A Novel Mutation in *FOXC1* in a Lebanese Family with Congenital Heart Disease and Anterior Segment Dysgenesis: Potential Roles for *NFATC1* and *DPT* in the Phenotypic Variations

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Congenital heart diseases (CHDs) are still the leading cause of death in neonates. Anterior segment dysgenesis is a broad clinical phenotype that affects the normal development of the eye, leading in most of the cases to glaucoma which is still a major cause of blindness for children and adolescents. Despite tremendous insights gained from genetic studies, a clear genotype–phenotype correlation is still difficult to draw. In Lebanon, a small country with still a high rate of consanguineous marriages, there are little data on the epidemiology of glaucoma amongst children with or without CHD. We carried out whole exome sequencing (WES) on a family with anterior segment dysgenesis, and CHD composed of three affected children with glaucoma, two of them with structural cardiac defects and three healthy siblings. The results unravel a novel mutation in *FOXC1* (p. R127H) segregating with the phenotype and inherited from the mother, who did not develop glaucoma. We propose a digenic model for glaucoma in this family by combining the *FOXC1* variant with a missense variant inherited from the father in the dermatopontin (*DPT*) gene. We also unravel a novel *NFATC1* missense mutation predicted to be deleterious and present only in the patient with a severe ocular and cardiac phenotype. This is the first report on *FOXC1* using WES to genetically characterize a family with both ocular and cardiac malformations. Our results support the usage of such technology to have a better genotype–phenotype picture for Mendelian-inherited diseases for which expressivity and penetrance are still not answered.

Keywords: anterior segment dysgenesis, congenital heart disease, forkhead box c1, digenic, whole exome sequencing

INTRODUCTION

Glaucoma is the second leading cause of blindness in the world, according to the World Health Organization (1, 2). It is characterized by a progressive damage of the eye's optic nerve, which is highly influenced by fluid building up in the front part of the eye leading to an increase in the intraocular pressure (IOP) (3, 4). Genetic predisposition, aging, and environmental factors do play essential roles

in the developing of the disease in adults; however, the financial, social, and familial burden is more sensed in the congenital and juvenile cases (3, 5–8). These cases are mostly linked to developmental defects in the formation and differentiation of the cells that make the ocular system in general, and in particular the optic nerve and the anterior segment which comprise the iris, the lens, and the cornea (3, 5, 6).

Pediatric glaucomas are divided into two major types: primary congenital glaucoma (PCG), which accounts for 50–70% of all childhood cases, and syndromic-associated glaucomas that include among others juvenile open angle glaucoma (JOAG) (OMIM#37750, #603383, and #137760), aniridia (OMIM#106210), and the Axenfeld–Rieger syndrome (ARS) (OMIM#180500, #601499, and #602482). Linkage analyses and genome wide association studies have identified many loci and genes implicated in PCG, mostly inherited in an autosomal recessive form, diagnosed in the first year of life, and largely prevalent in countries with high consanguinity. Among these, *MYOC* (myocilin) and *CYP1B1* (cytochrome P450) represent the frequently mutated genes linked to the phenotype (6, 9). Interestingly, a digenic mode of inheritance with both *CYP1B1* and *MYOC* mutations has been documented in patients with PCG highlighting the potential involvement of common genetic and molecular players in the disease (10). JOAG is inherited mainly as a dominant trait with an onset age ranging from 3 to 35 years and characterized by high IOP requiring in most of the cases early surgical treatment. The major genetic players are mutations in *MYOC* with high prevalence and penetrance, followed by *OPTN* (optineurin) and *WDR36* (WD repeat containing protein 36) (11). Aniridia is a very rare panocular disease whereby glaucoma is diagnosed in 50–70% of the cases at later ages (end of adolescent, early adulthood). Mostly inherited as autosomal dominant, it is mainly caused by mutations in *PAX6* (paired-box gene 6), the master regulator gene of eye development (12, 13). As for ARS, it is an autosomal dominant disorder characterized by iris stromal hypoplasia, prominent Schwalbe line (embryotoxon), adhesion between the iris and Schwalbe line, microcornea, corneal opacity, and increased IOP that leads to glaucoma in about 50% of the cases (3, 14–17). Patients with ARS can also have maxillary hypoplasia, dental anomalies, umbilical hernia, and/or hypospadias. More rarely, they may have hydrocephalus, hearing loss, cardiac and kidney abnormalities, and congenital hip dislocation in addition to the ocular abnormalities. Globally, mutations in two genes *FOXC1* (forkhead box c1) and *PITX2* (paired-like homeodomain transcription factor 2) have been shown to be responsible for most of the cases with complete penetrance but variable expressivity (14, 17–19).

FOXC1 belongs to the Forkhead box (FOX) family of transcription factors, which share an evolutionarily conserved DNA-binding domain known as the Forkhead domain (20–22). In addition to the eye, it plays a dose-dependent evolutionary conserved role in the early development of the blood vessels, the brain, the heart, and the somites (23). *Foxc1* homozygous null mutant mice have a lethal phenotype; they die pre- and perinatally with hemorrhagic hydrocephalus and multiple skeletal, ocular, and genitourinary defects. They also suffer from cardiovascular defects, most notably, interruption or coarctation of the aortic arch (24, 25). In addition to its critical role during ocular development, *Foxc1* has a protective role in the adult eye. It regulates antiapoptotic genes such as *Foxo1a* in order to maintain homeostasis in the adult trabecular meshwork (TM), which is constantly exposed to aqueous humor (26). By maintaining programmed cell death and thus proliferation of TM cells, *FOXC1* is a major player in the onset of glaucoma once deregulated. In humans, deleterious *FOXC1* mutations associated with ARS span the entire region of the protein including the DNA-binding and activation domains, resulting in reduced transcriptional activity due to haploinsufficiency. Despite the *in vitro* studies that assess these differential activities, a genotype–phenotype correlation map could not be established to explain the variable expressivity of the phenotype even among patients harboring the same mutation.

