Molecular Vision 2020; 26:757-765 <http://www.molvis.org/molvis/v26/757>
Received 16 May 2020 | Accepted 23 November 2020 | Published 25 November 2020

A mutation in *DOP1B* identified as a probable cause for autosomal recessive Peters anomaly in a consanguineous family

Ensieh Darbari,[1] Davood Zare-Abdollahi,[2] Afagh Alavi,[2] Mozhgan Rezaei Kanavi,[3] Sepehr Feizi,[4] Seyed Bagher Hosseini,[5] Alireza Baradaran-Rafii,[3] Hamid Ahmadieh,[4] Shohreh Issazadeh-Navikas,[6] Elahe Elahi[1]

[1]School of Biology, College of Science, University of Tehran, Tehran, Iran; [2]Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; [3]Ocular Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [4]Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [5]Ocular Pathology Unit, Central Eye Bank of Iran, Tehran, Iran; [6]Neuroinflammation Unit, Biotech Research and Innovation Centre (BRIC), Faculty of Health and Medical Sciences, Copenhagen Biocentre, University of Copenhagen, 2200 Copenhagen, Denmark

Purpose: Peters anomaly (PA) is a heterogeneous developmental disorder characterized by central corneal opacity and iridocorneal or corneolenticular adhesions. Although many causative genes have been identified, most screened patients do not have mutations in the known genes. We aimed to identify the genetic cause of Peters anomaly in a pedigree with three affected individuals.

Methods: Slit-lamp biomicroscopy and ultrasound biomicroscopy were performed for definitive diagnosis. Exome sequencing was conducted on the DNA of all three patients. After identification of a candidate causative gene, expression of the gene was assessed with real-time PCR in various ocular tissues of three human embryos and three adults.

Results: The patients were affected with isolated PA. The parents of the patients were related to one another. Inheritance of PA was autosomal recessive. After appropriate filtering of the exome data, a homozygous variation in *DOP1B* remained as the only candidate genetic cause of PA in the pedigree. The variant segregated with disease status in the pedigree and was absent among 800 control Iranians. The variant has been reported in various databases at frequencies of 0.006 or less only in the heterozygous state in some cohorts of African origin. The p.Val1660 amino acid affected by the mutation is completely conserved in mammals and birds during evolution. Expression of *DOP1B* was shown in all adult and embryonic lens, iris, cornea, sclera, and retina tissues that were tested.

Conclusions: *DOP1B* that encodes DOP1 leucine zipper like protein B was identified as the putative PA-causing gene in pedigree PA-101. As *DOP1B* is positioned within the Down syndrome chromosomal region on chromosome 21, until now this gene has mostly been studied with respect to brain functions. However, members of the *Dopey* gene family have been shown to have roles in development in other organisms. Evidence of the expression of *DOP1B* in various PA-relevant eye tissues, which, to the best of our knowledge, is shown here for the first time, is to be noted. However, this finding does not necessarily implicate a specific role for *DOP1B* in eye development as the gene is expressed in many tissues. Ultimately, definitive assessment of the contribution of *DOP1B* to PA pathology awaits identification of mutations in the gene in unrelated patients with PA and functional studies.

Anterior segment dysgenesis (ASD) includes various developmental disorders that affect the cornea, iris, and lens [1]. Peters anomaly (PA) is a form of ASD which itself is clinically heterogeneous. PA is characterized by central corneal opacity, defects in the posterior stroma, Descemet's membrane, and endothelium, and iridocorneal or keratolenticular adhesions [2]. The majority of PA cases are bilateral. The disease is usually associated with significant visual loss due to impairment of the central visual axis. Differential diagnosis with respect to related disorders such as sclerocornea is facilitated with ultrasound biomicroscopy [3]. In comparison to optical technologies, ultrasound can travel through opaque media and produce higher-quality images of ocular structures. The developmental mishaps that are thought to culminate in the PA phenotype may involve lens vesicle separation from the surface ectoderm, subsequent invasions of neural crest cells that normally give rise to the corneal endothelium, the corneal stroma, and the iris stroma, or anterior displacement of a normally developed lens [2,4]. Migration of mesenchyme cells initiate at about the sixth week of human gestation [2,5]. Although eye development is well advanced by the time of birth, completion is postnatal [6]. Peters anomaly presentation may be isolated, in conjunction with other ocular anomalies, or part of a broader syndrome; isolated cases are relatively rare (Table 1) [2,7]. Glaucoma is the most frequent accompanying ocular presentation, possibly present in more than 50% of cases [8]. The best-studied syndromic form of PA is Peters plus syndrome (OMIM: 261540) which, in addition

Correspondence to: Elahe Elahi, University College of Science, University of Tehran; Phone: 0098-9122181251; FAX: 0098-2166405141; email: elaheelahi@ut.ac.ir, elahe.elahi@gmail.com

