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Heterozygous mutation in *OTX2* associated with early-onset retinal dystrophy with atypical maculopathy

Maram EA Abdalla-Elsayed,[1] Patrik Schatz,[2,3] Christine Neuhaus,[4] Arif O. Khan[5]

[1]Jeddah Eye Hospital, Jeddah, Kingdom of Saudi Arabia; [2]King Khaled Eye Specialist Hospital, Riyadh, Kingdom of Saudi Arabia; [3]Department of Ophthalmology, Clinical Sciences, Skane County University Hospital, University of Lund, Lund, Sweden; [4]Bioscientia Center for Human Genetics, Ingelheim, Germany; [5]Eye Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates

Purpose: Heterozygous mutations in *OTX2* have been associated with a range of ocular and pituitary abnormalities. We report a novel heterozygous deletion in *OTX2* underlying early-onset retinal dystrophy with atypical maculopathy.
Methods: Clinical examination included electroretinography and multimodal retinal imaging. Molecular genetic testing was composed of next-generation sequencing of a panel of retinal dystrophy genes.
Results: A now 17-year-old boy presented 12 years earlier with a history of progressively poor vision since birth, nyctalopia, and early-onset retinal dystrophy with atypical maculopathy. He also had bilateral microphthalmos and a slim prepubertal appearance; growth hormone levels were within normal ranges. Next-generation sequencing of a retinal dystrophy gene panel revealed a heterozygous deletion c.485delC (p.Pro162G.Infs*24) in exon 5 of *OTX2*.
Conclusions: This second report of maculopathy associated with a heterozygous mutation in *OTX2* confirms that mutations in *OTX2* should be considered in the differential diagnosis of atypical hereditary maculopathy, with or without rod-cone dystrophy.

Macular pattern dystrophies are a genetically and clinically heterogeneous group of autosomal dominant eye diseases characterized by abnormal bilateral accumulation of lipofuscin-containing pigment in the RPE in the macular area. *Macular pattern dystrophy-1* (*MDPT1*; OMIM 169150) is caused by a heterozygous mutation in the *photoreceptor peripherin* gene (*PRPH2*; OMIM 179605) on chromosome 6p21.1 [1,2]. *Macular pattern dystrophy-2* (*MDPT2*; OMIM 608970) is caused by heterozygous mutations in the *alpha-E-catenin-cadherin associated protein* (*CTNNA1*; OMIM 116805) on chromosome 5q31 [3]. Adult onset foveomacular dystrophy (OMIM 608161), which is caused by heterozygous mutations in *PRPH2* or in *BEST1* (OMIM 607854) is sometimes also included among the macular pattern dystrophies [4-6].

In addition, in a historical context, several other morphological patterns have been described, which may have specific genetic associations, such as reticular (fishnet-like) dystrophy, macroreticular (spider-shaped) dystrophy, and butterfly-shaped pigment dystrophy. The last has been described in association with *PRPH2,* as well as mutations in *CTNNA1* [3,7].

Correspondence to: Patrik Schatz, Vitreoretinal Division, King Khaled Eye Specialist Hospital, Al- Oruba Street, PO Box 7191 Riyadh 11462, Kingdom of Saudi Arabia, Phone: +966 11 482 1234 ext. 3773; FAX+966 11 4821234 ext. 3773; email: pschatz@kkesh.med.sa

A recent study reported two families whose members presented with either "grouped pigmentation macular dystrophy" or macular pattern dystrophy, associated with heterozygous mutations in the homeobox gene *Orthodenticle, Drosophila, homolog of, 2* (*OTX2*; OMIM 600037) [8]. *OTX2* encodes a transcription factor that plays a critical role in forebrain and eye development. Heterozygous mutations in *OTX2* were originally linked to a heterogeneous phenotype associated with severe ocular malformations or abnormalities with or without brain and pituitary abnormalities. The latter is considered to result from lack of *OTX2*-mediated regulation of *HESX1* (homeobox gene expressed in ES cells 1, OMIM 601802), which is one of the transcription factors involved in pituitary development. In addition, there is a role for *OTX2* in the mature retina as evidenced by the fact that loss of Otx2 protein causes slow degeneration of photoreceptor cells [9]. It is thought that bipolar cells import Otx2 protein which is relocated to the mitochondria to support ATP synthesis, highlighting a therapeutic potential of Otx2 protein transduction in retinal dystrophy [10]. In addition, recent studies have even established the *OTX2* gene as an oncogene for medulloblastoma [11].

