out of 93 (8.6%) variants were found to be noncoding (all were splice-site mutations); these were found in one cataract patient (HSF4), two nystagmus patients (both FRMD7), and five albinism patients (OCA2 [two patients], GPR143, SLC38A8, and TYR). Noncoding variants were only found in splice regions due to the limitations of the UK Genomics England 100,000 Genomes Project diagnostic pipeline. As with clinically accredited diagnostic targeted gene panels (e.g., the Oculome), the focus was on the detection of class 4 and 5 variants. So while WGS has the capacity to detect all noncoding variation, only those with a canonical splicing effect or those previously identified and/or functionally proven variants will be regarded as class 4 or 5. For unsolved cases, the discovery of novel noncoding variants is undertaken by further data mining in the research setting.
| Family ID | Disease group | Test | Gene | Confirmed phenotype (OMIM) | Zygosity | Variant | Variant type | Inheritance |
|-----------|---------------|------|------|-----------------------------|----------|---------|-------------|-------------|
| 1         | MAC           | Panel | KMT2D | Kabuki syndrome 1           | Het      | c.6354del; p.Ala2119Leufs*25 | Frameshift deletion | AD          |
| 4         | MAC           | WGS  | MAB21L2 | Microphthalmia/coloboma and skeletal dysplasia syndrome | Het | c.379A>T; p.Lys127Ter | Nonsense | AD |
| 9         | MAC           | WGS  | ALDH1A3 | Microphthalmia, isolated 8 | Hom | c.104T>C; p.phe35Ser | Missense | AR |
| 16        | ASDA          | Panel | SLC4A11 | Corneal endothelial dystrophy, autosomal recessive | Het | c.2321+1G>A; p.(Glu170Lys) | Missense | AR |
| 17        | ASDA          | WGS  | KERA  | Cornea plana 2, autosomal recessive | Hom | c.809C>T; p.(Ser270Leu) | Missense | AR |
| 20        | ASDA          | Single gene | PAX6 | Aniridia | Het | c.1253_1262del10; p.(Trp418TyrfsTer104) | Frameshift deletion | AD |
| 21        | ASDA          | Panel | KERA  | Cornea plana 2, autosomal recessive | Hom | c.528C>G; p.(Arg176Lys) | Missense | AR |
| 22        | ASDA          | Panel | TGFBI | Reis–Bucklers corneal dystrophy | Het | c.1.664G>A; p.(Arg555Gln) | Missense | AR |
| 25        | ASDA          | Panel | MYOC  | Glaucoma 1A, primary open angle | Het | c.760C>A; p.(Pro254Thr) | Missense | AR |
| 27        | Cataract      | WGS  | CRYBB2 | Cataract 3, multiple types | Het | c.463C>T; p.(Gln155*) | Missense | AD |
| 28        | Cataract      | WGS  | EPFA2 | Cataract 6, multiple types | Het | c.1.751C>T; p.Pro584Leu | Missense | AR |
| 32        | Cataract      | WGS  | HSF4  | Cataract 5, multiple types | Het | c.360+1G>A | Noncoding (splice) | AR |
| 36        | Cataract      | WGS  | BCOR  | Congenital cataract | Het | c.856del; p.(Ser286Alafs*92) | Frameshift deletion | XD |
| 40        | Nystagmus     | WGS  | CACNA1A | Idiopathic infantile nystagmus | Het | c.2566G>T; p.(Glu856*) | Missense | AR |
| 46        | Nystagmus     | WGS  | FRMD7 | Nystagmus 1, congenital, X-linked | Het | c.383+1G>A | Noncoding (splice) | AR |
| 51        | Albinism      | WGS  | OCA2  | Albinism, oculocutaneous, type II | Hemi | c.619_636del; p.(Leu207_Leu212del) | Inframe deletion | AR |
| 52        | Albinism      | WGS  | GPR143 | Nystagmus 6, congenital, X-linked | Hemi | c.11C>G; p.Pro4Arg | Missense | XR |
| 53        | Albinism      | WGS  | HPS6  | Hermansky–Pudlak syndrome 6 | Hom | c.1228_1252del; p.(Tyr410Valfs*9) | Frameshift deletion | AR |
| 56        | Albinism      | WGS  | SLC38A8 | Foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis | Het | c.435G>A; p.Trp145Ter | Nonsense | AR |
| 58        | Albinism      | WGS  | SLC38A8 | Foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis | Hom | c.264C>G; p.Tyr88* | Nonsense | AR |
| Family ID | Ethnicity       | Disease group | Gene     | Confirmed phenotype (OMIM)                           | Zygosity | Variant       | Variant type     | Inheritance |
|-----------|----------------|---------------|----------|-----------------------------------------------------|----------|---------------|------------------|-------------|
| 9         | Asian Pakistani| MAC           | ALDH1A3  | Microphthalmia, isolated 8                          | Hom      | c.104T>C; p.(Phe35Ser) | Missense       | AR          |
| 26105     | White British  | MAC           | CREBBP   | Rubinstein–Taybi syndrome 1                          | Het      | c.2308,2315dup p. (Pro773Leufs*6) | Frameshift deletion | AD          |
| 26334     | White British  | MAC           | GJA8     | Cataract 1, multiple types                           | Het      | c.29OT>G; p.(Val97Gly) | Missense       | AD          |
| 17        | Asian Pakistani| MAC           | KERA     | Cornea plana 2, autosomal recessive                  | Hom      | c.809C>T; p.(Ser270Leu) | Missense       | AR          |
| 4         | Asian Indian   | MAC           | MAB21L2  | Microphthalmia/coloboma and skeletal dysplasia syndrome | Het      | c.379A>T; p.Lys127Ter | Nonsense       | AR          |
