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

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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 *BFSP1*- 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

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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 ≤95%, a minor allele frequency ≤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 × 150 cycles) on the HiSeqX platform to an average depth of ≥40X, 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.

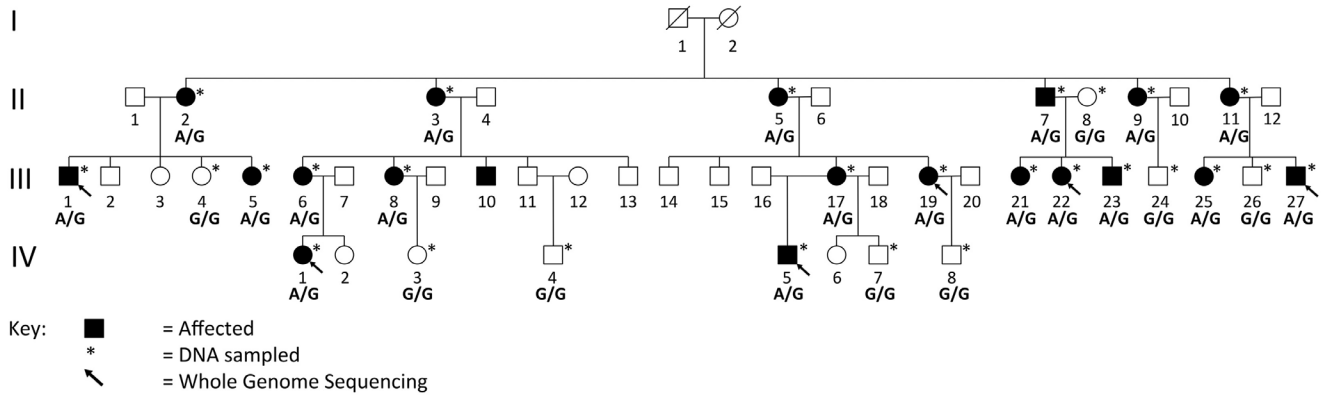

Figure 1. Family pedigree. Family pedigree illustrating the affected individuals, those who had DNA sampled, the six family members chosen for whole genome sequencing, and the genotype of each individual based on Sanger sequencing for the pathogenic R208W mutation in *PAX6*.

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

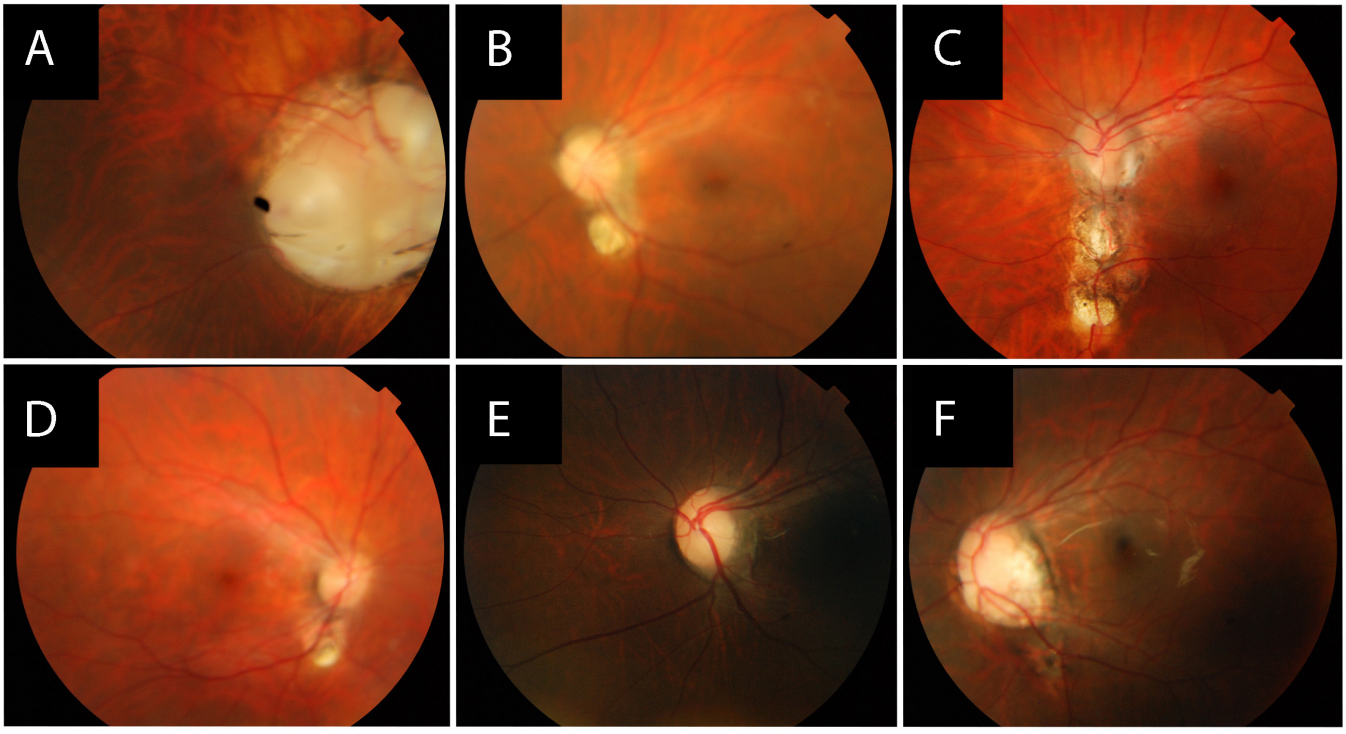

Figure 2. Illustrative images of posterior segment colobomas. Fundus photographs of some of the posterior segment colobomas present in affected family members. The following key uses the pedigree notation from Figure 1 to identify the individuals in whom the fundus photographs were taken. **A** = II:3, **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"

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