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Research article

Genetic analysis of consanguineous families presenting with congenital ocular defects

Ehsan Ullah [a, b], Muhammad Arif Nadeem Saqib [a, c], Sundus Sajid [a], Neelam Shah [a], Muhammad Zubair [d], Muzammil Ahmad Khan [d, e], Iftikhar Ahmed [f], Ghazanfar Ali [g], Atanu Kumar Dutta [h], Sumita Danda [h], Richard Lao [i], Paul Ling-Fung Tang [i], Pui-yan Kwok [i], Muhammad Ansar [a, *, 1], Anne Slavotinek [b, **, 1]

[a] Department of Biochemistry, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[b] Department of Pediatrics, University of California, San Francisco, CA, USA
[c] Pakistan Medical Research Council, Islamabad, Pakistan
[d] Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Pakistan
[e] Genomic Core Facility, Interim Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar
[f] Department of Community Medicine, Gomal Medical College, Dera Ismail Khan, Khyber- Pakhtoonkhuwa, Pakistan
[g] Department of Biotechnology, University of Azad Jammu and Kashmir, Muzaffarabad, Azad Jammu & Kashmir, Pakistan
[h] Medical Genetics Unit, Christian Medical College, Vellore, India
[i] Cardiovascular Research Institute, University of California, San Francisco, CA, USA

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ABSTRACT

Anophthalmia and microphthalmia (A/M) are a group of rare developmental disorders that affect the size of the ocular globe. A/M may present as the sole clinical feature, but are also frequently found in a variety of syndromes. A/M is genetically heterogeneous and can be caused by chromosomal aberrations, copy number variations and single gene mutations. To date, A/M has been caused by mutations in at least 20 genes that show different modes of inheritance. In this study, we enrolled eight consanguineous families with A/M, including seven from Pakistan and one from India. Sanger and exome sequencing of DNA samples from these families identified three novel mutations including two mutations in the Aldehyde Dehydrogenase 1 Family Member A3 (*ALDH1A3*) gene, [c.1310_1311delAT; p.(Tyr437Trp*fs**44) and c.964G > A; p.(Val322Met)] and a single missense mutation in Forkhead Box E3 (*FOXE3*) gene, [c.289A > G p.(Ile97Val)]. Additionally two previously reported mutations were identified in *FOXE3* and in Visual System Homeobox 2 (*VSX2*). This is the first comprehensive study on families with A/M from the Indian subcontinent which provides further evidence for the involvement of known genes with novel and recurrent mutations.

1. Introduction

Anophthalmia (clinical absence of the ocular globe) and microphthalmia (small ocular globe below expected size for chronological age) are rare and represent the most severe forms of developmental eye defects. Anophthalmia/Microphthalmia (A/M) constitutes a spectrum of developmental ocular disorders that can involve coloboma, or abnormal closure of optic fissure (Morrison et al., 2002). Microphthalmia may be observed with additional associated ocular features affecting the anterior segment of the eye, including the sclera, cornea and lens, and/or the posterior segment of the eye (Verma and Fitzpatrick, 2007). A/M has also been reported with extra-ocular features including cardiac abnormalities,

Abbreviations: A/M, Anophthalmia/Microphthalmia; AR, Autosomal Recessive; AD, Autosomal Dominant; PCR, Polymerase Chain Reaction; SIFT, Sorting Intolerant From Tolerant; PolyPhen2.0, Polymorphism Phenotyping Version 2.0; PROVEAN, Protein Variation Effect Analyzer; NHLBI, National Heart, Lung, and Blood Institute; EVS, Exome Variant Server; dbSNP, database of Single Nucleotide Polymorphism; ExAC, Exome Aggregation Consortium; RA, Retinoic Acid; NMD, Nonsense-Mediated Decay; 3D, 3 Dimensional.