| 21143     | Asian Pakistani| MAC           | MFRP     | Microphthalmia, isolated 5                          | Hom      | c.1150del; p.(His384Thrfs*94) | Frameshift deletion | AR          |
| 25272     | White other    | MAC           | PAX6     | Coloboma, ocular                                    | Het      | c.413A>G; p.(Asn138Ser) | Missense       | AD          |
| 16899     | Black African  | MAC           | PRSS56   | Microphthalmia, isolated 6                          | Het      | c.320G>A; p.(Gly107Glu) | Missense       | AR          |
| 25356     | White British  | MAC           | PRSS56   | Microphthalmia, isolated 6                          | Het      | c.1439G>C; p.(Arg480Pro) | Missense       | AR          |
| 13302     | Not stated     | MAC           | PTPN11   | Noonan syndrome 1                                   | Het      | c.1573del; p.(Val525Cysfs*55) | Frameshift deletion | AD          |
| 23756     | White other    | MAC           | TFAP2A   | Branchiooculo facial syndrome                       | Het      | c.890C>A; p.(Ala297Asp) | Missense       | AD          |
| 25755     | Mixed: White/Asian | ASDA          | (aniridia) | Aniridia                                           | Het      | c.112delG; p.Arg38GlyfsTer30 | Frameshift deletion | AD          |
| 16024     | Asian Pakistani| ASDA          | (glaucoma)| Anterior segment dysgenesis 6, multiple subtypes    | Hom      | c.1169G>A; p.(Arg390His) | Missense       | AR          |
| 23974     | White British  | ASDA          | (glaucoma)| Anterior segment dysgenesis 6, multiple subtypes    | Het      | c.171G>A; p.(Trp57*) | Nonsense       | AR          |
| 24231     | White British  | ASDA          | (glaucoma)| Anterior segment dysgenesis 6, multiple subtypes    | Het      | c.840C>A; p.(Cys280*) | Nonsense       | AR          |
| 26098     | White British  | ASDA          | (glaucoma)| Anterior segment dysgenesis 6, multiple subtypes    | Het      | c.1168C>T; p.(Arg390Cys) | Nonsense       | AR          |
| 25760     | White British  | ASDA          | (glaucoma)| Anterior segment dysgenesis 3, multiple subtypes    | Het      | c.1009_1012dup p. (Ala338GlyfsTer191) | Frameshift duplication | AD          |
| 27        | Asian other    | Cataract      | CRYBB2   | Cataract 3, multiple types                           | Het      | c.463C>T; p.(Gln155*) | Nonsense       | AD          |
| 26275     | White British  | Cataract      | CRYBB2   | Cataract 3, multiple types                           | Het      | c.547C>T; p.(Gln183*) | Nonsense       | AD          |
| 23145     | Asian Pakistani| Cataract      | CRYBB3   | Cataract 22                                         | Het      | c.466G>A; p.(Gly156Arg) | Missense       | AD          |
| 23076     | White British  | Cataract      | CRYGD    | Cataract 4, multiple types                           | Het      | c.418C>T; p.(Arg140*) | Nonsense       | AD          |
| 26301     | Black African  | Cataract      | CRYGD    | Cataract 4, multiple types                           | Het      | c.70C>A; p.Pro24Thr | Missense       | AD          |
| 28        | White British  | Cataract      | EPHA2    | Cataract 6, multiple types                           | Het      | c.1751C>T; p.Pro584Leu | Missense       | AD          |
| 26333     | Other          | Cataract      | GJA8     | Cataract 1, multiple types                           | Het      | c.134G>T; p.(Trp45Leu) | Missense       | AD          |
| Family ID | Ethnicity        | Disease group | Gene   | Confirmed phenotype (OMIM)                                                                 | Zygosity | Variant       | Variant type                 | Inheritance |
|-----------|------------------|---------------|--------|------------------------------------------------------------------------------------------|----------|---------------|------------------------------|-------------|
| 32        | Other            | Cataract      | HSF4   | Cataract 5, multiple types                                                                | Het      | c.360+1G>A    | Noncoding (splice)           | AD          |
| 25141     | White British    | Cataract      | MAF    | Cataract 21, multiple types                                                                | Het      | c.782T>C; p.(Phe261Ser) | Missense                        | AD          |
| 26351     | White British    | Cataract      | MIP    | Cataract 15, multiple types                                                                | Het      | c.519del; p.(Phe174Leufs*10) | Frameshift deletion             | AD          |
| 12720     | Asian Bangladeshi| Cataract      | TDRD7  | Cataract 36                                                                                | Hom      | c.1591C>T; p.(Arg531Trp) | Missense                        | AR          |
| 36        | White British    | Cataract      | BCOR   | Congenital cataract                                                                       | Het      | c.856del; p.(Ser286Alafs*92) | Frameshift deletion             | XD          |
| 22026     | White other      | Cataract      | CHMP4B | Cataract 31, multiple types                                                                | Het      | c.481G>C; p.Glu161Gln | Missense                        | AD          |
