

# Familial congenital cataract, coloboma, and nystagmus phenotype with variable expression caused by mutation in *PAX6* in a South African family

Saadiah Goolam,<sup>1</sup> Nadia Carstens,<sup>2,3</sup> Mark Ross,<sup>4</sup> David Bentley,<sup>4</sup> Margarida Lopes,<sup>4</sup> John Peden,<sup>4</sup> Zoya Kingsbury,<sup>4</sup> Eleni Tsogka,<sup>4</sup> Robyn Barlow,<sup>2</sup> Trevor R. Carmichael,<sup>1</sup> Michèle Ramsay,<sup>2,3</sup> Susan E. Williams<sup>1</sup>

(The first two authors have contributed equally to this work)

<sup>1</sup>Division of Ophthalmology, Department of Neurosciences, University of Witwatersrand, Johannesburg, South Africa; <sup>2</sup>Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, South Africa; <sup>3</sup>Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; <sup>4</sup>Illumina Cambridge Ltd., Great Chesterford, United Kingdom

**Purpose:** To report on a clinical and genetic investigation of a large, multigenerational South African family of mixed ancestry with autosomal dominant congenital cataracts, coloboma, and nystagmus.

**Methods:** Ophthalmic examination was performed in 27 individuals from the same admixed South African family. DNA was sampled from either peripheral blood or buccal swabs in all 27 individuals, and whole genome sequencing was performed in six individuals. Sanger sequencing was used to validate the probable mutation in the remaining family members.

**Results:** Twenty-seven family members with 19 affected individuals were included in the study. The predominant phenotype, with highly variable expression, was congenital cataract (14 individuals), posterior segment coloboma (17 individuals), and nystagmus (18 individuals). Other features present included high myopia, microcornea, and strabismus. An R208W mutation in *PAX6* (dbSNP rs757259413; HGMD CM930572; NM\_000280.3:c.622G>A; NP\_000271.1:p.Arg208Trp) was identified as being the most probable pathogenic mutation. Cosegregation of the mutation with the phenotype was confirmed in all 27 family members.

**Conclusions:** *PAX6* is a highly conserved gene crucial for normal oculogenesis, and although mutations within the gene may cause an array of ocular developmental abnormalities, most are associated with aniridia and aniridia-related ocular defects. The observation that *PAX6* aniridia phenotypes are largely associated with nonsense mutations and milder non-aniridia phenotypes with missense mutations suggested that there may be specific genotype–phenotype correlations for the gene. The R208W mutation in *PAX6* identified in this family challenges this theory as it has previously been reported in three unrelated families and is associated with aniridia and non-aniridia phenotypes across the four families. *PAX6* with its wide phenotypic associations and highly variable expression should be considered a candidate gene in the diagnostic screen for any ocular developmental abnormality.

Congenital cataracts are an important treatable cause of visual disability in children and account for approximately 10% of childhood blindness worldwide [1,2]. Congenital cataracts may occur in isolation or with other ocular abnormalities and may have syndromic associations. Associated ocular abnormalities include microcornea, microphthalmia, anterior segment dysgenesis including aniridia, and glaucoma [1]. In non-consanguineous populations, most non-syndromic hereditary forms of cataract demonstrate an autosomal dominant pattern of inheritance, but autosomal recessive and X-linked

inheritance have also been described [3]. More than 30 genes have been implicated in non-syndromic congenital cataracts, with most mutations found in the crystallin and gap junction groups of genes; however, transcription factor (*PAX6*- Gene ID: 5080; OMIM 607108 and *MAF*- Gene ID: 4094; OMIM 177075) and lens structural protein (*VIM*- Gene ID: 7431; OMIM 193060, *MIP*- Gene ID: 4284; OMIM 154050, and *BFSPI*- Gene ID: 631; OMIM 603307) genes also account for a considerable proportion [1,3].