Several malformations of the eye have been associated with heterozygous mutations in *OTX2*, including bilateral anophthalmia, optic nerve hypoplasia, and ocular coloboma. The retinal phenotypes are summarized im Appendix 1 and have been described as early-onset retinal dystrophy

(one patient) [12], Leber congenital amaurosis (one patient) [13], pigmentary retinopathy (one patient) [13], and pattern dystrophy (seven patients) [8]. In the current study, we describe a case of early onset retinal dystrophy with atypical maculopathy, microphthalmos, and small stature without growth hormone abnormality.

METHODS

Informed consent was obtained for this case report. Institutional Review Board (IRB)/Ethics Committee approval was obtained at King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia. The research adhered to the tenets of the Declaration of Helsinki. The study adhered to the ARVO statement on human subjects.

Clinical examination: All clinical work was carried out at King Khaled Eye Specialist Hospital. The retinal structure was analyzed qualitatively with transfoveal horizontal spectral domain optical coherence tomography (OCT) scans (Heidelberg Engineering, Inc., Heidelberg, Germany) and wide-field and macular fundus photography (Optos PLC, Dunfermline, UK, and Topcon Medical Systems, Inc. Oakland, NJ). Retinal function was evaluated with full-field electroretinography (ffERG, Nicolet Biomedical Instruments, Madison, WI), in dark- and light-adapted states according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards, with a few modifications as described previously [14].

Molecular genetic analysis: Molecular genetic testing included next-generation sequencing (NGS, performed by the Center for Human Genetics, Bioscientia, Ingelheim, Germany) [15]. Sequencing was performed for 62 known genes involved in retinal dystrophies. These genes (Appendix 2) were enriched and sequenced in parallel as follows. Genomic DNA was fragmented, and the coding exons of the analyzed genes, as well as the corresponding exon–intron boundaries, were enriched using the Roche NimbleGen sequence capture approach and then amplified and sequenced simultaneously with NGS using an Illumina HiSeq 1500 system (San Diego, CA). Genomic DNA was isolated from EDTA blood as described previously. In brief, 0.5–1 mg of genomic DNA per sample was sheared using the Covaris S2 AFA system (Covaris Inc., Woburn, MA) and ligated to barcoded adaptors for multiplexing. Pre-capture amplified samples were pooled and hybridized to the customized in-solution capture library for 72 h, subsequently eluted and post-capture amplified by ligation-mediated (LM-) PCR. This amplified enriched DNA was used as input for emulsion PCR (emPCR) and subsequent sequencing on the Illumina system [15]. For more than 99% of the regions of interest, 20-fold coverage was obtained. NGS data analysis was performed using bioinformatic analysis tools, as well as JSI Medical Systems software (Ettenheim, Germany; version 4.1.2). Identified variants and indels were filtered against external and internal databases and depending on their allele frequency, focusing on rare variants with a minor allele frequency (MAF) of 1% or less. Nonsense, frameshift, and canonical splice-site variants were primarily considered likely pathogenic. Variants that have been annotated as common polymorphisms in databases or in the literature were not considered further.

Putatively pathogenic differences between the wild-type sequence (human reference genome according to UCSC Genome Browser: hg19, GRCh37) and the patient's sequence were validated using PCR amplification followed by conventional Sanger sequencing. The resulting sequence data for the *cyclic nucleotide-gated channel, beta-1* (*CNGB1*, OMIM 600724; locus: chromosome 16q21) gene were compared to the reference sequence NM_001297.4 for the *OTX2* gene (OMIM: 600037; locus: chromosome 14q22.3) and to the reference sequence NM_021728.3.