| 23303     | Other            | Cataract      | CRYBB2 | Cataract 3, multiple types                                                                 | Het      | c.355G>A; p.(Gly119Arg) | Missense                        | AD          |
| 24533     | White British    | Cataract      | NHS    | Nance–Horan syndrome                                                                      | Hemi     | c.245dup; p.(Pro83Alafs*100) | Frameshift duplication         | XR          |
| 23139     | White British    | Cataract      | PITX3  | Cataract 11, multiple types                                                                | Het      | c.785C>A; p.(Ser262Glu) | Nonsense                        | AD          |
| 26297     | Asian Indian     | Cataract      | CRYBB1 | Cataract 17, multiple types                                                                | Het      | c.667C>T; p.(Gln223*) | Missense                        | AD          |
| 40        | Mixed White/Black African | Nystagmus | CACNA1A | Idiopathic infantile nystagmus                                                              | Het      | c.2566G>T; p.(Glu856*) | Missense                        | AD          |
| 10282     | White British    | Nystagmus     | FRMD7  | Nystagmus 1, congenital, X-linked                                                          | Hemi     | c.206-5T>A    | Noncoding (splice)           | XR          |
| 18284     | White British    | Nystagmus     | FRMD7  | Nystagmus 1, congenital, X-linked                                                          | Hemi     | c.1003C>T; p.(Arg335*) | Nonsense                        | XR          |
| 26131     | Mixed: Other     | Nystagmus     | FRMD7  | Nystagmus 1, congenital, X-linked                                                          | Hemi     | c.875T>C; p.(Leu292Pro) | Missense                        | XR          |
| 46        | Other            | Nystagmus     | FRMD7  | Nystagmus 1, congenital, X-linked                                                          | Hemi     | c.383-1G>A    | Noncoding (splice)           | XR          |
| 25824     | Black Caribbean  | Nystagmus     | OCA2   | Albinism, oculocutaneous, type II                                                          | Hemi     | c.1103C>T; p.(Ala368Val) | Missense                        | AR          |
| 26350     | Asian Pakistani  | Nystagmus     | SLC38A8| Foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis | Hom      | c.264C>G; p.Tyr88* | Nonsense                        | AR          |
| 52        | Black Caribbean  | Albinism      | GPR143 | Nystagmus 6, congenital, X-linked                                                          | Hemi     | c.11C>G; p.(Pro4Arg) | Missense                        | XR          |
| 14259     | White British    | Albinism      | GPR143 | Ocular albinism, type I                                                                    | Hemi     | c.238-240del; p.Leu80del | Inframe deletion                | XR          |
| 17466     | Asian Indian     | Albinism      | GPR143 | Ocular albinism, type I                                                                    | Hemi     | c.251-1G>C    | Noncoding (splice)           | XR          |
| 18142     | Asian Indian     | Albinism      | GPR143 | Ocular albinism, type I                                                                    | Hemi     | c.499del; p.(Leu167Cysfs*51) | Frameshift deletion             | XR          |
| 18524     | White British    | Albinism      | GPR143 | Ocular albinism, type I                                                                    | Hemi     | c.874T>G; p.Trp292Gly | Missense                        | XR          |
| 20738     | White British    | Albinism      | GPR143 | Ocular albinism, type I                                                                    | Hemi     | c.703G>A; p.Glu235Lys | Missense                        | XR          |
| 24336     | White British    | Albinism      | GPR143 | Ocular albinism, type I                                                                    | Hemi     | c.180dup; p.(Ala61Argfs*40) | Frameshift duplication         | XR          |
| 25692     | Black African    | Albinism      | GPR143 | Ocular albinism, type I                                                                    | Hemi     | c.691T>C; p.Tyr231His | Missense                        | XR          |
| 53        | Other            | Albinism      | HPS6   | Hermansky–Pudlak syndrome 6                                                                | Hom      | c.1228,1252del; p.(Tyr410Valfs*9) | Frameshift deletion             | AR          |
| Family ID | Ethnicity       | Disease group | Gene | Confirmed phenotype (OMIM)                   | Zygosity | Variant | Variant type          | Inheritance |
|-----------|----------------|---------------|------|---------------------------------------------|----------|---------|-----------------------|-------------|