Ocular colobomas are rarer developmental abnormalities, with most resulting from failed closure of the choroidal fissure during early embryogenesis. They may involve any of the ocular structures along the fissure line from the cornea to the optic nerve [4]. They, too, can occur in isolation or in association with other ocular abnormalities (particularly microphthalmia and anophthalmia), and they may also have

Correspondence to: Susan E Williams, University of the Witwatersrand Division of Ophthalmology, Department of Neurosciences, 87 Craigtowne, 1, Richmond avenue, Johannesburg, Gauteng 2196, South Africa; Phone: +27824676999; FAX: +27114473725; email: susanwil@mweb.co.za or Susan.Williams@wits.ac.za

syndromic associations [4-6]. Inherited forms predominantly display an autosomal dominant mode of inheritance, but autosomal recessive and less frequently X-linked forms have been documented [5,6]. Most hereditary forms of coloboma are associated with non-ocular systemic features [6]. Several genes have been associated with the coloboma phenotype, and some are regulators of other coloboma-associated genes [6,7]. Congenital cataracts may be associated with ocular coloboma, particularly in the setting of an anterior segment coloboma involving either the iris, zonules, or ciliary body.

We report a clinical and genetic investigation of a large, multigenerational South African family of mixed ancestry with autosomal dominant congenital cataracts, coloboma, and nystagmus. The phenotype shows variably expressed congenital cataract and predominantly isolated posterior segment coloboma phenotypes in members of this family, and a next-generation sequencing approach was used to elucidate its genetic etiology.

## METHODS

*Patients:* Following the initial evaluation of a mother (III:17) and son (IV:5) presenting for management of congenital cataracts in 2014, an additional 25 individuals from the same admixed South African family were identified and invited to participate in the study between May and December 2015. The study received approval from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa (protocol number M131125) and adhered to the Association for Research and Vision in Ophthalmology (ARVO) statement on human subjects. Written informed consent was obtained from all participating family members for the use of their DNA and clinical data for research purposes after a genetic counselor explained the nature and possible consequences of the study. All family members underwent ophthalmic evaluation which consisted of slit-lamp, indirect ophthalmoscopic, optometric, and biometric assessments where appropriate.

*Genotyping and whole genome sequencing:* DNA was sampled using either peripheral blood (drawn from the antecubital vein) or saliva in 27 family members. The samples were refrigerated until extraction. Genomic DNA was isolated from whole blood using a salting-out procedure adapted from Miller et al. (1988) or saliva using the *Oragene* Saliva Collection and DNA extraction system (DNA Genotek, Inc., Ontario, Canada) [8]. The approach was to identify the most distantly related affected individuals in the pedigree as the most informative for sequencing as they would have

the smallest degree of haplotype sharing. Genotyping was performed in the pedigree using the Illumina Omni 2.5–8.v1 array (Illumina, San Diego, CA). As part of quality control, single nucleotide polymorphisms (SNPs) were excluded if they had a call rate  $\leq 95\%$ , a minor allele frequency  $\leq 0.01$ , or significant deviations from Hardy–Weinberg equilibrium ( $p \leq 10^{-7}$ ). The coefficient of relatedness ( $\pi$ -hat) was calculated using PLINK1.9 [9] and the number of shared haplotypes that are identical by descent (IBD) using Beagle4 [10]. The six affected individuals with the lowest degree of kinship, which also shared, as expected, more IBD segments than unrelated pedigree members, were selected for whole genome sequencing (WGS). Application of sequencing by synthesis (SBS) to human WGS was previously described [11]. PCR-free libraries of blood and buccal scrape samples were prepared using the TruSeq® DNA PCR-free sample preparation kit (Illumina). The libraries were sequenced as paired-ends ( $2 \times 150$  cycles) on the HiSeqX platform to an average depth of  $\geq 40\times$ , following alignment to the human reference sequence (GRCh37) and removal of duplicate read-pairs. Variant calling was performed using the Illumina IsaacVariantCaller (2.1.4.2).