RESULTS

A now 17-year-old boy was referred 12 years previously at age 5 years with a history of retinal dystrophy from birth which was progressive and accompanied by nyctalopia. There was no family history of any ophthalmic disorders, and the patient was the youngest of 12 siblings. His parents were first cousins. The patient's best-corrected visual acuity was 20/200 oculi uterque (OU), and he was bilaterally hyperopic at +6.50 diopters (D). Macular pigmentary changes were noted in both fundi, but fundus imaging was not performed at this stage. There was a history of congenital hip dislocation, and he had undergone surgery with titanium screws in the left side of the hip. He started to walk at about 3 years of age, but no cognitive impairment was reported. On examination at age 17 years, the patient had microphthalmic eyes with axial length 17.98 mm and 17.88 mm in the right and left eyes, respectively. Best-corrected visual acuity was 20/300 OU. Fundus photography revealed dense intraretinal macular pigmentation as can be seen in pattern dystrophy and mid-peripheral pigmentation with mild arteriolar attenuation (Figure 1A,B). OCT transfoveal scans showed unusual intraretinal pigment scars, which may represent RPE hyperplasia and migration; however, there was no sign of lipofuscin deposition such as that seen in macular pattern dystrophy (Figure 1C, lower panel). FfERG performed at age 5 years (under sedation) and at 14 years showed non-recordable rod responses and severely reduced and delayed cone responses, atypical for

pattern dystrophy, compatible with rod-cone dystrophy with atypical maculopathy (Figure 2). At the most recent follow-up at age 17 years, his height was 165 cm (8th percentile), weight was 60 kg (32nd percentile), and head circumference was 53.5 cm. The human growth hormone (0.8 µg/l), insulin-like growth factor 1 (IGF-1; 254 ng/ml), and insulin-like growth factor binding protein 3 (IGFBP-3; 5,251 ng/ml) levels were all normal. Brain magnetic resonance imaging (MRI) was not performed, because of the potential risks associated with applying a strong magnetic field in the presence of titanium screws in the patient's hip.

NGS analysis revealed the heterozygous deletion c.485delC (p.Pro162G.Infs*24) in exon 5 of *OTX2*, leading to a frameshift and predicted premature protein truncation. This variant was not annotated in the Single Nucleotide Polymorphism Database (dbSNP) or the literature; there was no allele frequency. The variant was not listed in the Exac Browser, access date November 17, 2016. A literature review of *OTX2*-related pathology (Appendix 1), such as rod-cone dystrophy with maculopathy and microphthalmos, and the fact that the *OTX2* mutation is a frameshift mutation support pathogenicity.