Table 1. Genes associated with Peters anomaly*

Gene	Chromosome position	Selected biologic functions	Associated anomalies	Inheritance	Peters anomaly-related references
B3GLCT	13q12	Beta-1,3-glucosyltransferase	Peters Plus syndrome	AR	[9,10,20-22]
CDH2	18q12	Cell-cell adhesion, member of the cadherin superfamily	Eye, heart, brain, and skeletal anomalies, Peters anomaly	AD	[11]
COL4A1	13q34	Collagen component of basement membrane	Cerebrovascular disease presentations with ocular, kidney, & muscle anomalies	AD	[23]
CYP1B1	2p22	Cytochrome P450 monooxygenase	Primary congenital glaucoma	AR	[42,43]
DOP1B	21q22	Developmental roles in various organisms, brain development, member of Dopey gene family	Association with Down syndrome	AR	Present study
FLNA	Xq28	Actin-binding protein	Otopalatodigital spectrum	XLR	[12]
FOXC1	6p25	Transcription factor	Anterior segment dysgenesis, Axenfeld–Rieger syndrome	AD	[24-27]
FOXE3	1p33	Transcription factor	Microphthalmia, cataract, anterior segment dysgenesis	AD	[44,45]
HCCS	Xp22	Holocytochrome C-type synthetase cytidylyltransferase-like protein	Microphthalmia, MLS syndrome, Peters anomaly, Walker-Warburg syndrome	XLD	[12]
NCAPG2	7q36	Chromosome condensation, member of condensin II complex	Khan-Khan-Katsanis syndrome, severe neurodevelopmental defects including ocular abnormalities	AR	[13]
NDP	Xp11	Member of canonical Wnt signaling pathway	Norrie disease, familial exudative vitreoretinopathy	XLR	[12]
PAX6	11p13	Transcription factor	Anterior segment dysgenesis, cataract	AD	[46]
PITX2	4q25	Transcription factor	Anterior segment dysgenesis Axenfeld–Rieger syndrome	AD	[28,29]
POMT2	14q24.3	O-mannosyltransferase2	Muscular dystrophy-dystroglycanopathy, Walker-Warburg syndrome	AR	[14]
PITX3	10q24	Transcription factor	Anterior segment dysgenesis, cataract	AD	[30]
PROC	2q14	Inactivator of coagulation factors Va and VIIIa	Hereditary thrombophilia	AR	[15]
PTCH1	9q22	Receptor for secreted hedgehog ligands	Basal cell Nevus syndrome, holoprosencephaly 7	AD	[16]
RERE	1p36.23	Transcriptional regulation, regulator of retinoic acid signaling	Neurodevelopment disorder with or without anomalies of brain, eye or heart	AD	[17]
SLC4A11	20p13	SLC4 bicarbonate transporter	Corneal endothelial dystrophy 2	AR	[12]
TFAP2A	6p24	Transcription factor	Branchiooculofacial syndrome	AD	[12]
WDR37	10p15	Member of the WD repeat protein family	Neurooculocardiogenitourinary syndrome	AD/AR	[18]
WFS1	4p16	Cation-selective ion channel	Wolfram-like Syndrome	AD	[19]

*From NCBI and the literature; AD, autosomal dominant, AR, autosomal recessive, AD/AR, both autosomal dominant and autosomal recessive; XLD, X-linked dominant; XLR, X-linked recessive

to PA features, is characterized by growth retardation, short stature, brachydactyly, and distinctive facial features [9,10].

In terms of inheritance, Peters anomaly incidence is most often apparently sporadic, although recessive inheritance and dominant inheritance have also been observed (Table 1) [2,8]. Twenty-one PA causative genes that have been reported to date are listed in Table 1. Many of these, including *CDH2* (OMIM: 114020; Gene ID: 1000) [11], *FLNA* (OMIM: 300017; Gene ID: 2316) [12], *HCCS* (OMIM: 300056; Gene ID: 3052) [12], *NCAPG2* (OMIM: 608532; Gene ID: 54892) [13], *NDP* (OMIM: 300658; Gene ID: 4693) [12], *POMT2* (OMIM: 607439; Gene ID: 29954) [14], *PROC* (OMIM: 612283; Gene ID: 5624) [15], *PTCH1* (OMIM: 601309; Gene ID: 5727) [16], *RERE* (OMIM: 605226; Gene ID: 473) [17], *SLC4A11* (OMIM: 610206; Gene ID: 83959) [12], *TFAP2A* (OMIM: 107580; Gene ID: 7020) [12], *WDR37* (OMIM: 618586; Gene ID: 22884 [18], and *WFS1* (OMIM: 606201; Gene ID: 7466) [19], have been reported only in single patients or families. The majority of the genes, including *B3GLCT (*OMIM: 610308; Gene ID: 145173) [9,10,20-22], *CDH2* [11], *COL4A1 (*OMIM: 120130; Gene ID: 1282) [23], *FLNA* [12], *FOXC1* (OMIM: 601090; Gene ID: 2296) [24-27], *NCAPG2* [13], *PITX2* (OMIM: 601542; Gene ID: 5308) [28,29], *PITX3* (OMIM: 602669; Gene ID: 5309) [30], *RERE* [17], *SLC4A11* [12], *TFAP2A* [12], *WDR37* [18], and *WFS1* [19], are associated with syndromic forms of PA. Many of the reported PA causative genes (*COL4A1, CYP1B1* (OMIM: 601771; Gene ID: 1545), *FOXC1*, *FOXE3* (OMIM: 601094; Gene ID: 2301), *PAX6* (OMIM: 607108; Gene ID: 5080)*, PITX2*, and *PITX3*) are primarily associated with other conditions that are usually ophthalmic conditions [12]. *B3GLCT*, the genetic cause of Peters plus syndrome, is an exception in this regard. Notably, mutations in the known PA-associated genes were not observed in most patients of various genetic screenings studies [11-14,16-19]. We report identification of a mutation in *DOP1B* (OMIM: 604803; Gene ID: 9980) as the possible cause of Peters anomaly in three affected individuals of a pedigree and show expression of the gene in various human embryonic and adult ocular tissues.