*Variant ranking:* Using a tiered approach, we first prioritized rare variants (minor allele frequency below 1% in ExAC [12]) that were predicted by bioinformatic functional predictors [13] to affect the protein structure and function in genes known to be associated with cataracts from the Cat-Map database [14] or developmental eye disease phenotypes [15]. This filtering approach was then repeated for genes not registered on the Cat-Map database. The most probable disease-causing variant was then selected from the prioritized variants based on a Human Gene Mutation Database (HGMD [16]) search.

*Validation and segregation analysis:* Sanger sequencing was used to validate the most probable disease-causing variant in each of the 27 participating family members. Primers were designed to span the location of the variant and produce a fragment with PCR amplification. Each PCR was performed using 50 ng of purified genomic DNA as a template in a reaction mix containing 1X final concentration of KAPA Taq ReadyMix (Kapa Biosystems, Wilmington, MA). The following PCR conditions were used: Step 1, 95 °C for 10 min; Step 2, 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, repeated for 35 cycles; and Step 3, 72 °C for 10 min. These primers were then used to sequence the amplicons with the BigDye® Terminator v3.1 Cycle Sequencing chemistry (Applied Biosystems, Life Technologies, Thermo Fisher Scientific Corp., Carlsbad, CA) and a 3130 XL Genetic Analyzer.

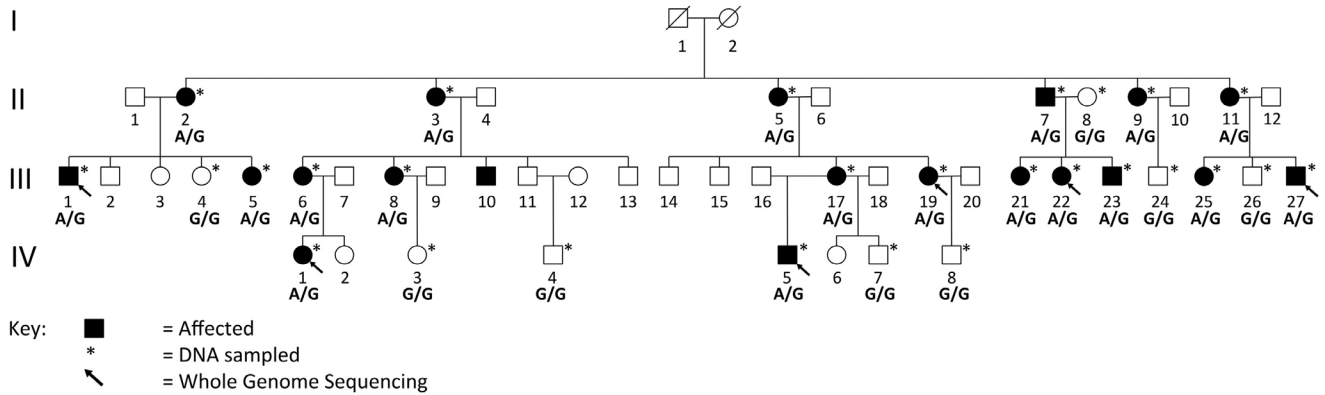


Figure 1. Family pedigree. Family pedigree illustrating the affected individuals, those who had DNA sampled, the six family members chosen for whole genome sequencing, and the genotype of each individual based on Sanger sequencing for the pathogenic R208W mutation in *PAX6*.

## RESULTS

Twenty-seven individuals were included in the study with 19 affected family members. The multigenerational pedigree was consistent with an autosomal dominant pattern of inheritance (Figure 1).