In Lebanon, a small country with still frequent consanguineous marriages, only one recent study addressed the genetic basis of congenital glaucoma (27). We hereby describe the first *FOXC1* missense mutation using whole exome sequencing (WES) in a Lebanese family with anterior segment dysgenesis and cardiac phenotypes. We showed that this novel mutation has different expressivity, but we propose a digenic model of inheritance that includes *NFATC1* to explain the severe cardiac phenotype in one of the patients. Our findings explicitly call for a revision of the expressivity and penetrance terms in genetic inheritance by using WES as a tool to explain genotype–phenotype correlations.

MATERIALS AND METHODS

Patients Recruitment

The study was approved by the institutional review board at the American University of Beirut. All patients, their legal guardians, and family members signed an informed consent form before being enrolled in the study. Patients presenting to the Department of Pediatrics and Adolescent Medicine with Congenital Heart Disease (CHD) were serially recruited in the study under IRB approved protocol BioCh.Gn.01. Blood samples were collected from a family consisting of six children and their parents. Two children presented with glaucoma and VSD and one with glaucoma only. Standard clinical evaluation included a complete physical examination, electrocardiography and two-dimensional transthoracic echocardiography with color Doppler were obtained. Family consanguinity history was utilized in constructing pedigrees after interviewing all patients and their parents.

Exome Sequencing

Blood samples were collected from all members, and the DNA was extracted using the Qiagen Blood-Midi kit (Qiagen Science Inc., Germantown, MD, USA), following the manufacturer's protocol. DNA quantification was performed using the NanoDrop (Thermo Fisher Scientific Inc., Waltham, MA, USA) at the molecular core facility at AUB. One microgram of coded DNA samples from both parents, and the six children were shipped to Macrogen (South Korea, www.dna.macrogen.com) where exome sequencing was performed using the V6 Sureselect target

enrichment capture system from Agilent on a HiSeq4000 platform from Illumina (San Diego, CA, USA).

Sanger Sequencing

Sanger sequencing was used to confirm the missense mutation in *FOXC1* by exome sequencing. Briefly, primers were designed to amplify partially the region on exon 1 of the gene that covers the mutation: 5′-CCTACGGGCCCTACACG-3′ (F) and 5′-GTTGTCCACGCTGAAGCC-3′ (R). The 749 bp PCR products were resolved on agarose gels, then purified using the QIAquick kits (Qiagen, Science Inc. Germantown, MD, USA). Sanger sequencing was carried out as previously described on an ABI3500 (Applied Biosystems, Foster City, CA, USA) platform at the American University of Beirut Molecular Core Facility.

Data Analysis

Primary analysis was done at Macrogen. The Fastq files were mapped to the reference genome using the Burrows–Wheeler Alignment tool. The Genome Analysis Toolkit was used for variants calling, and the SnEff software was used to annotate the variants. The Illumina Variant Studio was used to filter the variants as per their frequency and presence or absence in the affected family members versus the healthy individuals.
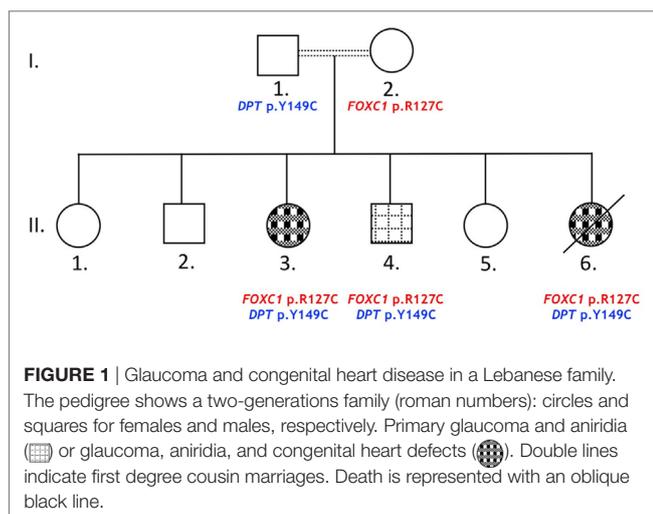
RESULTS

Clinical Evaluation: Is Glaucoma Separate from CHD?

Index patient II.3 (**Figure 1**) was referred to the Pediatrics Heart Center at the American University of Beirut Medical Center for consultation at the age of 8 years. Echocardiography showed a small perimembranous ventricular septal defect (VSD) with aneurysmal tissue formation. The color Doppler study was indicative of normal pulmonary arterial pressure with no valvar stenosis. There was no need for cardiac medications, and the parents were advised to a follow-up visit after 2 years. Concomitant with this date, the parents had a newborn girl, patient II.6 who was admitted to the emergency room for congestive heart failure

at 4 months of age. Echocardiography results showed a mild biventricular hypertrophy, with a more prominent right ventricle phenotype. A large subaortic VSD was noted with an overriding aorta, and pulmonary atresia reminiscent of a Tetralogy of Fallot (TOF)-like phenotype. The color Doppler imaging showed a trace tricuspid regurgitation, and a patent ductus arteriosus (PDA) like structure arising most likely from the transverse aorta. A right-modified Blalock–Taussig shunt was performed, and in parallel the patient was diagnosed with aniridia and glaucoma. Her ocular examination at the age of 6 months showed partial aniridia in both eyes with corneal edema on the right and corneal opacification with neovascularization on the left. Examination under anesthesia revealed elevated IOP at 21 mmHg with enlarged corneas measuring 11 mm on the right and 13 mm on the left. Dilated fundoscopy showed a normal posterior pole on the right with a cup to disk ratio of 0.4. No view could be obtained of the left fundus. She underwent partial trabeculotomy in the right eye as it was as discovered intraoperatively that she had incomplete development of her Schlemm's canal. This finding prohibited complete trabeculotomy and confirmed an anterior segment dysgenesis diagnosis. Two months later, she underwent Ahmed tube implantation in the right eye due to persistent glaucoma. Her postoperative course was complicated by a right corneal ulcer and an infection of the shunt that necessitated its removal 3 months after placement. She subsequently did well postoperatively with a limited hyphema and was lost to follow-up later. The same ocular finding was also detected in two of her siblings (II.3 and II.4), but was absent from the remaining four. The parents were not examined at our facilities, but they denied having any cardiac or ocular problem. The family was enrolled in our ongoing genetic study on CHDs, but patient II.6 passed away few months later, and no follow-up was made with the rest of the family members.