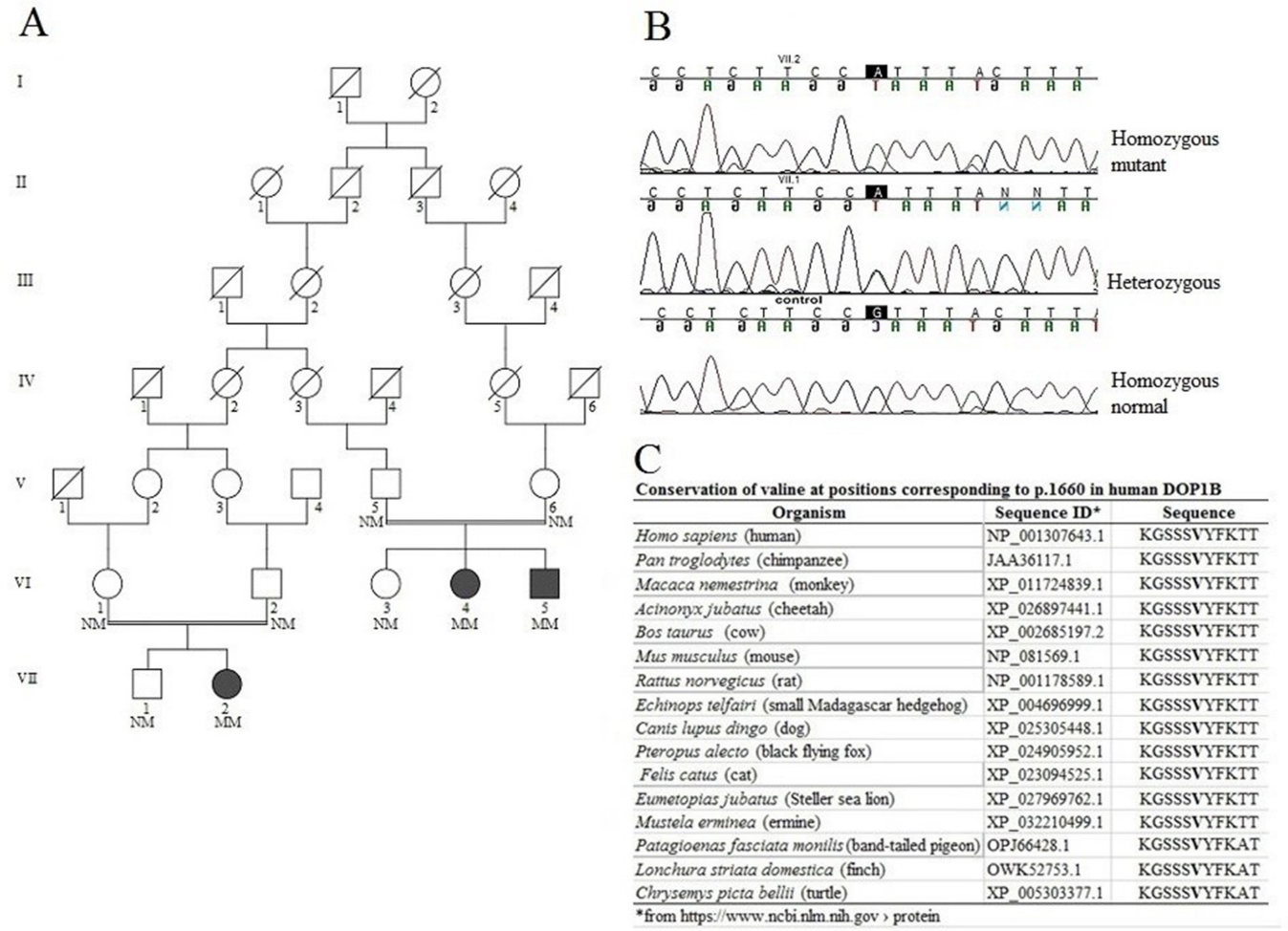
METHODS

This research was performed with adherence to the Declaration of Helsinki and the ARVO statement on human subjects. Participants or responsible guardians gave informed consent, and the research was approved by the ethics board of the University of Tehran. Eye globes were obtained from the Central Eye Bank of Iran, a non-profit organization established three decades ago and dedicated mainly to the procurement of eye globes from deceased individuals to be used for corneal transplants and related procedures. Responsible individuals of globe donors give signed permission for use of the globes or parts for research purposes. Far less frequently, globes of aborted embryos are obtained for research purposes through affiliations of the Eye Bank with the Shahid Beheshti University of Medical Sciences and with informed written consent of the parents. The embryos whose eye globes were used in this study had no gross or apparent anatomic abnormality.

Subjects and eye examinations: Three members of a pedigree diagnosed with PA were referred to us for genetic analysis. Slit-lamp biomicroscopy was performed for all three patients, and ultrasound biomicroscopy (UBM; Eye Cubed ™, Ellex, Adelaide, Australia) was performed for two patients. UBM scanning was conducted to a depth of 5 mm, using a 40 MHz transducer and an anterior B-scan time gain control (TGC) probe.

Genetic analysis: Genetic analysis was initiated with whole exome sequencing of the DNA of the three patients using the Sure Select V6-POST kit and an Illumina HiSeq 4000 system (Illumina, Foster City, CA). Exome sequence alignment for each patient was against human reference genome GRCh37/hg19, and variant callings were done using wANNOVAR. Subsequently, filtering was performed by removing single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) of >0.01 in the dbSNP database, the TransOmics for Precision Medicine program, the 1000 Genomes database, the NHLBI Exome Sequencing Project, the Exome Aggregation Consortium database, the Genome Aggregation Database, the Greater Middle East Variome Project, ENSEMBL, the Healthy Exomes database, the Sequencing Initiative Suomi database, the VarCards database, or the Iranome database. SNPs observed in in-house exome data belonging to approximately 110 unrelated Iranians affected with non-ocular diseases were also removed. Among the variations that remained, those that did not affect amino acid change or splicing were not considered. Subsequently, a file of homozygous variations and a file of compound heterozygous variations were prepared. The files of the patients were scrutinized to identify retained variations common to the three patients. The single variation identified was screened for segregation with disease status in the pedigree with Sanger sequencing of available members. It was also sought in the Iranome database that contains exome sequence data on 800 healthy Iranians.

Gene expression analysis: Expression of the putative PA-causing gene *DOP1B* was assessed in lens, iris, cornea, sclera, and retina tissues isolated from the eye globes of three adults and three embryos. The isolation procedure was



C

Conservation of valine at positions corresponding to p.1660 in human DOP1B

Organism	Sequence ID*	Sequence
Homo sapiens (human)	NP_001307643.1	KGSSSVYFKTT
Pan troglodytes (chimpanzee)	JAA36117.1	KGSSSVYFKTT
Macaca nemestrina (monkey)	XP_011724839.1	KGSSSVYFKTT
Acinonyx jubatus (cheetah)	XP_026897441.1	KGSSSVYFKTT
Bos taurus (cow)	XP_002685197.2	KGSSSVYFKTT
Mus musculus (mouse)	NP_081569.1	KGSSSVYFKTT
Rattus norvegicus (rat)	NP_001178589.1	KGSSSVYFKTT
Echinops telfairi (small Madagascar hedgehog)	XP_004696999.1	KGSSSVYFKTT
Canis lupus dingo (dog)	XP_025305448.1	KGSSSVYFKTT
Pteropus alecto (black flying fox)	XP_024905952.1	KGSSSVYFKTT
Felis catus (cat)	XP_023094525.1	KGSSSVYFKTT
Eumetopias jubatus (Steller sea lion)	XP_027969762.1	KGSSSVYFKTT
Mustela erminea (ermine)	XP_032210499.1	KGSSSVYFKTT
Patagioenas fasciata monilis (band-tailed pigeon)	OPJ66428.1	KGSSSVYFKAT
Lonchura striata domestica (finch)	OWK52753.1	KGSSSVYFKAT
Chrysemys picta bellii (turtle)	XP_005303377.1	KGSSSVYFKAT

*from https://www.ncbi.nlm.nih.gov › protein

Figure 1. Peters anomaly pedigree PA-101. **A**: Pedigree PA-101. Filled square and circles: PA-affected. Unfilled shapes: not affected with PA. **B**: Chromatograms showing homozygous and heterozygous mutation c.4978G>A (p.Val1660Ile) in *DOP1B*, and the wild-type genotype. **C**: Amino acid sequence alignments showing conservation of Val at positions corresponding to p.1660 in the human *DOP1B*-encoded protein in orthologous proteins of other organisms.