*Clinical evaluation:* Appendix 1 provides a summary of the clinical features in each of the 19 affected family members. There was variable expressivity of the phenotypes in affected members, each of whom showed at least one feature, but up to seven features in one of the individuals. Cataracts were present in 14 family members with the predominant type being anterior polar cataracts, and pupillary membrane remnants were present in four of these family members. Two family members were pseudophakic bilaterally at the time of assessment, and we were unable to ascertain what the cataract morphology had been before the surgery. Colobomas were present in 17 family members; 16 of them were isolated posterior segment colobomas (Figure 2). Bilateral temporally located iris colobomas without any posterior extension were present in one family member (IV:5), who also had large bilateral optic disc colobomas. Apart from this one individual with iris colobomas and the pupillary membrane remnants observed in four of the individuals with anterior polar cataracts, there were no other abnormalities detected in the iris architecture in the affected members of this family. Eighteen family members had nystagmus; the character was highly variable, ranging from mild gaze-evoked to prominent with horizontal and torsional components. Twelve family members were myopic; eight of them had either a refractive error of greater than 6 diopters (D) or an axial length of greater than 26.5 mm. Microcornea was present in three family members. Although foveal hypoplasia was not noted clinically in any

of the affected individuals, it was difficult to exclude this in some family members with nystagmus and posterior staphylomas, and optical coherence tomography (OCT) was attempted in these patients. An OCT image could be acquired in one eye of II:5 which did not suggest foveal hypoplasia. There were no neurodevelopmental or other systemic abnormalities detected in any of the 27 family members examined.

*Whole genome sequencing and variant analysis:* Appendix 2 contains a list of prioritized variants that had a minor allele frequency of less than 1% in public frequency databases and were present in a heterozygous state in all six of the affected individuals who had undergone whole genome sequencing. An R208W mutation in *PAX6* (dbSNP rs757259413; HGMD CM930572; NM\_000280.3:c.622G>A; NP\_000271.1:p.Arg208Trp) was identified with this approach. The variant had a combined annotation dependent depletion (CADD) score of 29.3 and was consistently predicted to be deleterious across all the bioinformatic functional predictors within ANNOVAR (Appendix 2).

*Segregation analysis:* Primers (sense 5'-CCA GGG CCT CAA TTT GCT CT-3' and antisense 5'-TGG GAA TGT TTT GGT GAG GCT-3') were designed to span the region of the R208W mutation in *PAX6*. A total of 27 (19 affected and eight unaffected) family members were evaluated, and Sanger sequencing results revealed that the R208W A allele cosegregated with the disease phenotype in all affected family members (Figure 1).

## DISCUSSION

The variably expressed cataract, coloboma, and nystagmus phenotype seen in this admixed family is attributed to an R208W mutation in the *PAX6* gene. *PAX6* is classically

known as the aniridia gene, with most pathogenic mutations resulting in a phenotype associated with the aniridia spectrum of ocular abnormalities [17,18]. These include variable iris defects or hypoplasia, glaucoma, corneal opacity, limbal stem cell deficiency, cataracts, foveal hypoplasia, and nystagmus [18,19]. *PAX6*, however, is a highly conserved gene encoding a transcriptional regulatory protein that is expressed in the developing eye, as well as the central nervous system and the pancreas [18,20]. *PAX6* is a “master control gene” crucial for normal oculogenesis, and mutations in the gene have also been linked to a broad range of non-aniridia phenotypes, including myopia, microcornea, coloboma and Peter’s anomaly, as well as neurodevelopmental defects [17-20]. The ocular features seen in this family (cataract, coloboma, nystagmus, myopia, and microcornea) all fall within the spectrum of aniridia and non-aniridia phenotypes previously associated with mutations in *PAX6*, although this particular constellation of ocular abnormalities appears to be novel.

The *PAX6* gene located on chromosome 11p13 consists of two DNA-binding domains, the paired domain and the homeodomain, which are found in the first two thirds of the open reading frame and are separated from each other by a 79-amino acid linker region [17,21,22]. The remaining third