Genetic Analysis (I): A Novel FOXC1 Missense Variant with Variable Expressivity

The results of WES yielded an average of 98,521 variants enclosing both single nucleotide polymorphisms and insertions/deletions in the six children and their parents. A first round of variants' filtering consists of keeping only variants from an inclusive list of 65 genes (Table S1 in Supplementary Material) implicated in glaucoma, anterior segment dysgenesis, microcornea, and microphthalmia. We use an arbitrary model of inheritance that includes X-linked recessive, or autosomal dominant, or autosomal recessive with a minor allele frequency (MAF) less than 5%, and excluding synonymous and in-frame insertions/deletions variants. Only one novel variant in the coding region of *FOXC1* was detected in the three affected children (**Table 1**); the chr 6:1611059C>T variant leads to a missense mutation p.R127C (NM_001453.2) in the DNA-binding domain of the protein. While being absent from the father and the three non-affected children, this missense mutation is inherited from the healthy mother and is predicted to be deleterious and damaging as ascertained by Sanger sequencing (**Figure 2**). Additionally, a missense variant in *PCMTD1* was encountered in all family members, affected and unaffected, therefore excluding it from being responsible for the ocular phenotype (**Table 1**).

FIGURE 1 | Glaucoma and congenital heart disease in a Lebanese family. The pedigree shows a two-generations family (roman numbers): circles and squares for females and males, respectively. Primary glaucoma and aniridia (▦) or glaucoma, aniridia, and congenital heart defects (▨). Double lines indicate first degree cousin marriages. Death is represented with an oblique black line.

TABLE 1 | Variants in genes implicated in glaucoma, anterior segment dysgenesis, microcornea, and microphthalmia in the affected patients.

Sample	Gene	Variant	Coordinate	Chr	Type	Filters	Quality	Inherited from	Allelic depths	Transcript	Consequence	Protein position	Amino acids	Sift	PolyPhen	dbSNP ID	Allele freq global minor	Allele Freq Evs	EVS Coverage	EVS Samples
II.6	CNTNAP2	C>C/CTG	148106477	7	Insertion	PASS	658.77	Father	21,18	NM_014141.5	splice_region_variant, intron_variant, feature_elongation	0					0	0	0	0
II.4	CNTNAP2	G>G/T	146805232	7	Snv	PASS	36.77	None	2,3	NM_014141.5	splice_region_variant, intron_variant	0					0	0	54	6503
II.6	COL11A1	GA>G/G	103496805	1	Deletion	PASS	78.03	Both	0,4	NM_080629.2	splice_region_variant, intron_variant, feature_truncation	0				rs67059272, rs36076089	0	0	20	6498
II.3	COL11A1	GA>G/G	103496805	1	Deletion	PASS	52.28	Both	0,3	NM_080629.2	splice_region_variant, intron_variant, feature_truncation	0				rs67059272, rs36076089	0	0	20	6498
II.4	COL11A1	GA>G/G	103496805	1	Deletion	PASS	52.28	Both	0,3	NM_080629.2	splice_region_variant, intron_variant, feature_truncation	0				rs67059272, rs36076089	0	0	20	6498
II.6	FOXC1	C>C/T	1611059	6	Snv	PASS	615.77	Mother	28,20	NM_001453.2	missense_variant	127	R/C	deleterious(0)	probably_damaging(1)		0	0	96	6503
II.3	FOXC1	C>C/T	1611059	6	Snv	PASS	896.77	Mother	27,28	NM_001453.2	missense_variant	127	R/C	deleterious(0)	probably_damaging(1)		0	0	96	6503
II.4	FOXC1	C>C/T	1611059	6	Snv	PASS	704.77	Mother	15,22	NM_001453.2	missense_variant	127	R/C	deleterious(0)	probably_damaging(1)		0	0	96	6503
II.6	GALC	GA>G/G	88417095	14	Deletion	PASS	282.1	Both	1,12	NM_000153.3	splice_region_variant, intron_variant, feature_truncation	0				rs11300320	0	0	11	5784
II.3	GALC	GA>G/G	88417095	14	Deletion	PASS	799.77	Both	0,30	NM_000153.3	splice_region_variant, intron_variant, feature_truncation	0				rs11300320	0	0	11	5784
II.4	GALC	GA>G/G	88417095	14	Deletion	PASS	477.77	Both	0,18	NM_000153.3	splice_region_variant, intron_variant, feature_truncation	0				rs11300320	0	0	11	5784
II.6	PCMTD1	A>A/C	52733079	8	Snv	PASS	76.77	None	20,4	NM_052937.2	missense_variant	302	S/R	tolerated(0.09)	probably_damaging(0.975)	rs75865149	0	0	115	6503
II.6	PCMTD1	T>T/G	52733128	8	Snv	PASS	238.77	Ambiguous	15,9	NM_052937.2	missense_variant	286	N/T	tolerated(0.56)	benign(0.012)	rs62506083	0	0	188	6503
II.3	PCMTD1	T>T/G	52733128	8	Snv	PASS	81.77	Ambiguous	14,5	NM_052937.2	missense_variant	286	N/T	tolerated(0.56)	benign(0.012)	rs62506083	0	0	188	6503
II.4	PCMTD1	T>T/G	52733128	8	Snv	PASS	187.77	Ambiguous	19,11	NM_052937.2	missense_variant	286	N/T	tolerated(0.56)	benign(0.012)	rs62506083	0	0	188	6503