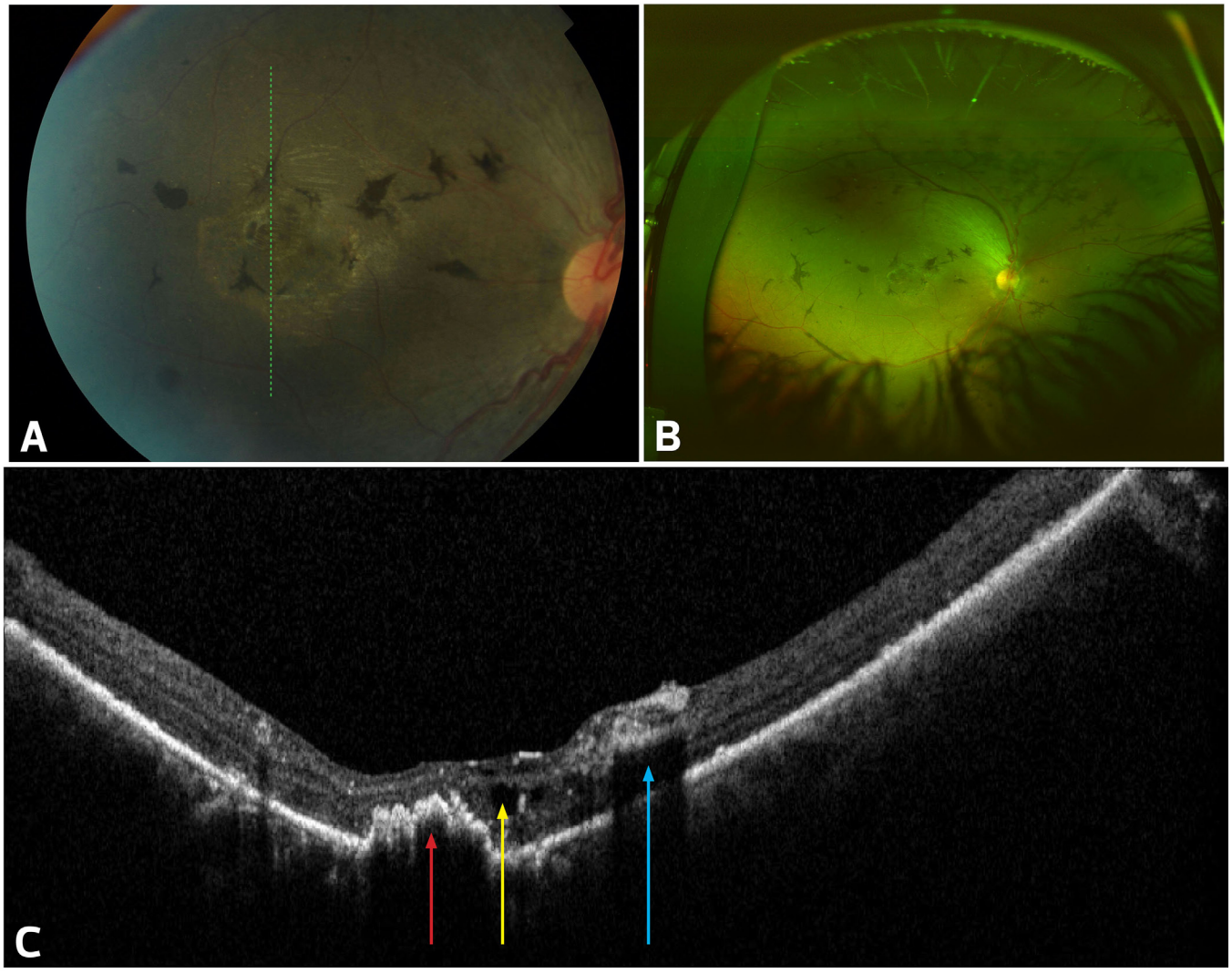

Figure 1. Fundus and optical coherence tomography findings in a 17-year-old patient with heterozygous deletion in *OTX2*. Color fundus (**A**: Central fundus. **B**: Wide field imaging including the peripheral retina) images of the right eye show atypical maculopathy (**A**), and mid-peripheral pigmentation with mild arteriolar attenuation (**B**). **C**: Transfoveal optical coherence tomography scan shows intraretinal hyperreflective scar (blue arrow), intraretinal cyst-like spaces (yellow arrow), RPE changes (red arrow), and foveal atrophy. The hyperreflective lesions may represent RPE hyperplasia and migration; however, there is no sign of lipofuscin deposition such as that seen in macular pattern dystrophy.

The primers used for the sequencing of exon 5 of *OTX2* and the chromatogram of the region that includes the mutation in *OTX2* are presented in Appendix 3. Additional variants that were detected with NGS are given in Table 1.

DISCUSSION

We provide the second report of atypical maculopathy associated with a heterozygous mutation in *OTX2*. The phenotype included strikingly dense macular pattern-like intraretinal pigment abnormalities, midperipheral retinal pigment clumping, microphthalmos, and rod-cone dystrophy. Brain MRI was not performed; however, there was no accompanying growth hormone deficiency, or abnormal IGF-1, or insulin-like growth factor binding protein 3 (IGFBP-3) levels.