| 51        | White British  | Albinism      | OCA2 | Albinism, oculocutaneous, type II           | Het      | c.619_636del; p. (Leu207_Leu212del) | Inframe deletion | AR          |
|           |                |               |      |                                             | Het      | c.1327G>A; p.(Val443Ile)        | Missense               |             |
| 14205     | White British  | Albinism      | OCA2 | Albinism, oculocutaneous, type II           | Hom      | c.1320G>C; p.(Leu440Phe)       | Missense               | AR          |
| 22452     | Black African  | Albinism      | OCA2 | Albinism, oculocutaneous, type II           | Het      | c.1182+1G>A                  | Noncoding (splice)     | AR          |
| 24075     | Black African  | Albinism      | OCA2 | Albinism, oculocutaneous, type II           | Hom      | c.619_636del; p. (Leu207_Leu212del) | Inframe deletion | AR          |
| 25242     | White British  | Albinism      | OCA2 | Albinism, oculocutaneous, type II           | Het      | c.1320G>C; p.(Leu440Phe)       | Missense               | AR          |
| 25246     | White other    | Albinism      | OCA2 | Albinism, oculocutaneous, type II           | Het      | c.1286T>C; p.(Leu429Pro)       | Missense               | AR          |
| 26332     | White British  | Albinism      | OCA2 | Albinism, oculocutaneous, type II           | Het      | c.1465A>G; p.(Asn489Asp)       | Missense               | AR          |
| 20483     | Asian Pakistani| Albinism      | SLC24A5 | Albinism, oculocutaneous, type VI          | Hom      | c.568_572del; p.(Ile190*)     | Nonsense               | AR          |
| 56        | White British  | Albinism      | SLC38A8 | Foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis | Het      | c.435G>A; p.Trp145Ter       | Nonsense               | AR          |
| 58        | Asian Indian   | Albinism      | SLC38A8 | Foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis | Hom      | c.264C>G; p.Tyr88*       | Nonsense               | AR          |
| 26364     | Asian other    | Albinism      | SLC38A8 | Foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis | Hom      | c.698A>G; p.(Glu233Gly)     | Missense               | AR          |
| 12189     | Asian Indian   | Albinism      | TYR  | Albinism, oculocutaneous, type IA           | Hom      | c.1255G>A; p.(Gly419Arg)     | Missense               | AR          |
| 12676     | White British  | Albinism      | TYR  | Albinism, oculocutaneous, type IA           | Het      | c.832C>T; p.Arg278*         | Nonsense               | AR          |
| 18849     | White other    | Albinism      | TYR  | Albinism, oculocutaneous, type IA           | Hom      | c.371T>G; p.(Phe124Cys)     | Missense               | AR          |
| 20104     | Mixed: White/Black Caribbean | Albinism | TYR  | Albinism, oculocutaneous, type IA          | Het      | c.241C>T; p.Pro81Ser      | Missense               | AR          |
| 22206     | White British  | Albinism      | TYR  | Albinism, oculocutaneous, type IA           | Het      | c.1118C>A; p.(Thr373Lys)    | Missense               | AR          |
| 22894     | White Irish    | Albinism      | TYR  | Albinism, oculocutaneous, type IA           | Het      | c.823G>T; p.(Val275Phe)     | Missense               | AR          |
| 23431     | Asian Indian   | Albinism      | TYR  | Albinism, oculocutaneous, type IA           | Hom      | c.832C>T; p.(Arg278*)       | Nonsense               | AR          |

(Continues)
The spectrum of genetic and phenotypic heterogeneity, and copresentation of developmental eye disorders is a major challenge in obtaining a molecular diagnosis. A mixed testing approach in the clinic using targeted gene panels, single gene and WGS yielded a higher overall molecular diagnostic rate of 33.9% (20 out of 59 families) compared to WGS alone. Arguably, only 24.9% received a result from the 100,000 Genomes Project (73 out of 293 families), but this involved a larger sample size and was in-line with other studies. The development of a next generation sequencing (NGS) panel assay for ocular conditions known as the Oculome panel test (http://www.labs.gosh.nhs.uk/media/764794/oculome_v8.pdf) screened 277 pediatric patients across several panels (including MAC, ASDA, cataract, retinal and albinism) with 68 individuals (24.5%) receiving a definitive diagnosis (Patel et al., 2019). For disease subgroups, there are significant differences between the published outcomes and what we determined. Having small sample sizes can impact on the accuracy of these genetic diagnostic rates, as demonstrated with the ASDA subgroup; for the Oculome panel test 24.8% (28 of 113 cases) received a molecular diagnosis, but from our clinic this reached 60% (6/10) cases solved, however the larger WGS cohort of 46 patients found only 13.0% (six cases) were solved, demonstrating the variability in outcomes. Conversely, in the congenital cataract group undergoing the Oculome panel test, eight of nine cases (88.9%) received a molecular diagnosis. We had a similar number of patients from our clinics but only solved four out of nine families (44.4%), and with WGS 44.7% (17/38) were solved.