* Corresponding author.
** Corresponding author.
E-mail addresses: ansar@qau.edu.pk (M. Ansar), slavotia@peds.ucsf.edu (A. Slavotinek).
[1] Equally contributing authors.

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facial anomalies, renal anomalies, microcephaly and hydrocephalus (Bessant et al., 1999; Kallen et al., 1996; Morle et al., 2000; Morrison et al., 2002). The incidence of microphthalmia and anophthalmia has been estimated to be 1 in 7000 and 1 in 30,000 births, respectively (Morrison et al., 2002).

Though A/M can result from non-genetic causes including viral infections, exposure to drugs and alcohol during early pregnancy and deficiency of vitamin A (Khorshidi et al., 2012), heritable factors remain the major determinant of the phenotype. Causative genetic factors include chromosome aberrations and copy number variations and single gene mutations. Single gene mutations causing A/M may be inherited in autosomal recessive (AR), autosomal dominant (AD) or X-linked patterns (Bardakjian and Schneider, 2011). The genes reported to be associated with A/M include SRY-Box 2 (*SOX2*; MIM#184429), Orthodenticle, Drosophila, Homolog of, 2 (*OTX2*; MIM#600037), Paired Box Gene 6 (*PAX6*; MIM#607108), Stimulated by Retinoic Acid 6 (*STRA6*; MIM#610745), Aldehyde Dehydrogenase 1 Family Member A3 (*ALDH1A3*; MIM#600463), Retinoic Acid Receptor, Beta (*RARB*; MIM#180220), Visual System Homeobox 2 (*VSX2*; MIM#142993), Retinal and Anterior Neural Fold Homeobox Gene (*RAX*; MIM#601881), Forkhead Box E3 (*FOXE3*; MIM#601094), Bone Morphogenetic Protein 4 (*BMP4*; MIM#112262), Bone Morphogenetic Protein 7 *BMP7* (MIM#112267), Growth/Differentiation Factor 3 (*GDF3*; MIM#606522), Growth/Differentiation Factor 6 (*GDF6*; MIM#601147), ATP-binding Cassette, Subfamily B, Member 6 (*ABCB6*; MIM#605452), Atonal, Drosophila, Homolog of, 7 (*ATOH7*; MIM#609875), Chromosome 12 Open Reading Frame 57 (*C12orf57*; MIM#615140), Teneurin Transmembrane Protein 3 (*TENM3*, also known as *ODZ3*; MIM#610083), Ventral Anterior Homeobox 1 (*VAX1*; MIM#604294), Sal-like 2 (*SALL2*; MIM#602219) and Yes-associated protein 1 (*YAP1*; MIM#606608) (Williamson and FitzPatrick, 2014).

Mutations in *SOX2* contribute to 15–20% of individuals with A/M, but all other known genes are more rarely affected (Fares-Taie et al., 2013). Interestingly, in ~50–60% of A/M cases, the underlying genetic factors are still unknown, indicating a requirement for further studies to decipher the genetics of A/M (Slavotinek et al., 2014). For the current study, we recruited 8 families and used Sanger and exome sequencing to identify novel mutations in A/M genes.

2. Materials and methods

2.1. Family enrollment and study design

This study was designed in compliance with the tenets of the Declaration of Helsinki and patient enrollment had approval by the Institutional Review Board of Quaid-i-Azam University Islamabad, Pakistan, (families MA75, MA90, MA101, MA115, MA119, MA143, OPH1), Institutional Ethics Committee, Christian Medical College, Vellore, India and Committee on Human Research, University of California, San Francisco (family MCA463). Seven families (MA75, MA90, MA101, MA115, MA119, MA143, OPH1) were identified from remote areas of Pakistan and enrolled after obtaining informed consent. All families enrolled in present study were consanguineous. The authors visited the families at their places of residences, pedigrees were drawn and blood samples of available affected and normal family members were collected. The diagnosis was confirmed after collecting clinical and family history as well as on-spot examination by a local ophthalmologist. When possible, ophthalmoscope and B-scan ultrasonography (at 7.5 MHz) were used to obtain further clinical information.