This case supports the role of *OTX2* in retinal development and highlights the retinal dystrophy phenotype that should raise suspicion of a mutation in the gene. Vincent et al. described seven cases, with ages ranging from 8 to 46 years, from two different families with the same heterozygous missense mutation c.235G>A in exon 4 of *OTX2*. They presented with grouped macular pigments or macular pattern dystrophy [8]. These cases described by Vincent et al. were less severe than the present case and differed from the present case in several aspects [8]. First, all of the cases described by Vincent et al. were either mildly or highly myopic and had normal to long eyes, whereas the present case was hyperopic and had microphthalmos. All the cases had recordable rod and cone responses, and most had completely normal ffERGs, whereas the present case had a nearly extinguished ffERG compatible with rod-cone dystrophy. Finally, all the cases presented with discrete areas of RPE–photoreceptor outer segment separation, whereas this patient had pigmented intraretinal scars and advanced atrophy in the maculas and no

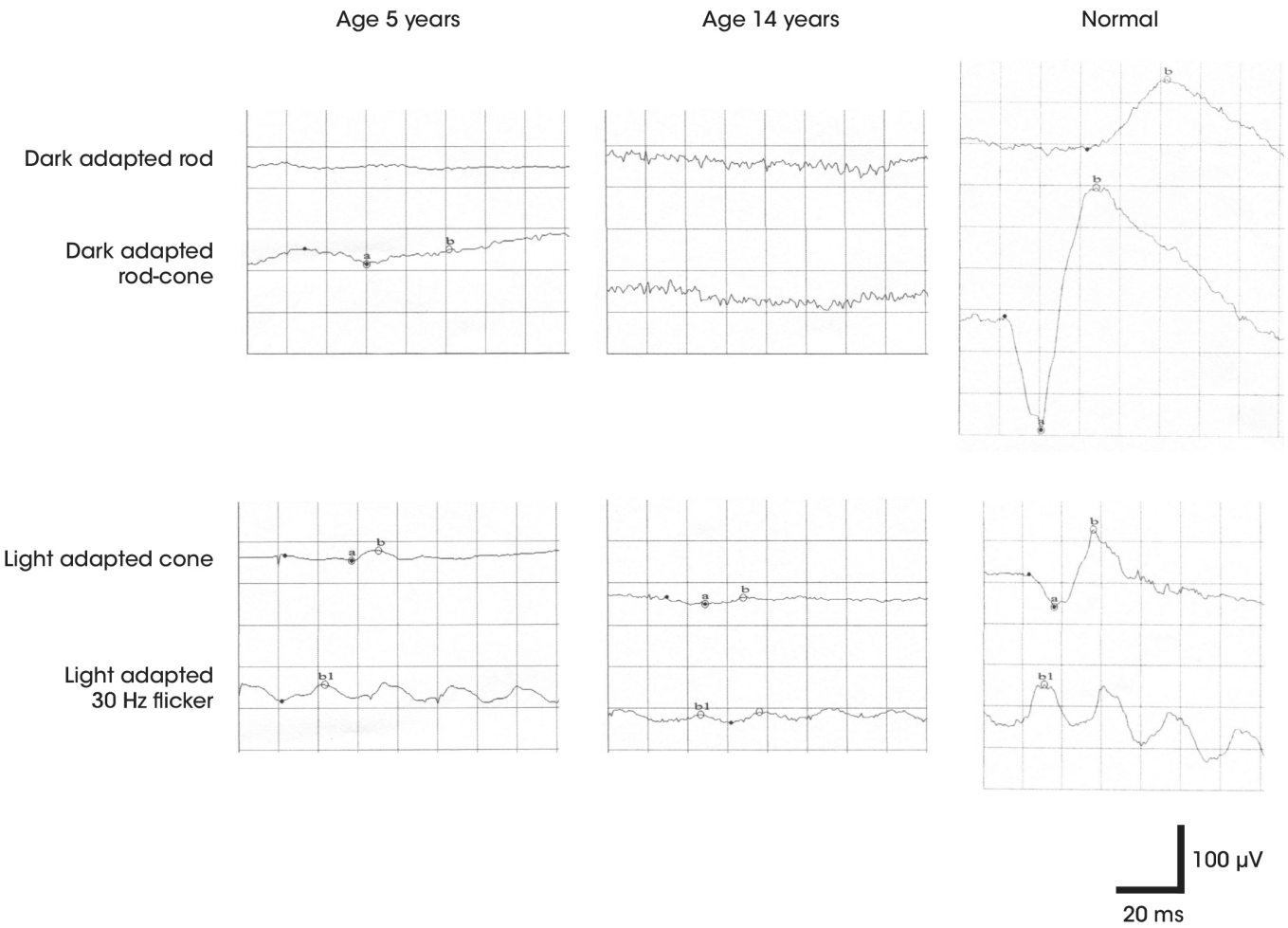

Figure 2. Full-field electroretinogram in a now 17-year-old patient with a heterozygous deletion in *OTX2*. The full-field electroretinogram of the right eye at age 5 years and 14 years shows nonrecordable rod responses and severely reduced cone responses, compared to normal). ms = milliseconds; µV = microvolts.

Table 1. Additional genetic variants identified by Next generation sequencing of retinal dystrophy genes in a patient with heterozygous *OTX2* mutation associated with early onset retinal dystrophy with atypical maculopathy.

Gene	Mutation	Allele frequency (ExAC)	Conclusion about likely pathogenicity
Cyclic nucleotide-gated channel, beta-1 gene (*CNGB1* MIM#600724)	Exon 29, heterozygous missense variant c.2957 A>T (p.Asn986Ile)	0.001	Bioinformatic analysis tools predict pathogenicity, however the gene is not known to cause any dominant disease and a second pathogenic mutation was not found in this gene