Genetic Analysis (II): A DPT Missense Variant Inherited from the Father Exclusively in the Affected Glaucomatous Patients

In order to understand the absence of ocular manifestations in the mother, we hypothesized that an additional variant inherited from the father with no effect on its own might be needed in conjunction with the *FOXC1* variant to cause Glaucoma in the affected children. Using the Illumina variant studio, we filtered the variants that are only shared by the three affected individuals (II.3, 4, and 6) and their father I.1 (**Figure 1**). The filtering process included all coding variants with an MAF < 5% and excluded synonymous and in-frame insertions/deletions variants. Among the six variants (**Table 2**), two are missense variants in the RNA Binding Motif Protein 43 (*RBM43*) gene (p.V34L) and the dermatopontin (*DPT*) gene (p.Y149C). The latter is novel, not reported previously in any database, and predicted to be deleterious and damaging (**Table 2**), while the former is reported in the SNP database, and the *RBM43* gene was not shown to be expressed in the eye. On the contrary, previous reports do support a role for *DPT* in the eye and potentially in glaucoma, thus reinforcing its potential role in the underlying phenotype especially that it is not expressed in the mother. The remaining four variants do not have an effect on the coding sequences or splicing, thus excluding them from any role in the ocular phenotype.

Genetic Analysis (III): A Novel Missense Variant in NFATC1 Responsible for the Severe Cardiac Phenotype?

To delineate the cardiac phenotype observed exclusively in probands II.3 and II.6, a variant filtering approach was conducted using a two-arm strategy. The variants should have the following characteristics; first, they must have an MAF < 5%, exclusively shared by the two individuals and absent from the others, and second, they m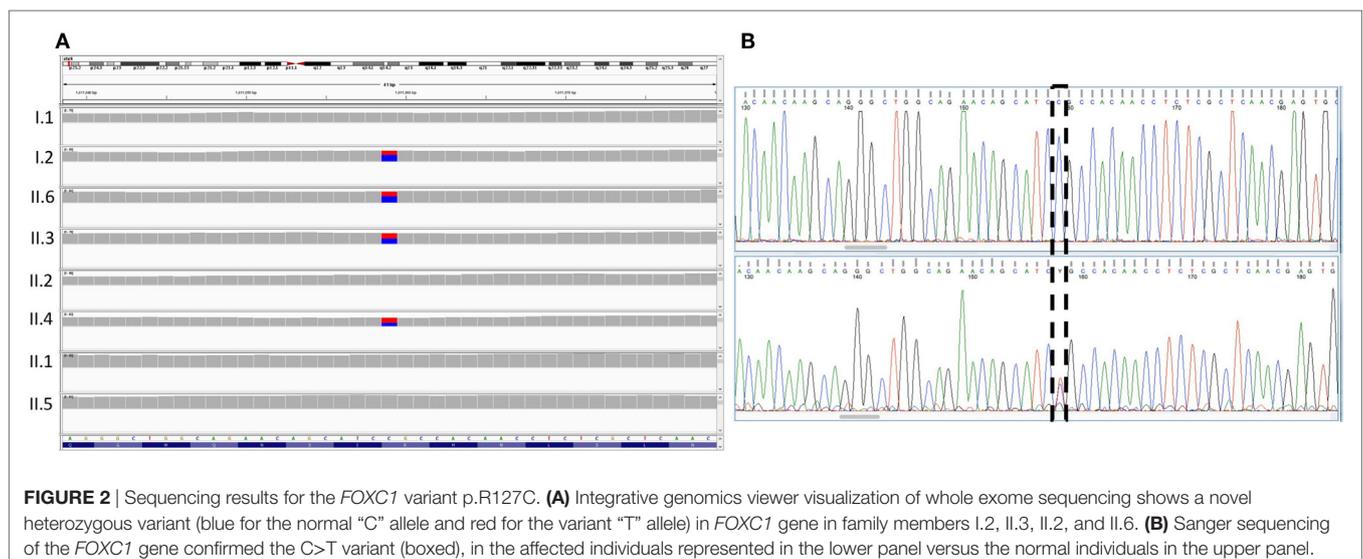ust be inherited from both parents (assuming a recessive model of inheritance) or from the father assuming a combinatorial effect with the *FOXC1* variant. In the former strategy, only eight variants were shared (**Table 3**), but none could explain the cardiac phenotype alone or in conjunction with *FOXC1* since there are no published data about their role neither in cardiac development or in CHD. In the latter, 18 shared variants were detected with no cardiac relevance for anyone of them; of note a nonsense variant in *ZNF28* inherited from the father was not encountered in any database (**Table 3**). We moved then to assess each individual with cardiac defect alone by using the same strategy above. In patient II.3, 13 variants in a total of 7 genes were inherited from the father, however, none were previously implicated in CHD, nor were they implicated in a *FOXC1* partnership (**Table 4**). In contrast, patient II.6 had 17 variants in 15 genes inherited from her father but not encountered in the other siblings. Two of these variants were in genes implicated in heart development and cardiac pathology, *NFATC1* and *OBSCN* (**Table 4**). The two variants chr18:77170979 G>A for *NFATC1* and chr1:228462101 G>A for *OBSCN* lead to novel missense variants, p.R222Q and p.C1880Y respectively. They were not reported previously and are predicted to be deleterious and damaging, potentially explaining the severe cardiac phenotype in the patient (**Table 4**).

Genetic Analysis (IV): A Family at Risk of Sudden Death?