For MAC conditions, the Oculome panel test was able to solve 8.2% of cases but through our clinics and the 100,000 Genomes Project (using WGS), our patients had at least a twofold increase with 21.4% (3/14) and 15.7% (11/70) diagnostic rates, respectively. This confirms that WGS has the capacity to increase diagnostic rates. The most prevalent gene was \texttt{PRSS56} (OMIM #613858), which causes autosomal recessive microphthalmia, isolated 6 (OMIM #613517), found in two unrelated families (16899 and 25356). All other MAC families had their own individual disease-causing gene, which can make genotype–phenotype correlations hard to determine as many more cases need to be identified to strengthen associations and prognosis. As only 11 out of 70 cases were solved, this suggests that many deep intronic variants may be as yet undetected due to the diagnostic pipeline limitations. This low solve rate also implies that more variants and novel genes remain undiscovered, and/or possible alternate non-Mendelian disease aetiologies, for example epigenetic or complex genetic causes. In a recent paper using the wildtype zebrafish as a model of optic fissure morphogenesis, ocular tissue from the site of the unfused, fusing and post-fusion optic fissure was taken for comparative global transcriptomic profiling against dorsal retina at the same timepoint (Richardson et al., 2019). Overall, 322 differentially expressed genes were found to be involved in optic fissure morphogenesis, these can be further interrogated through knockdown/out studies for resultant MAC phenotypes, comparative analysis in other animal models looking for conserved genes, and in larger patient
coHORTS such as those recruited to the 100,000 Genomes Project (Caulfield et al., 2019) and the NIHBIoresource (NBR-RD, 2019).

The use of human induced pluripotent stem cells (hiPSCs) has been manipulated to generate models of human eye development, and could be used to identify more candidate genes by revealing underlying molecular mechanisms (Hung, Khan, Lo, Hewitt, & Wong, 2017; Llonch, Carido, & Ader, 2018). Optic vesicle-like structures derived from a microphthalmia patient with a VSX2 null variant (p.Arg200Gln) showed upregulation of WNT pathway components and misexpression of retinal pigment epithelium (RPE) markers at the expense of the neural retina (NR) lineage, which was rescued by pharmacological inhibition of WNT signaling (Capowski et al., 2016). This supports an important role for VSX2 in WNT signaling and maintenance of the NR through WNT pathway suppression. One potential problem of in vitro modeling is the lack of surrounding embryological tissue and external stimuli that may provide cues for in vivo development, this is particularly important given the involvement of multiple ocular structures in patients. Utilization of data from both cell-based systems and animal models will continue to provide a more complete and accurate representation.

The given primary diagnosis can influence the choice of gene panel and subsequent diagnostic rates. For example, in this study family 36 with congenital cataracts was found to harbor a heterozygous frameshift deletion (c.856del, p.[Ser286Alafs*92]) in BCR (OMIM #300485), and this causes X-linked dominant microphthalmia, syndromic 2 (OMIM #300166). The female patient presented with bilateral congenital cataracts, unilateral left microphthalmia and persistent primary teeth, and thus could fall into both MAC and cataract cohorts, but was counted under congenital cataract. Similarly, family 17 had unilateral left microphthalmia with cornea plana, anterior segment dysgenesis, left exotropia and high hypermetropia, and was identified to have a homozygous missense variant (c.809C>T, p.[Ser287Leu]) in the KERA gene (OMIM #603288). KERA is known to cause autosomal recessive corneal plana 2 (OMIM #217300) and this variant is rare in healthy population databases, although not previously reported to cause disease. The unaffected father was confirmed as a heterozygous carrier, and the unaffected mother did not undergo genetic testing. KERA is not found in the MAC targeted gene panel despite several case reports suggesting its association (Hu et al., 2019; Kumari et al., 2016; Lehmann et al., 2001). This variant was identified from WGS, which has more flexibility to screen novel genes or variants. The drawback of targeted gene panels is that they are predesigned to cover given regions of the genome, and if updates are required, a new panel must be designed which incurs costs for time and validation. In contrast, WGS covers the entire genome, over 3 billion nucleotides and 20,000 genes, and can identify previously noncovered variants such as CNVs, structural variations, intergenic and deep intronic variants (Lionel et al., 2018; Van Cauwenbergh et al., 2017; Vaz-Drago, Custodio, & Carmo-Fonseca, 2017). This is important for developmental eye disorders, such as aniridia where ~15% of all PAX6 variants are located in intronic regions, with the majority at the intron–exon border (Lima Cunha, Arno, Corton, & Moosajee, 2019), although only 8.6% were noncoding variants from the WGS group in this study.