Cyclic nucleotide-gated channel, beta-1 gene (*CNGB1* MIM#600724)	Exon 17, heterozygous synonymous variant c.1479G>A	0.01	Unlikely pathogenic
Cyclic nucleotide-gated channel, beta-1 gene (*CNGB1* MIM#600724)	Intron 7, heterozygous c.458+7C>T (rs368819628)	0.0005	Splice prediction programs do not predict impaired splicing
Microsomal triglyceride transfer protein gene (*MTTP* MIM#157147)	Heterozygous intronic variant c.394–7 C>T, 7 base pairs upstream to the exon that begins at position 394	0.0002	Unlikely pathogenic

apparent separation between the RPE and the photoreceptor outer segments (Figure 1C).

As noted by Ragge et al. [13], genetic counseling for families with mutations in *OTX2* may be challenging, due to factors such as the possibility of gonosomal mosaicism, de novo mutations, and uncertainty regarding the factors that determine the penetrance. A limitation of this study is that the NGS panel did not include the *membrane-type frizzled-related protein* (*MFRP*, MIM #606227) gene, in which biallelic mutations also have been associated with short axial length and a rod-cone dystrophy [16]. Further limitations are that no relatives were available for examination; thus, segregation analysis was not possible. Whole exome sequencing was not performed; thus, we cannot rule out the possibility of the presence of a homozygous mutation in a different untested gene. However, a literature review of *OTX2*-related pathology (Appendix 1), such as rod-cone dystrophy with maculopathy and microphthalmos, and the fact that the mutation in *OTX2* in this study was a frameshift mutation support pathogenicity. However, although the frameshift variant in *OTX2* is likely pathogenic, functional studies are needed to definitively prove this. To summarize, the presence of microphthalmos, early-onset retinal dystrophy, and atypical maculopathy support *OTX2* as a putative causative gene, although the presentation differs in many aspects from the classical pattern dystrophy associated with mutations in *OTX2* as described by Vincent et al. [8].