A final thorough analysis of all predicted deleterious variants in the exome sequencing of patient II.6 showed a novel nonsense mutation in desmoglein (*DSG2*) (**Figure 3A**), a gene implicated in arrhythmogenic right ventricular dysplasia. The variant is inherited from the father and is shared between four siblings (**Figure 3B**), including all three affected children with either glaucoma and/or CHD, in addition to a healthy sibling (II.1).

DISCUSSION

Anterior segment dysgenesis englobes a wild spectrum of ocular defects that include among others the ARS, which is frequently

FIGURE 2 | Sequencing results for the *FOXC1* variant p.R127C. **(A)** Integrative genomics viewer visualization of whole exome sequencing shows a novel heterozygous variant (blue for the normal "C" allele and red for the variant "T" allele) in *FOXC1* gene in family members I.2, II.3, II.2, and II.6. **(B)** Sanger sequencing of the *FOXC1* gene confirmed the C>T variant (boxed), in the affected individuals represented in the lower panel versus the normal individuals in the upper panel.

TABLE 2 | Variants shared by affected patients inherited exclusively from the father.

Sample	Gene	Variant	Coordinate	Chr	Type	Filters	Quality	Inherited from	Allelic depths	Transcript	Consequence	Protein position	Amino acids	Sift	PolyPhen	dbSNP ID	Allele freq global minor	Allele freq EVS	EVS coverage	EVS samples
II.3	DPT	T>T/C	16870348	1	Snv	PASS	805.77	Father	34,28	NM_001937.4	missense_variant	149	Y/C	deleterious(0.04)	possibly_damaging(0.628)		0	0	172	6,503
II.4	DPT	T>T/C	16870348	1	Snv	PASS	997.77	Father	23,30	NM_001937.4	missense_variant	149	Y/C	deleterious(0.04)	possibly_damaging(0.628)		0	0	172	6,503
II.6	DPT	T>T/C	16870348	1	Snv	PASS	548.77	Father	28,21	NM_001937.4	missense_variant	149	Y/C	deleterious(0.04)	possibly_damaging(0.628)		0	0	172	6,503
II.3	INHBA	C>C/T	41729843	7	Snv	PASS	798.77	Father	28,27	NR_027118.1	upstream_gene_variant	0				rs138819536	0.09	0.26	51	6,503
II.4	INHBA	C>C/T	41729843	7	Snv	PASS	988.77	Father	29,33	NR_027118.1	upstream_gene_variant	0				rs138819536	0.09	0.26	51	6,503
II.6	INHBA	C>C/T	41729843	7	Snv	PASS	774.77	Father	21,27	NR_027118.1	upstream_gene_variant	0				rs138819536	0.09	0.26	51	6,503
II.3	POLR2J4, SPDYE1	G>G/A	44046965	7	Snv	PASS	1,115.77	Father	63,42	NR_003655.2	intron_variant, nc_transcript_variant	0				rs141407881	0.55	1.05	174	6,503
II.4	POLR2J4, SPDYE1	G>G/A	44046965	7	Snv	PASS	978.77	Father	54,37	NR_003655.2	intron_variant, nc_transcript_variant	0				rs141407881	0.55	1.05	174	6,503
II.6	POLR2J4, SPDYE1	G>G/A	44046965	7	Snv	PASS	966.77	Father	44,38	NR_003655.2	intron_variant, nc_transcript_variant	0				rs141407881	0.55	1.05	174	6,503
II.3	RBM43	C>C/G	152112161	2	Snv	PASS	907.77	Father	21,37	NM_198557.2	missense_variant	34	V/L	tolerated(0.8)	benign(0)	rs147060862	1.56	2.39	130	6,503
II.4	RBM43	C>C/G	152112161	2	Snv	PASS	680.77	Father	22,28	NM_198557.2	missense_variant	34	V/L	tolerated(0.8)	benign(0)	rs147060862	1.56	2.39	130	6,503
II.6	RBM43	C>C/G	152112161	2	Snv	PASS	278.77	Father	26,12	NM_198557.2	missense_variant	34	V/L	tolerated(0.8)	benign(0)	rs147060862	1.56	2.39	130	6,503
II.3	RTEL1, RTEL1-TNFRSF6B	G>G/A	62324290	20	Snv	PASS	1,215.77	Father	46,47	NM_003823.3	upstream_gene_variant	0				rs61736615	1.69	2.81	118	6,492
II.4	RTEL1, RTEL1-TNFRSF6B	G>G/A	62324290	20	Snv	PASS	941.77	Father	44,35	NM_003823.3	upstream_gene_variant	0				rs61736615	1.69	2.81	118	6,492
II.6	RTEL1, RTEL1-TNFRSF6B	G>G/A	62324290	20	Snv	PASS	1,239.77	Father	34,47	NM_003823.3	upstream_gene_variant	0				rs61736615	1.69	2.81	118	6,492
II.3	TIPRL	C>C/T	1.68E+08	1	Snv	PASS	1,016.77	Father	36,32	NM_152902.3	splice_region_variant, intron_variant	0					0	0.01	107	6,503
II.4	TIPRL	C>C/T	1.68E+08	1	Snv	PASS	573.77	Father	41,20	NM_152902.3	splice_region_variant, intron_variant	0					0	0.01	107	6,503
II.6	TIPRL	C>C/T	1.68E+08	1	Snv	PASS	893.77	Father	30,30	NM_152902.3	splice_region_variant, intron_variant	0					0	0.01	107	6,503

EVS, Exome databases.

TABLE 3 | Variants shared by patients with both glaucoma and congenital heart disease inherited either from the father alone or from both parents.