comprises a proline, serine, and threonine-rich (PST) domain with transcriptional trans-regulatory function [17,21,22]. A review of the Online Human *PAX6* Allelic Variant Database revealed that most mutations in *PAX6* are found in the first two thirds of the open reading frame, with most of these resulting in an aniridia-associated phenotype [17,21]. Overall, as well as among the aniridia-associated mutations, the most common type of mutation is a single nucleotide substitution resulting in premature truncation or nonsense mediated RNA decay (a nonsense mutation) [17,18,21]. A smaller proportion of the mutations in *PAX6* are single nucleotide substitutions which result in the coding of a different amino acid (a missense mutation) [17,18]. The majority of non-aniridia-associated phenotypes appear to be caused by the less frequently observed missense mutations, an observation that gave rise to the idea that there may be specific genotype–phenotype correlations for the *PAX6* gene, with nonsense mutations resulting in the aniridia phenotypes, and missense mutations causing the milder non-aniridia phenotypes [17,20-22].

The R208W (c.622G>A) mutation responsible for the variable cataract and coloboma phenotype seen in this family is a missense mutation that results in the substitution of arginine for tryptophan. It is located in exon 8 in the region

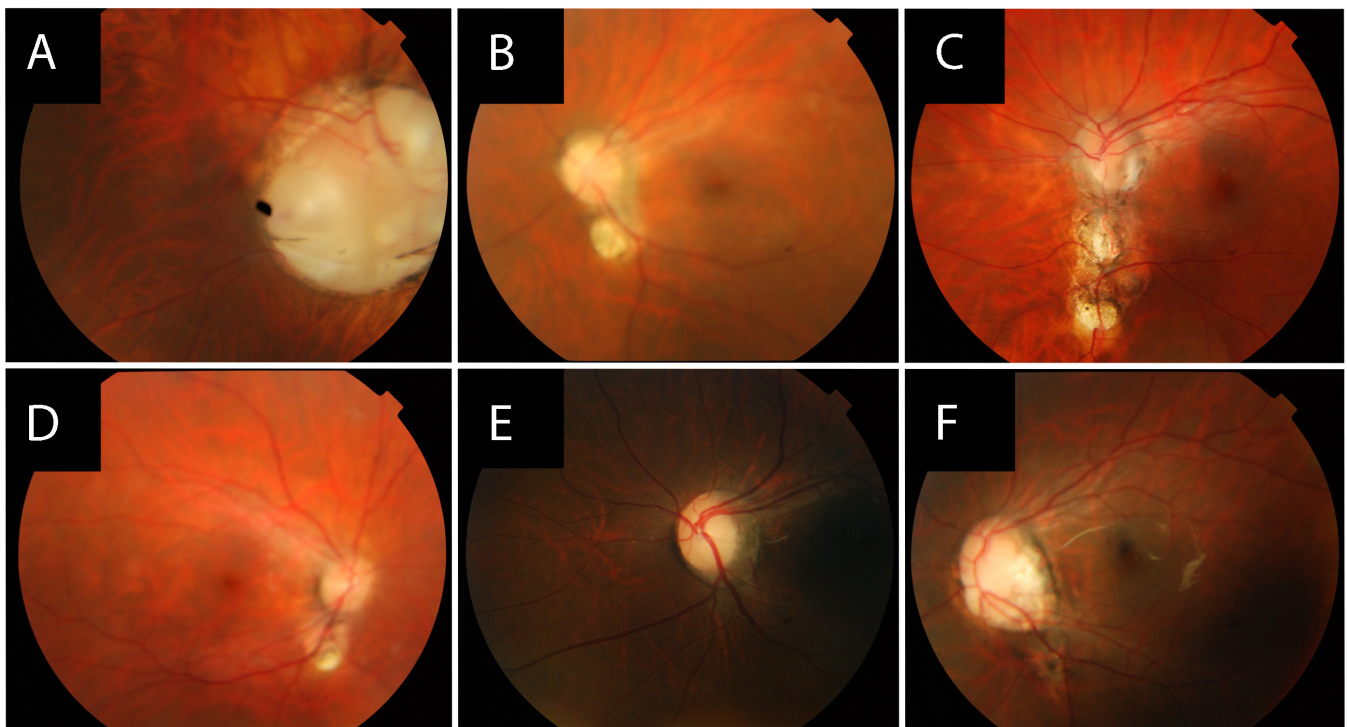


Figure 2. Illustrative images of posterior segment colobomas. Fundus photographs of some of the posterior segment colobomas present in affected family members. The following key uses the pedigree notation from Figure 1 to identify the individuals in whom the fundus photographs were taken. A = II:3, B = II:11, C = III:17, D = II:9, E = III:25, and F = III:29.