2.2. Sanger sequencing of A/M genes

DNA samples from one affected individual from each family first underwent Sanger sequencing of selected A/M genes, *ALDH1A3*, *OTX2*, *VSX2*, *SOX2*, *RAX* and *FOXE3*, prior to consideration for exome sequencing. For this purpose, primers were designed to cover coding exons and splice sites of *ALDH1A3* (NM_000693.2), *OTX2* (NM_021728.2), *VSX2* (NM_182894.2), *SOX2* (NM_003106.2), *RAX* (NM_013435.2) and *FOXE3* (NM_012186.2) genes. The exons were amplified using polymerase chain reaction (PCR) and the products were cleaned up by adding 2 μL Exo-Sap (USB Corp, USA) mixture according to the manufacturer's recommendations. The purified product was sequenced with Big Dye Terminator Cycling Sequencing Kit v3.1 (Applied Biosystem, Foster City, CA) and the data was analyzed on Sequencher 4.7 (Gene Codes, USA). Bioinformatics tools, including MutationTaster (http://www.mutationtaster.org/), PolyPhen2.0 (http://genetics.bwh.harvard.edu/pph2/), SIFT and PROVEAN (http://provean.jcvi.org/), were used to predict the pathogenicity of the identified variants. The frequency of sequence variants was checked with 1000 Genomes Browser (http://www.1000genomes.org/; Sep 2015), NHLBI Exome Sequencing Project (ESP) Exome Variant Server (EVS) (http://evs.gs.washington.edu/EVS/; Sep 2015), Exome Aggregation Consortium Browser (Beta) (ExAC; Cambridge, MA; http://exac.broadinstitute.org; Sep 2015) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/; Sep 2015).

2.3. Exome sequencing and validation

The families negative for mutations in the most commonly affected A/M genes with autosomal dominant and recessive inheritance were subjected to exome sequencing, which was carried out using a trio based strategy for MA75 (IV-6 and IV-7 & V-2) and MA101 (III-1, III-2 & IV-1) (Fig. 1). Exome sequencing was performed as previously described (Choi et al., 2015; Slavotinek et al., 2013; Yahyavi et al., 2013). Briefly, libraries were prepared using biotinylated DNA oligonucleotides (SeqCap EZ Human Exome Library v3.0; Roche Nimblegen, Madison, WI, USA) and sequencing was performed on a HiSeq2000 (Illumina, San Diego, CA, USA) for paired-end 100 cycles. Sequencing reads were aligned to the hg19 reference genome using the Burrows–Wheeler Alignment tool (BWAv0.5.9) using the default parameters that allow two mismatches. Indexing, realignment and duplicate removal were performed using Picard and Samtools and variants were subsequently called using Genome Analysis Toolkit v 1.3–21-gcb284ee. We then utilized wANNOVAR (http://wannovar.usc.edu/) with default parameters to analyze the sequence variants (Chang and Wang, 2012; Wang et al., 2010). Coding sequence variants that were homozygous were selected based on predictions of pathogenicity from SIFT (Ng and Henikoff, 2001), PolyPhen-2 (Adzhubei et al., 2010) and Mutation Taster (Schwarz et al., 2010). The allele frequencies used for public databases including 1000 Genomes Browser, NHLBI ESP EVS, ExAC and dbSNP were kept up to 0.01 for filtration. Finally shortlisted variants were tested for segregation using Sanger sequencing of additional family members.

3. Results

3.1. Clinical findings

All family pedigrees provided evidence of consanguinity and therefore autosomal recessive inheritance was first considered for variant prioritization (Fig. 1). Patients from four families (MA75, MA90, OPH1 and MCA463) were diagnosed with bilateral anophthalmia/microphthalmia (Fig. 2A–D), whilst patients from three