Sample	Gene	Variant	Coordinate	Chr	Type	Filters	Quality	Inherited from	Allelic depths	Transcript	Consequence	Protein position	Amino acids	Sift	PolyPhen	dbSNP ID	Allele freq global minor	Allele freq EVS	EVS coverage	EVS samples
II.3	CCDC155	C>C/G	49910139	19	Snv	PASS	816.77	Father	49,31	NM_144688.4	splice_region_variant, intron_variant	0				rs112074780	1.1	1.1	47	6,098
II.6	CCDC155	C>C/G	49910139	19	Snv	PASS	1,090.77	Father	54,39	NM_144688.4	splice_region_variant, intron_variant	0				rs112074780	1.1	1.1	47	6,098
II.3	CCDC9	T>T/C	47768055	19	Snv	PASS	529.77	Father	41,22	NM_015603.2	missense_variant	191	V/A	deleterious(0.01)	unknown(0)		0	0	19	6,496
II.6	CCDC9	T>T/C	47768055	19	Snv	PASS	630.77	Father	40,28	NM_015603.2	missense_variant	191	V/A	deleterious(0.01)	unknown(0)		0	0	19	6,496
II.3	DKKL1	G>G/A	49878275	19	Snv	PASS	1,317.77	Father	31,45	NM_014419.3	missense_variant	240	R/Q	tolerated(1)	benign(0.002)	rs35389403	0.73	0.54	25	6,444
II.6	DKKL1	G>G/A	49878275	19	Snv	PASS	882.77	Father	40,32	NM_014419.3	missense_variant	240	R/Q	tolerated(1)	benign(0.002)	rs35389403	0.73	0.54	25	6,444
II.3	KLK11	C>C/T	51527970	19	Snv	PASS	781.77	Father	51,34	NM_144947.1	missense_variant	73	E/K	tolerated(0.31)	benign(0.178)	rs117268623	1.74	1.94	26	6,503
II.6	KLK11	C>C/T	51527970	19	Snv	PASS	772.77	Father	34,31	NM_144947.1	missense_variant	73	E/K	tolerated(0.31)	benign(0.178)	rs117268623	1.74	1.94	26	6,503
II.3	LAMC2	G>G/A	183177132	1	Snv	PASS	808.77	Father	25,28	NM_005562.2	missense_variant	66	E/K	deleterious(0)	probably_damaging(0.995)	rs146325169	0	0.12	275	6,503
II.6	LAMC2	G>G/A	183177132	1	Snv	PASS	627.77	Father	21,27	NM_005562.2	missense_variant	66	E/K	deleterious(0)	probably_damaging(0.995)	rs146325169	0	0.12	275	6,503
II.3	OR8U1	C>C/A	56143976	11	Snv	PASS	61.77	Father	25,4	NM_001005204.1	missense_variant	293	Q/K	deleterious(0.03)	benign(0.003)		0	0	123	6,047
II.6	OR8U1	C>C/A	56143976	11	Snv	PASS	43.77	Father	17,3	NM_001005204.1	missense_variant	293	Q/K	deleterious(0.03)	benign(0.003)		0	0	123	6,047
II.3	PDILT	A>A/C	20371972	16	Snv	PASS	511.77	Father	14,18	NM_174924.1	missense_variant	475	L/R	tolerated(0.31)	benign(0.002)	rs4500734	1.37	2.41	138	6,503
II.6	PDILT	A>A/C	20371972	16	Snv	PASS	283.77	Father	17,12	NM_174924.1	missense_variant	475	L/R	tolerated(0.31)	benign(0.002)	rs4500734	1.37	2.41	138	6,503
II.3	PIH1D1	G>G/A	49949912	19	Snv	PASS	354.77	Father	15,14	NM_017916.2	missense_variant	243	R/C	tolerated(0.07)	possibly_damaging(0.866)	rs149419497	0.41	0.34	61	6,503
II.6	PIH1D1	G>G/A	49949912	19	Snv	PASS	304.77	Father	19,14	NM_017916.2	missense_variant	243	R/C	tolerated(0.07)	possibly_damaging(0.866)	rs149419497	0.41	0.34	61	6,503
II.3	PRX	G>G/A	40900763	19	Snv	PASS	1,056.77	Father	37,36	NM_181882.2	missense_variant	1166	P/S	tolerated(0.55)	benign(0.002)	rs147826200	0	0.07	72	6,503
II.6	PRX	G>G/A	40900763	19	Snv	PASS	1,039.77	Father	30,39	NM_181882.2	missense_variant	1166	P/S	tolerated(0.55)	benign(0.002)	rs147826200	0	0.07	72	6,503
II.3	RYR1	G>G/A	38997024	19	Snv	PASS	717.77	Father	26,25	NM_000540.2	splice_region_variant, intron_variant	0				rs200023171	0.05	0.03	50	6,503
II.6	RYR1	G>G/A	38997024	19	Snv	PASS	529.77	Father	20,18	NM_000540.2	splice_region_variant, intron_variant	0				rs200023171	0.05	0.03	50	6,503
II.3	SHCBP1L	A>A/G	182909488	1	Snv	PASS	928.77	Father	15,30	NM_030933.2	missense_variant	249	I/T	tolerated(0.74)	probably_damaging(0.996)	rs116513797	1.28	0.43	114	6,503
II.6	SHCBP1L	A>A/G	182909488	1	Snv	PASS	798.77	Father	15,26	NM_030933.2	missense_variant	249	I/T	tolerated(0.74)	probably_damaging(0.996)	rs116513797	1.28	0.43	114	6,503
II.3	SIX5	C>C/A	46269196	19	Snv	PASS	1,634.77	Father	57,59	NM_175875.4	missense_variant	595	V/L	tolerated(0.16)	possibly_damaging(0.658)	rs114060947	2.24	0	14	6,478
II.6	SIX5	C>C/A	46269196	19	Snv	PASS	897.77	Father	62,35	NM_175875.4	missense_variant	595	V/L	tolerated(0.16)	possibly_damaging(0.658)	rs114060947	2.24	0	14	6,478
II.3	STK10	G>G/A	171481667	5	Snv	PASS	585.77	Father	33,25	NM_005990.3	missense_variant	853	S/L	tolerated(0.07)	benign(0.146)	rs56066852	0.55	0.99	132	6,503
II.6	STK10	G>G/A	171481667	5	Snv	PASS	419.77	Father	32,19	NM_005990.3	missense_variant	853	S/L	tolerated(0.07)	benign(0.146)	rs56066852	0.55	0.99	132	6,503
II.3	ZNF229	G>G/A	44934187	19	Snv	PASS	974.77	Father	53,39	NM_014518.2	missense_variant	257	R/C	deleterious(0.01)	benign(0.059)	rs144097942	0.55	1.24	106	6,015
II.6	ZNF229	G>G/A	44934187	19	Snv	PASS	534.77	Father	41,23	NM_014518.2	missense_variant	257	R/C	deleterious(0.01)	benign(0.059)	rs144097942	0.55	1.24	106	6,015