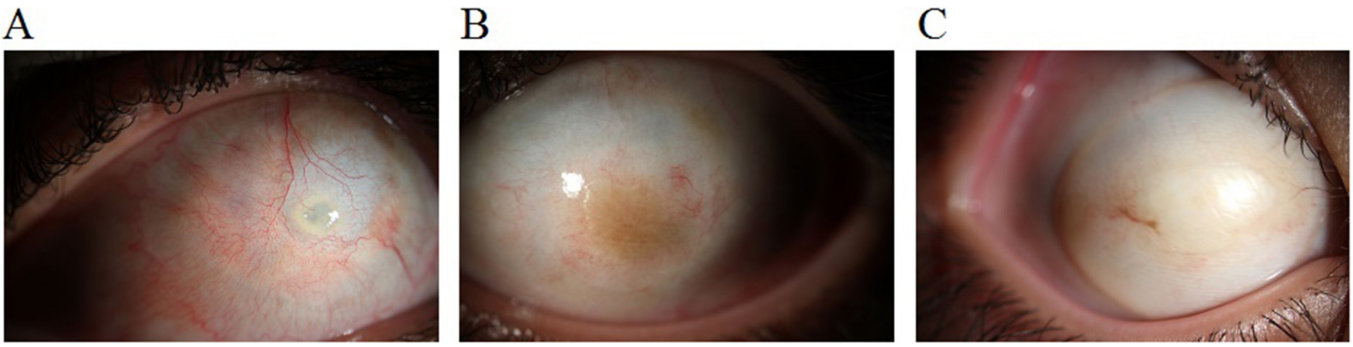


Figure 2. Slit-lamp biomicroscopy images show the Peters anomaly. **A**: PA-101-VI4. **B**: PA-101-VI5. **C**: PA-101-VII2. The images of PA-101-VI4 and PA-101-VI5 show total corneal opacity and vascularization. The image of PA-101-VII2 shows total corneal opacity and superficial keratinization.

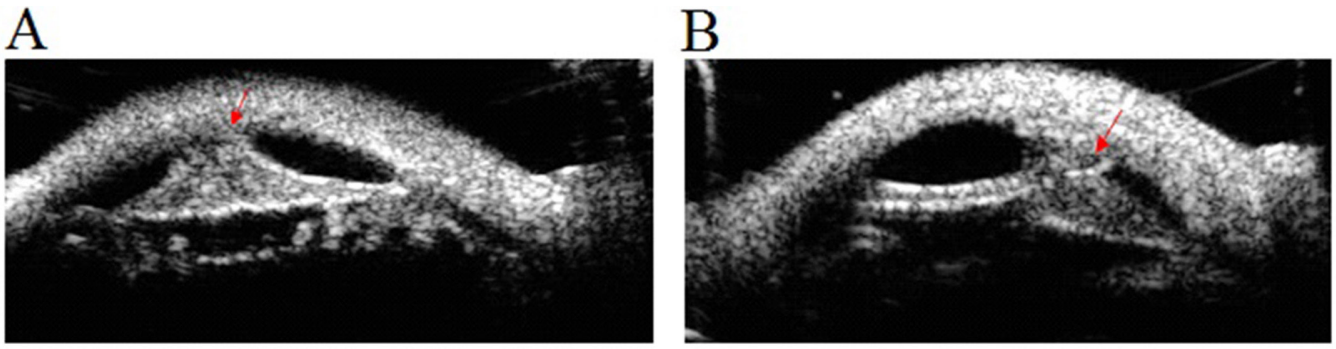


Figure 3. Ultrasound biomicroscopy images show the Peters anomaly. **A**: PA-101-VI4. **B**: PA-101-VI5. Images of both patients show irido-corneal adhesion (arrows).