A notable finding for patients with query albinism and infantile nystagmus, was that a number of genes were found in both subgroups, including OCA2 and SLC38A8. This highlights the diagnostic challenges of young children with nystagmus as their presenting complaint. There is significant phenotypic variability in albinism patients, which can make it hard to delineate from infantile nystagmus. Visual evoked potentials are excellent at detecting intracranial misrouting, but this can be inconclusive in young infants. Hence, for some patients their diagnosis will be revised based on their molecular result, for example one nystagmus family (25824) was found to have biallelic variants in OCA2 (OMIM #611409) causing autosomal recessive oculocutaneous albinism type II (OMIM #203200). Hence further clinical phenotyping can be required to confirm the correct clinical diagnosis. Four unrelated families (56, 26350, 58, and 26364) were identified with biallelic SLC38A8 (OMIM #615585) variants through WGS (as this gene is not present on targeted gene panels for nystagmus or albinism). It causes autosomal recessive foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis (OMIM #609218), and thus its features can pose clinical diagnostic challenges. This reinforces the need to undertake genetic testing in order to reach a definitive diagnosis that can guide the ongoing care and management of the patient. As more genes are identified, it is likely that we will begin to see more extensive spectrums of disease rather than distinct disease entities. Classifications of disease using eponymous names must also be superseded by a gene-based system where we can build our knowledge of genotype–phenotype relationships.

Patient access to genetic testing has been a barrier to accurate, timely diagnoses and appropriate management. In countries with insurance-based systems, agencies are reluctant to fund genetic testing unless there is clear evidence the results will accurately determine the clinical status of the patient and directly influence management (Capasso, 2014). In the United Kingdom, the 100,000 Genomes Project was established to provide an infrastructure that allows NHS patients with rare disease to access WGS through a genomic medicine service with centralized funding (Patch & Middleton, 2019; Turnbull et al., 2018). This transition is underway, with designated laboratories undertaking ophthalmic genetic testing and oversight from Genomic Hubs permitting equity of genomics services (Royal College of Ophthalmologists, 2020). The National Genomic Test Directory for rare and inherited diseases has been formed detailing which test is available for each clinical indication, with the Genomics England Panel app listing the genes included in each panel, reviewed by experts to ensure there is evidence for its inclusion (Genomics England Panel App, n.d.). Such advances will accelerate time to diagnosis, improve diagnostic rates, permit precision management and cost saving on prolonged, multidisciplinary team assessments and investigations (Gillespie et al., 2014; Musleh et al., 2016). For patients and families with developmental eye disorders, an accurate molecular diagnosis earlier in the patient pathway potentially provides several benefits by addressing uncertainty, improving decision-making and elucidation of any systemic manifestations, a number of which are can be potentially life threatening. Research into developmental eye disorders will also greatly benefit from larger solved patient datasets.
CONCLUSION

Understanding the etiology of developmental eye disorders remains challenging given their diverse phenotypes and genetic heterogeneity. Diagnostic rates remain variable and relatively lower than the progress made with IRDs. NGS technologies allow variants to be screened in parallel and at relatively low cost. Expanding WGS from the research setting to an accredited clinical service will allow for more accurate diagnosis and improved management of patients. Ensuring precise human phenotype ontology is used to document each clinical feature (not just the primary diagnosis) will enable clinical scientists to best apply the relevant diagnostic gene panel. Being able to gain a molecular diagnosis will further our understanding of the natural history of gene/variant-specific cohorts, reveal potential therapeutic targets and establish outcome measures for prospective future treatments.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The datasets generated for this study were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) through the Rare & Inherited Disease Genomic Laboratory at Great Ormond Street Hospital (London, UK).