(*Continued*)

TABLE 3 | Continued

Sample	Gene	Variant	Coordinate	Chr	Type	Filters	Quality	Inherited from	Allelic depths	Transcript	Consequence	Protein position	Amino acids	Sift	PolyPhen	dbSNP ID	Allele freq global minor	Allele freq EVS	EVS coverage	EVS samples
II.3	ZNF28	G>G/A	53311347	19	Snv	PASS	896.77	Father	34,33	NM_006969.3	stop_gained	19	Q/*				0	0	0	0
II.6	ZNF28	G>G/A	53311347	19	Snv	PASS	410.77	Father	22,18	NM_006969.3	stop_gained	19	Q/*				0	0	0	0
II.3	ZNF43	T>T/TC	21992330	19	Insertion	PASS	125.77	Father	11,6	NM_001256653.1	frameshift_variant, feature_elongation	179					0	0	0	0
II.6	ZNF43	T>T/TC	21992330	19	Insertion	PASS	185.77	Father	9,7	NM_001256653.1	frameshift_variant, feature_elongation	179					0	0	0	0
II.3	ZNF616	T>T/C	52618555	19	Snv	PASS	581.77	Father	26,20	NM_178523.3	missense_variant	621	N/S	tolerated(0.3)	benign(0.007)	rs116130534	0.05	0	69	6,503
II.6	ZNF616	T>T/C	52618555	19	Snv	PASS	775.77	Father	18,24	NM_178523.3	missense_variant	621	N/S	tolerated(0.3)	benign(0.007)	rs116130534	0.05	0	69	6,503
II.3	ZNF765	T>T/G	53912045	19	Snv	PASS	1,518.77	Father	103,59	NM_001040185.1	missense_variant	413	C/G	deleterious(0)	probably_ damaging(0.997)		0	0.02	96	6,503
II.6	ZNF765	T>T/G	53912045	19	Snv	PASS	903.77	Father	47,36	NM_001040185.1	missense_variant	413	C/G	deleterious(0)	probably_ damaging(0.997)		0	0.02	96	6,503
II.3	CNTLN	A>C/C	17462985	9	Snv	PASS	1,683.77	Both	0,52	NM_017738.2	missense_variant	1126	E/D	tolerated(0.12)	benign(0.016)	rs142750793	0.37	0.52	84	5,895
II.6	CNTLN	A>C/C	17462985	9	Snv	PASS	981.77	Both	0,32	NM_017738.2	missense_variant	1126	E/D	tolerated(0.12)	benign(0.016)	rs142750793	0.37	0.52	84	5,895
II.3	DPP4	G>C/C	162903930	2	Snv	PASS	1,135.77	Both	0,36	NM_001935.3	missense_variant	59	S/C	deleterious(0.04)	benign(0.249)		0	0	79	6,503
II.6	DPP4	G>C/C	162903930	2	Snv	PASS	880.77	Both	1,26	NM_001935.3	missense_variant	59	S/C	deleterious(0.04)	benign(0.249)		0	0	79	6,503
II.3	KIR2DL1	A>G/G	55285072	19	Snv	PASS	1,063.77	Both	0,33	NM_014218.2	missense_variant	120	I/V	deleterious(0.04)	benign(0.061)	rs138345877	1.42	2.86	197	6,383
II.6	KIR2DL1	A>G/G	55285072	19	Snv	PASS	840.77	Both	0,23	NM_014218.2	missense_variant	120	I/V	deleterious(0.04)	benign(0.061)	rs138345877	1.42	2.86	197	6,383
II.3	LILRB5	G>A/A	54756415	19	Snv	PASS	890.77	Both	0,27	NM_001081442.1	splice_region_variant, intron_variant	0				rs149294774	0.09	0.13	75	6,503
II.6	LILRB5	G>A/A	54756415	19	Snv	PASS	650.77	Both	0,19	NM_001081442.1	splice_region_variant, intron_variant	0				rs149294774	0.09	0.13	75	6,503
II.3	NLRP2	G>A/A	55501424	19	Snv	PASS	1,580.77	Both	2,52	NM_001174081.1	missense_variant	801	A/T	tolerated(0.58)	benign(0.078)	rs117066658	0.78	1.06	113	6,503
II.6	NLRP2	G>A/A	55501424	19	Snv	PASS	1,047.77	Both	0,33	NM_001174081.1	missense_variant	801	A/T	tolerated(0.58)	benign(0.078)	rs117066658	0.78	1.06	113	6,503
II.3	SULT1C2	T>C/C	108921036	2	Snv	PASS	2,210.77	Both	0,65	NM_176825.2	missense_variant	139	Y/H	deleterious(0)	probably_ damaging(1)	rs17036091	0.09	0.26	174	6,503
II.6	SULT1C2	T>C/C	108921036	2	Snv	PASS	1,496.77	Both	0,45	NM_176825.2	missense_variant	139	Y/H	deleterious(0)	probably_ damaging(1)	rs17036091	0.09	0.26	174	6,503
II.3	TCP10L2	T>C/C	167592524	6	Snv	PASS	1,270.77	Both	0,37	NM_001145121.1	missense_variant	228	L/P	tolerated(0.13)	benign(0)	rs2989545	0	0	24	2,278
II.6	TCP10L2	T>C/C	167592524	6	Snv	PASS	1,366.77	Both	0,38	NM_001145121.1	missense_variant	228	L/P	tolerated(0.13)	benign(0)	rs2989545	0	0	24	2,278
II.3	UBAP2	A>G/G	33944445	9	Snv	PASS	3,558.77	Both	1,110	NM_018449.2	missense_variant	488	I/T	tolerated(0.28)	benign(0.015)	rs201283769	0	0.02	116	6,503
II.6	UBAP2	A>G/G	33944445	9	Snv	PASS	2,596.77	Both	0,75	NM_018449.2	missense_variant	488	I/T	tolerated(0.28)	benign(0.015)	rs201283769	0	0.02	116	6,503

EVS, Exome databases.