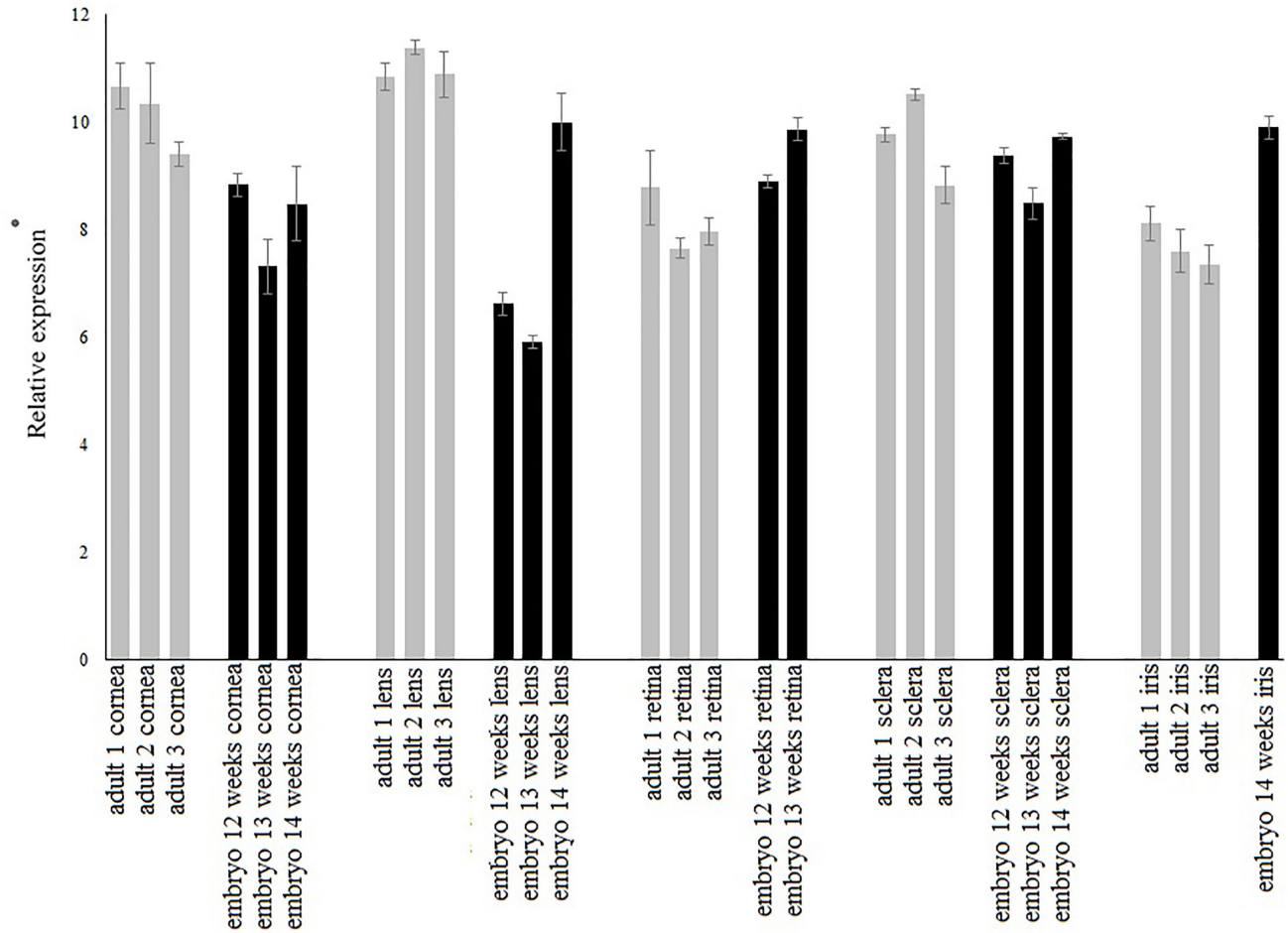


Figure 4. Expression of *DOP1B* in adult and embryonic ocular tissues as assessed with real-time PCR. Relative expression levels are presented as normalized to *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) expression. The average threshold cycle (CT) of *GAPDH* was 16.21. The expression of *DOP1B* was assessed in the cornea, lens, retina, sclera, and iris tissues of three adult men and three embryos. Standard deviations are shown.

performed by an expert in ocular pathology (MRK), taking great care to avoid contamination by adjacent tissues. One embryonic retina and two embryonic iris samples were not used because of unsuitable quality. The adults were men aged 20, 25, and 35 years old. The embryos were males aborted at 12, 13, and 14 weeks of gestation. Total RNA extraction was performed using RNX-Plus according to the manufacturer's instructions (Sinaclon, Tehran, Iran). Complementary DNA (cDNA) was synthesized using random hexamer primers. Real time PCR was performed on a Corbett Rotor-Gene 6000 machine (QIAGEN, Hilden, Germany) in 20 µl reactions that contained 1 µg of the cDNA, 10 µl SYBR green (BioFACT™, Daejeon, South Korea), and 10 pmol of forward and reverse primers. *GAPDH* (OMIM: 138400; Gene ID: 2597; glyceraldehyde-3-phosphate dehydrogenase) was used as the control gene. Sequences of forward and reverse primers used for amplification of *DOP1B* cDNA were 5′-ACT CAA CAA GGC TCT TCA GAG-3′ and 5′-GCA GCT GTA CAG AAA CAA GTC C-3′, respectively. These amplify the two largest alternatively spliced *DOP1B* cDNAs (NM_001320714.1 and NM_005128.3) that differ only in the 5′ untranslated region (UTR); the lengths of the two transcripts differ by 61 nucleotides. All expression analyses were performed in triplicate.

RESULTS

Subjects and eye examinations: The pedigree (PA-101) of the three related PA-affected individuals is shown in Figure 1A. Slit-lamp examination of PA-101-VI4, who was a 30-year-old woman, showed dense bilateral total corneal opacity and vascularization (Figure 2A). UBM images showed iridocorneal adhesion (Figure 3A). The patient did not have dysmorphic facial features or cognitive anomalies. Growth during childhood was reported to be normal. Her presentation was consistent with isolated PA diagnosis. PA-101-VI5 was a 19-year-old brother of PA-101-VI4. Results of his slit-lamp and UBM examinations were similar to those of his older sister (Figure 2B, Figure 3B). This patient had no other notable presentation. PA-101-VII2 was a 7-year-old girl and a distant relative of siblings. Her slit-lamp examination showed bilateral total corneal opacity and keratinization of the corneal epithelium and conjunctiva (Figure 2C). This young patient was not cooperative for the UBM examination. Nevertheless, considering affiliation with the described siblings, PA diagnosis is also justified for PA-101-VII2. Her facial and body appearance was normal. The mother reported that her daughter had hearing problems, and audiometric testing confirmed the presence of moderate sensorineural hearing loss. Hearing loss has also been reported for PA-affected patients with mutations in *TFAP2A* and *NDP* [12].