APPENDIX 1. RETINAL PHENOTYPES AND ASSOCIATED FINDINGS DESCRIBED WITH DIFFERENT MUTATIONS IN *OTX2*.

To access the data, click or select the words "Appendix 1" LCA=Leber congenital amaurosis. WNL=within normal limit. RE=Right eye. LE=Left eye.

APPENDIX 2. MOLECULAR GENETIC TESTING INCLUDED NEXT-GENERATION SEQUENCING (NGS, PERFORMED BY CENTER FOR HUMAN GENETICS BIOSCIENTIA, INGELHEIM, GERMANY), PERFORMED FOR THE FOLLOWING 62 GENES KNOWN TO BE INVOLVED IN RETINAL DYSTROPHIES.

To access the data, click or select the words "Appendix 2"

APPENDIX 3. SANGER SEQUENCING OF THE OTX2 GENE DETECTED THE DELETION C.485DELC (P.PRO162G.INFS*24) IN EXON 5 OF OTX2 IN A PATIENT WITH EARLY ONSET RETINAL DYSTROPHY WITH ATYPICAL MACULOPATHY.

To access the data, click or select the words "Appendix 3" Primers used for sequencing of Exon 5 of OTX2 are: F: agctgatctgcccatgtagg R: CTAAGGCCCTTCGTTTTTCC.

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REFERENCES

1. Kim RY, Dollfus H, Keen TJ, Fitzke FW, Arden GB, Bhattacharya SS, Bird AC. Autosomal dominant pattern dystrophy of the retina associated with a 4-base pair insertion at codon 140 in the peripherin/RDS gene. Arch Ophthal 1995; 113:451-5. [PMID: 7710395].

2. Vaclavik V, Tran HV, Gaillard MC, Schorderet DF, Munier FL. Pattern dystrophy with high intrafamilial variability associated with Y141C mutation in the peripherin/RDS gene and successful treatment of subfoveal CNV related to multifocal pattern type with anti-VEGF (ranibizumab) intravitreal injections. Retina 2012; 32:1942-9. [PMID: 22466463].

3. Saksens NTM, Krebs MP, Schoenmaker-Koller FE, Hicks W, Yu M, Shi L, Rowe L, Collin GB, Charette JR, Letteboer SJ, Neveling K, van Moorsel TW, Abu-Ltaif S, De Baere E, Walraedt S, Banfi S, Simonelli F, Cremers FP, Boon CJ, Roepman R, Leroy BP, Peachey NS, Hoyng CB, Nishina PM, den Hollander AI. Mutations in CTNNA1 cause butterfly-shaped pigment dystrophy and perturbed retinal pigment epithelium integrity. Nat Genet 2016; 48:144-51. [PMID: 26691986].

4. Wells J, Wroblewski J, Keen J, Inglehearn C, Jubb C, Eckstein A, Jay M, Arden G, Bhattacharya S, Fitzke F, Bird A. Mutations in the human retinal degeneration slow (RDS) gene can cause either retinitis pigmentosa or macular dystrophy. Nat Genet 1993; 3:213-8. [PMID: 8485576].

5. Yang Z, Li Y, Jiang L, Karan G, Moshfeghi DM, O'Connor ST, Li X, Yu Z, Lewis H, Zack DJ, Jacobson SG, Zhang K. A novel RDS/peripherin gene mutation associated with diverse macular phenotypes. Ophthalmic Genet 2004; 25:133-45. [PMID: 15370544].

6. Kramer F, White K, Pauleikhoff D, Gehrig A, Passmore L, Rivera A, Rudolph G, Kellner U, Andrassi M, Lorenz B,

Rohrschneider K, Blankenagel A, Jurklies B, Schilling H, Schutt F, Holz FG, Weber BHF. Mutations in the VMD2 gene are associated with juvenile-onset vitelliform macular dystrophy (Best disease) and adult vitelliform macular dystrophy but not age-related macular degeneration. Eur J Hum Genet 2000; 8:286-92. [PMID: 10854112].