directly preceding the homeodomain and has been reported previously in three unrelated probands [18,23,24]. The first report was by Hanson et al. in 1993 of a female patient with familial aniridia, one of the earliest cases to be associated with a mutation in *PAX6* [23]. Lim et al. in 2012 reported a second R208W-associated case of aniridia in a Korean patient with partial aniridia, nystagmus, foveal hypoplasia, microcornea, keratopathy, and cataract [18]. Thomas, in his 2012 thesis on idiopathic infantile nystagmus, identified a family with the R208W mutation where the predominant phenotype was optic disc colobomas, iris transillumination defects without aniridia, nystagmus, and cataract [24]. None of the 19 affected family members in the present study had aniridia or iris transillumination defects, and although one family member had bilateral iris defects, they appeared typical of iris colobomas within the inferotemporal quadrant of the globe. It is interesting that the same R208W missense mutation appears to be associated with aniridia and non-aniridia phenotypes across the four affected families. This challenges the suggestion of specific genotype–phenotype correlations with mutations in *PAX6*, and impresses the need to better understand the determinants of phenotypic expression.

Mutations in *PAX6* may be associated with a broad range of ocular developmental defects with marked phenotypic variability, as highlighted by the clinical findings in this family. We recommend that *PAX6* be considered a candidate gene in any genetic diagnostic screen performed for ocular developmental abnormalities, particularly when they are multiple.

#### **APPENDIX 1. OPHTHALMIC FEATURES PRESENT IN EACH OF THE AFFECTED FAMILY MEMBERS.**

To access the data, click or select the words “[Appendix 1](#)”

#### **APPENDIX 2. LIST OF PRIORITIZED VARIANTS THAT HAD A MINOR ALLELE FREQUENCY OF LESS THAN 1% IN PUBLIC FREQUENCY DATABASES AND WERE PRESENT IN A HETEROZYGOUS STATE IN ALL SIX OF THE AFFECTED INDIVIDUALS THAT UNDERWENT WHOLE GENOME SEQUENCING**

To access the data, click or select the words “[Appendix 2](#)”

#### **ACKNOWLEDGMENTS**

Research reported in this publication was supported by the Fogarty International Center of the National Institutes of Health (NIH) under Award Number D43TW008330. The content is solely the responsibility of the authors and does

not necessarily represent the official views of the NIH. The NGS sequencing was funded by Illumina as a prize awarded to NC at the Southern African Society for Human Genetics Congress in 2013. NC was a postdoctoral fellow on the Wits NCD Research Leadership Program (NIH grant: D43TW008330). We acknowledge support to TRC from the South African Medical Research Council. This study would not have been possible without the willing participation of all the family members.