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REFERENCES

Audo, I., Bujakowska, K. M., Leveillard, T., Mohand-Said, S., Lancelot, M. E., Germain, A., ... Zeitz, C. (2012). Development and application of a next-generation-sequencing (NGS) approach to detect known and novel gene defects underlying retinal diseases. Orphanet Journal of Rare Diseases, 7, 8. https://doi.org/10.1186/1750-1172-7-8
Bernardis, I., Chiesi, L., Tenenini, E., Artuso, L., Percesepe, A., Artusi, V., ... Tagliafico, E. (2016). Unravelling the complexity of inherited retinal dystrophies molecular testing: Added value of targeted next-generation sequencing. BioMed Research International, 2016, 6341870–6341874. https://doi.org/10.1155/2016/6341870
Busby, A., Dolk, H., & Armstrong, B. (2005). Eye anomalies: Seasonal variation and maternal viral infections. Epidemiology, 16(3), 317–322. https://doi.org/10.1097/01.ede.0000158817.43037.ab
Capasso, J. (2014). The cost of genetic testing for ocular disease: Who pays? Current Opinion in Ophthalmology, 25, 394–399.
Capowski, E. E., Wright, L. S., Liang, K., Phillips, M. J., Wallace, K., Petelinsek, A., ... Gamm, D. M. (2016). Regulation of WNT signaling by VSX2 during optic vesicle patterning in human induced pluripotent stem cells. Stem Cells, 34(11), 2625–2634. https://doi.org/10.1002/stem.2414
Caufield, M., Davies, J., Dennys, M., Elbahy, L., Fowler, T., Hill, S., ... Woods, K. (2019). The National Genomics Research and Healthcare Knowledgebase v5. London, England: Genomics England. https://doi.org/10.6084/m9.figshare.4530893.v5
Chassaing, N., Caussé, A., Vigouroux, A., Delahaye, A., Alessandri, J. L., Boespflug-Tanguy, O., ... Calvas, P. (2014). Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. Clinical Genetics, 86(4), 326–334. https://doi.org/10.1111/ceg.12275
Chassaing, N., Gilbert-Dussardier, B., Nicot, F., Fermeaux, V., Encharazvi, F., Fiorenza, M., ... Calvas, P. (2007). Germinal mosaicism and familial recurrence of a SOX2 mutation with highly variable phenotypic expression extending from AEG syndrome to absence of ocular involvement. American Journal of Medical Genetics. Part A, 143a(3), 289–291. https://doi.org/10.1002/ajmg.a.31524
Consugur, M. B., Navarro-Gomez, D., Place, E. M., Bujakowska, K. M., Sousa, M. E., Fonseca-Kelly, Z. D., ... Pierce, E. A. (2015). Panel-based genetic diagnostic testing for inherited eye diseases is highly accurate and reproducible, and more sensitive for variant detection, than exome sequencing. Genetics in Medicine, 17(4), 253–261. https://doi.org/10.1038/gim.2014.172
Ellingford, J. M., Barton, S., Bhaskar, S., O’Sullivan, J., Williams, S. G., Lamb, J. A., ... Black, G. C. M. (2016). Molecular findings from 537 individuals with inherited retinal disease. Journal of Medical Genetics, 53(11), 761–767. https://doi.org/10.1136/jmedgenet-2016-103837
Faiivre, L., Williamson, K. A., Faber, V., Laurent, N., Grimaldi, M., Thauvin-Robinet, C., ... Fitzpatrick, D. R. (2006). Recurrence of SOX2 anophthalmia syndrome with gonosomal mosaicism in a phenotypically normal mother. American Journal of Medical Genetics. Part A, 140(6), 636–639. https://doi.org/10.1002/ajmg.a.31114
Genomics England Panel App. (n.d.). Retrieved from https://panelapp.genomicsengland.co.uk/
Gilbert, C. F. A. (2001). Childhood blindness in the context of VISION 2020—The right to sight. Bull World Health Organ, 79(3), 227–232.
Gillespie, R. L., O’Sullivan, J., Ashworth, J., Bhaskar, S., Williams, S., Biswas, S., ... Lloyd, I. C. (2014). Personalized diagnosis and management of congenital cataract by next-generation sequencing. Ophthalmology, 121, 2124–2137.e2. https://doi.org/10.1016/j.ophtha.2014.06.006
Givens, K. T., Lee, D. A., Jones, T., & Istrup, D. M. (1993). Congenital rubella syndrome: Ophthalmic manifestations and associated systemic disorders. The British Journal of Ophthalmology, 77(6), 358–363. https://doi.org/10.1136/bjo.77.6.358
Harding, P., & Moosajee, M. (2019). The molecular basis of human anophthalmia and microphthalmia. Journal of Developmental Biology, 7(3), 16. https://doi.org/10.3390/jdb7030016
Hornby, S. J., Gilbert, C. E., Rahi, J. K., Sil, A. K., Xiao, Y., Dandona, L., & Foster, A. (2000). Regional variation in blindness in children due to microphthalmos, anophthalmos and coloboma. Ophthalmic Epidemiology, 7(2), 127–138.