7. Fossarello M, Bertini C, Galantuomo MS, Cao A, Serra A, Pirastu M. Deletion in the peripherin/RDS gene in two unrelated Sardinian families with autosomal dominant butterfly-shaped macular dystrophy. Arch Ophthalmol 1996; 114:448–56. Eur J Hum Genet 2000; 8:286-92. [PMID: 10854112].

8. Vincent A, Forster N, Maynes JT, Paton TA, Billingsley G, Roslin NM, Ali A, Sutherland J, Wright T, Westall CA, Paterson AD, Marshall CR. FORGE Canada Consortium. Héon E. OTX2 mutations cause autosomal dominant pattern dystrophy of the retinal pigment epithelium. J Med Genet 2014; 51:797-805. [PMID: 25293953].

9. Béby F, Housset M, Fossat N, Le Greneur C, Flamant F, Godement P, Lamonerie T. Otx2 Gene Deletion in Adult Mouse Retina Induces Rapid RPE Dystrophy and Slow Photoreceptor Degeneration. PLoS One 2010; 5:e11673-[PMID: 20657788].

10. Kim H, Prochiantz A, Kim J. Donating Otx2 to support neighbouring neuron survival. BMB Rep 2016; 49:69-70. [PMID: 26779998].

11. Bai R-Y, Staedtke V, Lidov HG, Eberhart CG, Riggins GJ. OTX2 Represses Myogenic and Neuronal Differentiation in Medulloblastoma Cells. Cancer Res 2012; 72:5988-6001. [PMID: 22986744].

12. Henderson RH, Williamson KA, Kennedy JS, Webster AR, Holder GE, Robson AG, FitzPatrick DR, van Heyningen V, Moore AT. A rare de novo nonsense mutation in OTX2 causes early-onset retinal dystrophy and pituitary dysfunction. Mol Vis 2009; 15:2442-7. [PMID: 19956411].

13. Ragge NK, Brown AG, Poloschek CM, Lorenz B, Henderson RA, Clarke MP, Russell-Eggitt I, Fielder A, Gerrelli D, Martinez-Barbera JP, Ruddle P, Hurst J, Collin JR, Salt A, Cooper ST, Thompson PJ, Sisodiya SM, Williamson KA, Fitzpatrick DR, van Heyningen V, Hanson IM. Heterozygous mutations of OTX2 cause severe ocular malformations. Am J Hum Genet 2005; 76:1008–22. Note: Erratum: Am J Hum Genet 2005; 77:334-.

14. Al Oreany AA, Al Hadlaq A, Schatz P. Congenital stationary night blindness with hypoplastic discs, negative electroretinogram and thinning of the inner nuclear layer. Graefes Arch Clin Exp Ophthalmol 2016; 254:1951-6. [PMID: 27084085].

15. Eisenberger T, Neuhaus C, Khan AO, Decker C, Preising MN, Friedburg C, Bieg A, Gliem M, Charbel Issa P, Holz FG, Baig SM, Hellenbroich Y, Galvez A, Platzer K, Wollnik B, Laddach N, Ghaffari SR, Rafati M, Botzenhart E, Tinschert S, Börger D, Bohring A, Schreml J, Körtge-Jung S, Schell-Apacik C, Bakur K, Al-Aama JY, Neuhann T, Herkenrath P, Nürnberg G, Nürnberg P, Davis JS, Gal A, Bergmann C, Lorenz B, Bolz HJ. Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. PLoS One 2013; 8:e78496-[PMID: 24265693].

16. Mukhopadhyay R, Sergouniotis PI, Mackay DS, Day AC, Wright G, Devery S, Leroy BP, Robson AG, Holder GE, Li Z, Webster AR. A detailed phenotypic assessment of individuals affected by MFRP-related oculopathy. Mol Vis 2010; 16:540-8. [PMID: 20361016].

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