Genetic analysis: The two affected siblings (PA-101-VI4 and PA-101-VI5) were born to consanguineous parents whose common ancestors were four generations removed (Figure 1A). An affected relative (PA-101-VII2) was also born to consanguineous parents whose common ancestors were two generations removed. In addition to these consanguinities, all four parents were related to one another. The consanguineous parents of the patients were not PA-affected, consistent with autosomal recessive inheritance of the disease. Although consanguinity of the parents suggested that a homozygous sequence variation would most likely be the PA-causative mutation, compound heterozygous variations were also considered for the sake of stringency. After the described filterings and data analysis, a candidate disease causing in known PA-associated genes was not identified. The only homozygous variation or compound heterozygous variations identified that were present in all three patients was the homozygous variation c.G4978A in *DOP1B* (alias *DOPEY2* and *C21orf5*; Figure 1B). The relatively distant familial relationship between the parents of the siblings and between the two branches of the family were likely important factors in reducing the number of candidate genes after the filterings. The human *DOP1B* gene is located on chromosome 21q22.12, within the Down syndrome chromosomal region (DSCR) [31-33]. The shared *DOP1B* variation was within a shared 3.9 Mb homozygous block bordered by positions 34,413,505 and 38,385,585 on this chromosome. *DOP1B* intragenic variations that define the haplotype associated with the c.G4978A variation in *DOP1B* on chromosome 21 are T>G, C>A, and G>A at positions 37,617,630, 37,617,724, and 37,661,405, respectively. These are relatively common variations with MAF ≥ 0.14. The specifications of the exome data of all three patients reflected high-quality sequencing (Supplementary Table S1). Sanger sequencing revealed segregation of the variation with disease status, as both pairs of parents and the unaffected sibling of each of the siblings were heterozygous carriers of the variation (Figure 1B). The variation was absent in 800 control Iranians. It was reported in various databases (rs139989297) only in the heterozygous state at low frequencies. The highest reported frequencies were in various cohorts of African origin (ANNOVAR reporting of 0.0015 in Africa cohort of 1000 Genome project, ENSEMBL reporting of 0.006 in cohort of Mende in Sierra Leone, and Exome Sequencing Project reporting of 0.0036 in an African American cohort). Therefore, c.G4978A in *DOP1B* that causes p.Val1660Ile in the encoded protein, DOP1 leucine zipper like protein B, was considered the best candidate PA-causing mutation in pedigree PA-101. Valine at position p.1660 in the human protein is well conserved among mammals and birds (Figure 1C). Five (fathmm-MKL; LRT; MutationTaster; Polyphen2_HDIV;

Polyphen2_HVAR) of 12 bioinformatics tools predict that the mutation is damaging.

Gene expression analysis: The expression of *DOP1B* was queried in the eye, as this is the target organ of Peters anomaly. Aborted embryos with gestations that correspond to the time point of the determination and differentiation of ocular tissues were not accessible. Instead, expression analysis at the level of transcription was performed in young male adults and in embryos that were aborted at 12, 13, and 14 weeks of gestation. Expression of *DOP1B* at the RNA level was shown in all adult and embryonic lens, iris, cornea, sclera, and retina tissues that were tested (Figure 4).

DISCUSSION

Peters anomaly is a clinically heterogeneous developmental eye disorder that has been associated with many genes which together are the cause of disease in a small proportion of patients with PA examined [11]. We have identified *DOP1B* as a novel putative PA-causing gene. Of course, the possibility that the disease in pedigree PA-101 was caused by mutations in regions of the genome not included in the exome sequencing protocol, including deep intronic variations, cannot be strictly ruled out. Because of the inclusion of *DOP1B* in the DSCR, the expression of this gene in humans has most often been examined in the brain and with respect to Down syndrome [32,34-36]. *DOP1B* was shown to be differentially expressed in different brain regions of the normal human fetus and overexpressed in Down syndrome fetal brain tissues. Studies in transgenic mice suggested involvement of *DOP1B* in brain morphogenesis and mental retardation [32]. An association between *DOP1B* copy number variations (CNVs) in DNA extracted from brain tissue and late-onset Alzheimer disease has also been reported [37]. In addition to the brain, the expression of *DOP1B* has been reported in many other tissues, including the eye (ENSG00000142197-DOPEY2/tissue; MGI:1917278). To the best of our knowledge, for the first time, we showed the expression of *DOP1B* in various specific embryonic and adult ocular tissues, including the cornea, lens, retina, sclera, and iris. These include tissues that are most relevant to Peters anomaly pathology. Although the expression of *DOP1B* in these tissues is consistent with its potential role in ocular development, the finding does not necessarily implicate such a role because the gene is expressed in many tissues. Ultimately, definitive assessment of the contribution of *DOP1B* to PA pathology awaits identification of mutations in the gene in unrelated patients with PA and functional studies.

DOP1B is a member of the *Dopey* gene family whose members contain leucine zipper-like domains with protein–protein interaction functions [32]. Several members of this family, including *DOP1B* homologous genes *DopA* in *Aspergillus nidulans*, *Dop1* in *Saccharomyces cerevisiae*, and *pad1* in *Caenorhabditis elegans*, have roles in cell differentiation, morphogenesis, and development in the respective organisms [38,39]. The orthologous gene in *Drosophila*, CG15099 (FlyBase), was suggested to be involved in cell cycle regulation [40]. In addition, as noted above, transgenic studies in mice implicated *DOP1B* in brain development [32]. Identification of a mutation in *DOP1B* in Peters anomaly–affected individuals of pedigree PA-101 also implicates the gene in eye development. The leucine-like zipper domains of the encoded protein could promote protein–protein interactions which may, in turn, affect transcriptional regulation. Although the p.Val1660Ile mutation in the PA-101 patients is not within these domains, it may indirectly affect their functions. Unfortunately, the crystalline structure of the DOP1B protein is not available. Interestingly, in a recent bioinformatics study, *DOP1B* was identified as one of several genes in which disease-causing variants have not been identified, but whose variants are strong candidates for disease causation [41]. It is likely that *DOP1B* and other yet-to-be-identified PA-causing genes, like several already identified, will each contribute to disease status in only a small minority of patients.

ACKNOWLEDGMENTS

We acknowledge the Iran National Science Foundation for funding the research and thank the patients and their family members for participating in the study.

REFERENCES

1. Reis LM, Semina EV. Genetics of anterior segment dysgenesis disorders. Curr Opin Ophthalmol 2011; 22:314-24. [PMID: 21730847].
2. Harissi-Dagher M, Colby K. Anterior segment dysgenesis: Peters anomaly and sclerocornea. Int Ophthalmol Clin 2008; 48:35-42. [PMID: 18427259].
3. Nischal KK, Naor J, Jay V, MacKeen LD, Rootman DS. Clinicopathological correlation of congenital corneal opacification using ultrasound biomicroscopy. Br J Ophthalmol 2002; 86:62-9. [PMID: 11801506].
4. Matsubara A, Ozeki H, Matsunaga N, Nozaki M, Ashikari M, Shirai S, Ogura Y. Histopathological examination of two cases of anterior staphyloma associated with Peters' anomaly and persistent hyperplastic primary vitreous. Br J Ophthalmol 2001; 85:1421-5. [PMID: 11734512].
5. Cvekl A, Tamm ER. Anterior eye development and ocular mesenchyme: new insights from mouse models and human diseases. BioEssays 2004; 26:374-86. [PMID: 15057935].

6. Davis-Silberman N, Ashery-Padan R. Iris development in vertebrates; genetic and molecular considerations. Brain Res 2008; 1192:17-28. [PMID: 17466284].