Huang, C., Long, X., Peng, C., Lin, P., Tan, H., Lv, W., & Wu, L. (2019). Novel variants in the KERA gene cause autosomal recessive cornea plana in a Chinese family: A case report. Medicine, 19(6), 4711–4718. https://doi.org/10.1082/mmr.2019.10153
Huang, S. C. C., Khan, S., Lo, C. Y., Hewitt, A. W., & Wong, R. C. B. (2017). Drug discovery using induced pluripotent stem cell models of
neurodegenerative and ocular diseases. Pharmacology & Therapeutics, 177, 32–43. https://doi.org/10.1016/j.phramthera.2017.02.026
Jiman, O. A., Taylor, R. L., Lenassi, E., Smith, J. C., Douzgou, S., Ellingford, J. M., ... Black, G. C. (2020). Diagnostic yield of panel-based genetic testing in syndrome inherited retinal disease. European Journal of Human Genetics, 28(3), 576–586. https://doi.org/10.1038/s41431-019-0548-5
Kumari, D., Tiwari, A., Choudhury, M., Kumar, A., Rao, A., & Dixit, M. (2016). A novel KERA mutation in a case of autosomal recessive corneal plana with primary angle-closure glaucoma. Journal of Glaucoma, 25(2), e106–e109. https://doi.org/10.1097/IJG.0000000000000258
Lehmann, O. J., El-shary, M. F., Ebenezer, N. D., Ocaña, L., Francis, P. J., Wilkie, S. E., ... Bhattacharya, S. S. (2001). A novel keratocan mutation causing autosomal recessive corneal plana. Investigative Ophthalmology & Visual Science, 42(13), 3118–3122.
Lima Cunha, D., Arno, G., Corton, M., & Moosajee, M. (2019). The spectrum of PAX6 mutations and genotype-phenotype correlations in the eye. Genes (Basel), 10(12), 1050. https://doi.org/10.3390/genes10121050
Lionel, A. C., Costain, G., Monfare, N., Walker, S., Reuter, M. S., Hosseini, S. M., ... Marshall, C. R. (2018). Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. Genetics in Medicine, 20(4), 435–443. https://doi.org/10.1038/s41436-018-0027-0
Lionch, S., Carido, M., & Ader, M. (2018). Organoid technology for retinal repair. Developmental Biology, 433(2), 132–143. https://doi.org/10.1016/j.ydbio.2017.09.028
Maguire, A. M., Sullivan, R., Wellman, J. A., Chung, D. C., Yu, Z.-F., Tillman, A., ... Maguire, A. M. (2017). Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: A randomised, controlled, open-label, phase 3 trial. Lancet (London, England), 390(10097), 849–860. https://doi.org/10.1016/S0140-6736(17)31868-8
Shah, S. P., Taylor, A. E., Crowe, N. K., Russell-Eggitt, I., Rahi, J. S., & Gilbert, C. E. (2011). Anophthalmos, microphthalmos, and typical coloboma in the United Kingdom: A prospective study of incidence and risk. Investigative Ophthalmology & Visual Science, 52(1), 558–564. https://doi.org/10.1167/iovs.10-5263
Tayebi, N., Akinrinade, O., Khan, M. I., Hejaifar, A., Dehghani, A., Cremers, F. P. M., & Akhlaghi, M. (2019). Targeted next generation sequencing reveals genetic defects underlying inherited retinal disease in Iranian families. Molecular Vision, 25, 106–117.
Turnbull, C., Scott, S. V., Thomas, E., Jones, L., Murugaesu, N., Pretty, F. B., ... Genomes Project. (2018). The 100 000 Genomes Project: Bringing whole genome sequencing to the NHS. BMJ, 361, k1687. https://doi.org/10.1136/bmj.k1687
Van Cauwenbergh, C., Van Schil, K., Cannooodt, R., Bauwens, M., Van Laethem, T., De Jaegere, S., ... De Baere, E. (2017). arEYE: A customized platform for high-resolution copy number analysis of coding and noncoding regions of known and candidate retinal dystrophy genes and retinal noncoding RNAs. Genetics in Medicine, 19(4), 457–466. https://doi.org/10.1038/gim.2016.119
Vaz-Drago, R., Custodio, N., & Camo-Fosseca, M. (2017). Deep intrinsic mutations and human disease. Human Genetics, 136(9), 1093–1111. https://doi.org/10.1007/s00439-017-1809-4
Williamson, K. A., & FitzPatrick, D. R. (2014). The genetic architecture of microphthalmia, anophthalmia and coloboma. European Journal of Medical Genetics, 57(8), 369–380. https://doi.org/10.1016/j.ejmg.2014.05.002

SUPPORTING INFORMATION

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

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