#### **REFERENCES**

1. Ma AS, Grigg JR, Ho G, Prokudin I, Farnsworth E, Holman K, Cheng A, Billson FA, Martin F, Fraser C, Mowat D, Smith J, Christodoulou J, Flaherty M, Bennetts B, Jamieson RV. Sporadic and Familial Congenital Cataracts: Mutational Spectrum and New Diagnoses Using Next-Generation Sequencing. *Hum Mutat* 2016; 37:371-84. [PMID: 26694549].
2. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. *Surv Ophthalmol* 2004; 49:300-15. [PMID: 15110667].
3. Jamieson RV, Perveen R, Kerr B, Carette M, Yardley J, Heon E, Wirth MG, van Heyningen V, Donnai D, Munier F, Black GC. Domain disruption and mutation of the bZIP transcription factor, MAF, associated with cataract, ocular anterior segment dysgenesis and coloboma. *Hum Mol Genet* 2002; 11:33-42. [PMID: 11772997].
4. Nakamura KM, Diehl NN, Mohny BG. Incidence, ocular findings, and systemic associations of ocular coloboma: a population-based study. *Arch Ophthalmol* 2011; 129:69-74. [PMID: 21220631].
5. Onwochei BC, Simon JW, Bateman JB, Couture KC, Mir E. Ocular colobomata. *Surv Ophthalmol* 2000; 45:175-94. [PMID: 11094243].
6. Gregory-Evans CY, Williams MJ, Halford S, Gregory-Evans K. Ocular coloboma: a reassessment in the age of molecular neuroscience. *J Med Genet* 2004; 41:881-91. [PMID: 15591273].
7. Reis LM, Semina EV. Conserved genetic pathways associated with microphthalmia, anophthalmia, and coloboma. *Birth Defects Res C Embryo Today* 2015; 105:96-113. [PMID: 26046913].
8. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16:1215-[PMID: 3344216].
9. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* 2015; 4:7-[PMID: 25722852].
10. Browning BL, Browning SR. A fast, powerful method for detecting identity by descent. *Am J Hum Genet* 2011; 88:173-82. [PMID: 21310274].

11. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, Boutell JM, Bryant J, Carter RJ, Keira Cheetham R, Cox AJ, Ellis DJ, Flatbush MR, Gormley NA, Humphray SJ, Irving LJ, Karbelashvili MS, Kirk SM, Li H, Liu X, Maisinger KS, Murray LJ, Obradovic B, Ost T, Parkinson ML, Pratt MR, Rasolonjatovo IM, Reed MT, Rigatti R, Rodighiero C, Ross MT, Sabot A, Sankar SV, Scally A, Schroth GP, Smith ME, Smith VP, Spiridou A, Torrance PE, Tzonev SS, Vermaas EH, Walter K, Wu X, Zhang L, Alam MD, Anastasi C, Aniebo IC, Bailey DM, Bancarz IR, Banerjee S, Barbour SG, Baybayan PA, Benoit VA, Benson KF, Bevis C, Black PJ, Boodhun A, Brennan JS, Bridgham JA, Brown RC, Brown AA, Buermann DH, Bundu AA, Burrows JC, Carter NP, Castillo N, Chiara ECM, Chang S, Neil Cooley R, Crake NR, Dada OO, Diakoumakos KD, Dominguez-Fernandez B, Earnshaw DJ, Egbujor UC, Elmore DW, Etchin SS, Ewan MR, Fedurco M, Fraser LJ, Fuentes Fajardo KV, Scott Furey W, George D, Gietzen KJ, Goddard CP, Golda GS, Granieri PA, Green DE, Gustafson DL, Hansen NF, Harnish K, Haudenschild CD, Heyer NI, Hims MM, Ho JT, Horgan AM, Hoschler K, Hurwitz S, Ivanov DV, Johnson MQ, James T, Huw Jones TA, Kang GD, Kerelska TH, Kersey AD, Khrebtukova I, Kindwall AP, Kingsbury Z, Kokko-Gonzales PI, Kumar A, Laurent MA, Lawley CT, Lee SE, Lee X, Liao AK, Loch JA, Lok M, Luo S, Mammen RM, Martin JW, McCauley PG, McNitt P, Mehta P, Moon KW, Mullens JW, Newington T, Ning Z, Ling Ng B, Novo SM, O'Neill MJ, Osborne MA, Osnowski A, Ostadan O, Paraschos LL, Pickering L, Pike AC, Pike AC, Chris Pinkard D, Pliskin DP, Podhasky J, Quijano VJ, Raczy C, Rae VH, Rawlings SR, Chiva Rodriguez A, Roe PM, Rogers J, Rogert Bacigalupo MC, Romanov N, Romieu A, Roth RK, Rourke NJ, Ruediger ST, Rusman E, Sanches-Kuiper RM, Schenker MR, Seoane JM, Shaw RJ, Shiver MK, Short SW, Sizto NL, Sluis JP, Smith MA, Ernest Sohna Sohna J, Spence EJ, Stevens K, Sutton N, Szajkowski L, Tregidgo CL, Turcatti G, Vandevondele S, Verhovsky Y, Virk SM, Wakelin S, Walcott GC, Wang J, Worsley GJ, Yan J, Yau L, Zuerlein M, Rogers J, Mullikin JC, Hurler ME, McCooke NJ, West JS, Oaks FL, Lundberg PL, Klenerman D, Durbin R, Smith AJ. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* 2008; 456:53-9. [PMID: 18987734].
12. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation C. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; 536:285-91. [PMID: 27535533].
13. Pabinger S, Dander A, Fischer M, Snajder R, Sperk M, Efreanova M, Krabichler B, Speicher MR, Zschocke J, Trajanoski Z. A survey of tools for variant analysis of next-generation genome sequencing data. *Brief Bioinform* 2014; 15:256-78. [PMID: 23341494].
14. Shiels A, Bennett TM, Hejtmanck JF. Cat-Map: putting cataract on the map. *Mol Vis* 2010; 16:2007-15. [PMID: 21042563].
15. Prokudin I, Simons C, Grigg JR, Storen R, Kumar V, Phua ZY, Smith J, Flaherty M, Davila S, Jamieson RV. Exome sequencing in developmental eye disease leads to identification of causal variants in GJA8, CRYGC, PAX6 and CYP11B1. *Eur J Hum Genet* 2014; 22:907-15. [PMID: 24281366].
16. Stenson PD, Mort M, Ball EV, Evans K, Hayden M, Heywood S, Hussain M, Phillips AD, Cooper DN. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet* 2017; 136:665-77. [PMID: 28349240].
17. Tzoulaki I, White IM, Hanson IM. PAX6 mutations: genotype-phenotype correlations. *BMC Genet* 2005; 6:1-12. [PMID: 15918896].
18. Lim HT, Seo EJ, Kim GH, Ahn H, Lee HJ, Shin KH, Lee JK, Yoo HW. Comparison between aniridia with and without PAX6 mutations: clinical and molecular analysis in 14 Korean patients with aniridia. *Ophthalmology* 2012; 119:1258-64. [PMID: 22361317].
19. Hewitt AW, Kearns LS, Jamieson RV, Williamson KA, van Heyningen V, Mackey DA. PAX6 mutations may be associated with high myopia. *Ophthalmic Genet* 2007; 28:179-82. [PMID: 17896318].
20. Dansault A, David G, Schwartz C, Jaliffa C, Vieira V, de la Houssaye G, Bigot K, Catin F, Tattu L, Chopin C, Halimi P, Roche O, Van Regemorter N, Munier F, Schorderet D, Dufier JL, Marsac C, Ricquier D, Menasche M, Penfornis A, Abitbol M. Three new PAX6 mutations including one causing an unusual ophthalmic phenotype associated with neurodevelopmental abnormalities. *Mol Vis* 2007; 13:511-23. [PMID: 17417613].
21. Park SH, Kim MS, Chae H, Kim Y, Kim M. Molecular analysis of the PAX6 gene for congenital aniridia in the Korean population: identification of four novel mutations. *Mol Vis* 2012; 18:488-94. [PMID: 22393275].
22. van Heyningen V, Williamson KA. PAX6 in sensory development. *Hum Mol Genet* 2002; 11:1611-1618. [PMID: 12015275].
23. Hanson IM, Seawright A, Hardman K, Hodgson S, Zaletayev D, Fekete G, van Heyningen V. PAX6 mutations in aniridia. *Hum Mol Genet* 1993; 2:915-20. [PMID: 8364574].

24. Thomas MG. Genotype-Phenotype Studies in Infantile Nystagmus with Emphasis on the Novel Frmd7 Gene:

University of Leicester; 2012.

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 9 June 2018. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.