7. Ito YA, Walter MA. Genomics and anterior segment dysgenesis: a review. Clin Experiment Ophthalmol 2014; 42:13-24. [PMID: 24433355].

8. Bhandari R, Ferri S, Whittaker B, Liu M, Lazzaro DR. Peters anomaly: review of the literature. Cornea 2011; 30:939-44. [PMID: 21448066].

9. Dassie-Ajdid J, Causse A, Poidvin A, Granier M, Kaplan J, Burglen L, Doummar D, Teisseire P, Vigouroux A, Malecaze F, Calvas P, Chassaing N. Novel B3GALTL mutation in Peters-plus Syndrome. Clin Genet 2009; 76:490-2. [PMID: 19796186].

10. Toton-Zuranska J, Kapusta P, Rybak-Krzyszkowska M, Lorenc K, Machlowska J, Skalniak A, Filipek E, Pawlik D, Wolkow PP. Contribution of a Novel B3GLCT Variant to Peters Plus Syndrome Discovered by a Combination of Next-Generation Sequencing and Automated Text Mining. Int J Mol Sci 2019; 20:6006-[PMID: 31795264].

11. Reis LM, Houssin NS, Zamora C, Abdul-Rahman O, Kalish JM, Zackai EH, Plageman TF Jr, Semina EV. Novel variants in CDH2 are associated with a new syndrome including Peters anomaly. Clin Genet 2020; 97:502-8. [PMID: 31650526].

12. Weh E, Reis LM, Happ HC, Levin AV, Wheeler PG, David KL, Carney E, Angle B, Hauser N, Semina EV. Whole exome sequence analysis of Peters anomaly. Hum Genet 2014; 133:1497-511. [PMID: 25182519].

13. Khan TN, Khan K, Sadeghpour A, Reynolds H, Perilla Y, McDonald MT, Gallentine WB, Baig SM, Davis EE, Katsanis N. Mutations in NCAPG2 Cause a Severe Neurodevelopmental Syndrome that Expands the Phenotypic Spectrum of Condensinopathies. Am J Hum Genet 2019; 104:94-111. [PMID: 30609410].

14. van Reeuwijk J, Janssen M, van den Elzen C, Beltran-Valero de Bernabe D, Sabatelli P, Merlini L, Boon M, Scheffer H, Brockington M, Muntoni F, Huynen MA, Verrips A, Walsh CA, Barth PG, Brunner HG, van Bokhoven H. POMT2 mutations cause alpha-dystroglycan hypoglycosylation and Walker-Warburg syndrome. J Med Genet 2005; 42:907-12. [PMID: 15894594].

15. Almarzouki HS, Tayyib AA, Khayat HA, Alsulami RE, Alzahrani SM, Alkahtani AS, Alghifees LS. Peters Anomaly in Twins: A Case Report of a Rare Incident with Novel Comorbidities. Case Rep Ophthalmol 2016; 7:186-92. [PMID: 27843434].

16. Chassaing N, Davis EE, McKnight KL, Niederriter AR, Causse A, David V, Desmaison A, Lamarre S, Vincent-Delorme C, Pasquier L, Coubes C, Lacombe D, Rossi M, Dufier JL, Dollfus H, Kaplan J, Katsanis N, Etchevers HC, Faguer S, Calvas P. Targeted resequencing identifies PTCH1 as a major contributor to ocular developmental anomalies and extends the SOX2 regulatory network. Genome Res 2016; 26:474-85. [PMID: 26893459].

17. Fregeau B, Kim BJ, Hernandez-Garcia A, Jordan VK, Cho MT, Schnur RE, Monaghan KG, Juusola J, Rosenfeld JA, Bhoj E, Zackai EH, Sacharow S, Baranano K, Bosch DGM, de Vries BBA, Lindstrom K, Schroeder A, James P, Kulch P, Lalani SR, van Haelst MM, van Gassen KLI, van Binsbergen E, Barkovich AJ, Scott DA, Sherr EH. De Novo Mutations of RERE Cause a Genetic Syndrome with Features that Overlap Those Associated with Proximal 1p36 Deletions. Am J Hum Genet 2016; 98:963-70. [PMID: 27087320].

18. Reis LM, Sorokina EA, Thompson S, Muheisen S, Velinov M, Zamora C, Aylsworth AS, Semina EV. De Novo Missense Variants in WDR37 Cause a Severe Multisystemic Syndrome. Am J Hum Genet 2019; 105:425-33. [PMID: 31327510].

19. Prochazkova D, Hruba Z, Konecna P, Skotakova J, Fajkusova L. A p.(Glu809Lys) Mutation in the WFS1 Gene Associated with Wolfram-like Syndrome: A Case Report. J Clin Res Pediatr Endocrinol 2016; 8:482-3. [PMID: 27217304].

20. Wang YE, Ramirez DA, Chang TC, Berrocal A. Peters plus syndrome and Chorioretinal findings associated with B3GLCT gene mutation - a case report. BMC Ophthalmol 2020; 20:118-[PMID: 32204707].

21. Lesnik Oberstein SA, Kriek M, White SJ, Kalf ME, Szuhai K, den Dunnen JT, Breuning MH, Hennekam RC. Peters Plus syndrome is caused by mutations in B3GALTL, a putative glycosyltransferase. Am J Hum Genet 2006; 79:562-6. [PMID: 16909395].

22. Reis LM, Tyler RC, Abdul-Rahman O, Trapane P, Wallerstein R, Broome D, Hoffman J, Khan A, Paradiso C, Ron N, Bergner A, Semina EV. Mutation analysis of B3GALTL in Peters Plus syndrome. Am J Med Genet A 2008; 146A:2603-10. [PMID: 18798333].

23. Deml B, Reis LM, Maheshwari M, Griffis C, Bick D, Semina EV. Whole exome analysis identifies dominant COL4A1 mutations in patients with complex ocular phenotypes involving microphthalmia. Clin Genet 2014; 86:475-81. [PMID: 24628545].

24. Honkanen RA, Nishimura DY, Swiderski RE, Bennett SR, Hong S, Kwon YH, Stone EM, Sheffield VC, Alward WL. A family with Axenfeld-Rieger syndrome and Peters Anomaly caused by a point mutation (Phe112Ser) in the FOXC1 gene. Am J Ophthalmol 2003; 135:368-75. [PMID: 12614756].

25. Weisschuh N, Wolf C, Wissinger B, Gramer E. A novel mutation in the FOXC1 gene in a family with Axenfeld-Rieger syndrome and Peters' anomaly. Clin Genet 2008; 74:476-80. [PMID: 18498376].

26. Nishimura DY, Searby CC, Alward WL, Walton D, Craig JE, Mackey DA, Kawase K, Kanis AB, Patil SR, Stone EM, Sheffield VC. A spectrum of FOXC1 mutations suggests gene dosage as a mechanism for developmental defects of the anterior chamber of the eye. Am J Hum Genet 2001; 68:364-72. [PMID: 11170889].

27. Nishimura DY, Swiderski RE, Alward WL, Searby CC, Patil SR, Bennet SR, Kanis AB, Gastier JM, Stone EM, Sheffield VC. The forkhead transcription factor gene FKHL7 is

responsible for glaucoma phenotypes which map to 6p25. Nat Genet 1998; 19:140-7. [PMID: 9620769].

28. Reis LM, Tyler RC, Volkmann Kloss BA, Schilter KF, Levin AV, Lowry RB, Zwijnenburg PJ, Stroh E, Broeckel U, Murray JC, Semina EV. PITX2 and FOXC1 spectrum of mutations in ocular syndromes. Eur J Hum Genet 2012; 20:1224-33. [PMID: 22569110].

29. Doward W, Perveen R, Lloyd IC, Ridgway AE, Wilson L, Black GC. A mutation in the RIEG1 gene associated with Peters' anomaly. J Med Genet 1999; 36:152-5. [PMID: 10051017].

30. Semina EV, Ferrell RE, Mintz-Hittner HA, Bitoun P, Alward WL, Reiter RS, Funkhauser C, Daack-Hirsch S, Murray JC. A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataracts and ASMD. Nat Genet 1998; 19:167-70. [PMID: 9620774].

31. Guipponi M, Brunschwig K, Chamoun Z, Scott HS, Shibuya K, Kudoh J, Delezoide AL, El Samadi S, Chettouh Z, Rossier C, Shimizu N, Mueller F, Delabar JM, Antonarakis SE. C21orf5, a novel human chromosome 21 gene, has a Caenorhabditis elegans ortholog (pad-1) required for embryonic patterning. Genomics 2000; 68:30-40. [PMID: 10950924].

32. Rachidi M, Lopes C, Costantine M, Delabar JM. C21orf5, a new member of Dopey family involved in morphogenesis, could participate in neurological alterations and mental retardation in Down syndrome. DNA Res 2005; 12:203-10. [PMID: 16303751].

33. Shapiro BL. The Down syndrome critical region. J Neural Transm Suppl 1999; 57:41-60. [PMID: 10666667].

34. Rachidi M, Delezoide AL, Delabar JM, Lopes C. A quantitative assessment of gene expression (QAGE) reveals differential overexpression of DOPEY2, a candidate gene for mental retardation, in Down syndrome brain regions. Int J Dev Neurosci 2009; 27:393-8. [PMID: 19460634].

35. Rachidi M, Lopes C, Delezoide AL, Delabar JM. C21orf5, a human candidate gene for brain abnormalities and mental retardation in Down syndrome. Cytogenet Genome Res 2006; 112:16-22. [PMID: 16276086].

36. Lopes C, Chettouh Z, Delabar JM, Rachidi M. The differentially expressed C21orf5 gene in the medial temporal-lobe system could play a role in mental retardation in Down syndrome and transgenic mice. Biochem Biophys Res Commun 2003; 305:915-24. [PMID: 12767918].

37. Swaminathan S, Huentelman MJ, Corneveaux JJ, Myers AJ, Faber KM, Foroud T, Mayeux R, Shen L, Kim S, Turk M, Hardy J, Reiman EM, Saykin AJ. Analysis of copy number variation in Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals. PLoS One 2012; 7:e50640-[PMID: 23227193].

38. Pascon RC, Miller BL. Morphogenesis in Aspergillus nidulans requires Dopey (DopA), a member of a novel family of leucine zipper-like proteins conserved from yeast to humans. Mol Microbiol 2000; 36:1250-64. [PMID: 10931277].

39. Dujon B. The yeast genome project: what did we learn? Trends Genet 1996; 12:263-70. [PMID: 8763498].

40. Bjorklund M, Taipale M, Varjosalo M, Saharinen J, Lahdenpera J, Taipale J. Identification of pathways regulating cell size and cell-cycle progression by RNAi. Nature 2006; 439:1009-13. [PMID: 16496002].

41. Alhuzimi E, Leal LG, Sternberg MJE, David A. Properties of human genes guided by their enrichment in rare and common variants. Hum Mutat 2018; 39:365-70. [PMID: 29197136].

42. Vincent A, Billingsley G, Priston M, Glaser T, Oliver E, Walter M, Ritch R, Levin A, Heon E. Further support of the role of CYP1B1 in patients with Peters anomaly. Mol Vis 2006; 12:506-10. [PMID: 16735991].

43. Vincent A, Billingsley G, Priston M, Williams-Lyn D, Sutherland J, Glaser T, Oliver E, Walter MA, Heathcote G, Levin A, Heon E. Phenotypic heterogeneity of CYP1B1: mutations in a patient with Peters' anomaly. J Med Genet 2001; 38:324-6. [PMID: 11403040].

44. Doucette L, Green J, Fernandez B, Johnson GJ, Parfrey P, Young TL. A novel, non-stop mutation in FOXE3 causes an autosomal dominant form of variable anterior segment dysgenesis including Peters anomaly. Eur J Hum Genet 2011; 19:293-9. [PMID: 21150893].

45. Ormestad M, Blixt A, Churchill A, Martinsson T, Enerback S, Carlsson P. Foxe3 haploinsufficiency in mice: a model for Peters' anomaly. Invest Ophthalmol Vis Sci 2002; 43:1350-7. [PMID: 11980846].

46. Hanson IM, Fletcher JM, Jordan T, Brown A, Taylor D, Adams RJ, Punnett HH, van Heyningen V. Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peters' anomaly. Nat Genet 1994; 6:168-73. [PMID: 8162071].

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 25 November 2020. 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.