TABLE 4 | Variants inherited from the father alone and not present in other siblings.

Sample	Gene	Variant	Coordinate	Chr	Type	Filters	Quality	Inherited from	Allelic depths	Transcript	Consequence	Protein position	Amino Acids	Sift	PolyPhen	dbSNP ID	Allele Freq Global Minor	Allele Freq EVS	EVS Coverage	EVS Samples
II.3	GXYLT1	C>C/A	42538340	12	Snv	PASS	130.77	Father	3,3	NM_173601.1	missense_variant	37	G/C	tolerated(0.1)	benign(0.186)		0	0	3	4104
II.3	GXYLT1	T>T/C	42538349	12	Snv	PASS	46.77	Father	3,2	NM_173601.1	missense_variant	34	T/A	tolerated(0.78)	benign(0)		0	0	4	4818
II.3	GXYLT1	C>C/A	42538352	12	Snv	PASS	46.77	Father	3,2	NM_173601.1	stop_gained	33	G/*				0	0	4	4931
II.3	GXYLT1	A>A/T	42538366	12	Snv	PASS	46.77	Father	3,2	NM_173601.1	missense_variant	28	V/E	tolerated(0.27)	benign(0.045)		0	0	5	5228
II.3	GXYLT1	C>C/T	42538367	12	Snv	PASS	46.77	Father	3,2	NM_173601.1	missense_variant	28	V/M	tolerated(0.22)	benign(0.161)		0	0	5	5232
II.3	MTFMT	CA>CA/C	65312614	15	Deletion	PASS	35.77	Father	2,3	NM_139242.3	splice_region_variant, intron_variant, feature_truncation	0					0	0	17	5906
II.3	MUC16	A>A/AG	9012894	19	Insertion	PASS	913.77	Father	95,30	NM_024690.2	frameshift_variant, feature_elongation	12,850					0	0	0	0
II.3	MUC16	AG>AG/A	9012897	19	Deletion	PASS	931.77	Father	96,28	NM_024690.2	frameshift_variant, splice_region_variant, feature_truncation	12,849					0	0	211	6170
II.3	PAXBP1	C>C/T	34133365	21	Snv	PASS	322.77	Father	12,13	NM_016631.3	splice_region_variant, intron_variant	0				rs111951332	1.01	1.71	179	6503
II.3	SDHAP1	A>A/G	195690163	3	Snv	PASS	659.77	Father	82,28	NR_003264.2	splice_region_variant, intron_variant, nc_transcript_variant	0				rs201372496	0	0	0	0
II.3	TUBB8	T>T/C	94018	10	Snv	PASS	213.77	Father	86,14	NM_177987.2	missense_variant	105	H/R	deleterious(0.02)	possibly_damaging(0.549)	rs9329307	0	0	68	6503
II.3	URB1	A>A/G	33726265	21	Snv	PASS	1,058.77	Father	67,45	NM_014825.2	missense_variant	798	L/P	tolerated(0.13)	benign(0)	rs189036928	0.92	1.14	116	2283
II.3	URB1	C>C/T	33738971	21	Snv	PASS	704.77	Father	31,28	NM_014825.2	missense_variant	431	V/M	tolerated(0.11)	benign(0.048)	rs117577554	1.14	1.16	92	2283
II.6	AP3B1	T>T/TA	77524068	5	Insertion	PASS	225.77	Father	9,11	NM_003664.3	splice_region_variant, intron_variant, feature_elongation	0				rs35569618, rs5868908	0	0	0	0
II.6	ATP6V1B2	G>G/A	20054928	8	Snv	PASS	438.77	Father	11,20	NM_001693.3	missense_variant	4	R/Q	tolerated(0.49)	benign(0.001)	rs116941637	0.6	0.56	17	6469
II.6	BDP1	A>A/T	70798553	5	Snv	PASS	141.77	Father	8,6	NM_018429.2	missense_variant	726	I/L	tolerated(0.51)	benign(0.002)	rs34588160	0.5	0.48	89	5959
II.6	CAST	C>C/G	96078410	5	Snv	PASS	427.77	Father	32,18	NM_001042440.2	missense_variant	343	R/G	tolerated(0.32)	possibly_damaging(0.66)		0	0	60	6503
II.6	DMGDH	T>T/C	78338202	5	Snv	PASS	882.77	Father	21,35	NM_013391.2	missense_variant	366	N/S	tolerated(0.56)	benign(0.004)	rs77116243	0.92	0.92	120	6503
II.6	FAM182A	C>C/T	26062032	20	Snv	PASS	96.77	Father	24,6	NR_026713.1	splice_region_variant, intron_variant, nc_transcript_variant	0				rs76871018	0	0	7	2190
II.6	gcnt4	G>G/C	74325772	5	Snv	PASS	488.77	Father	21,21	NM_016591.2	missense_variant	31	L/V	tolerated(0.34)	probably_damaging(0.946)		0	0	118	6499
II.6	HOMER1	T>T/A	78692723	5	Snv	PASS	494.77	Father	22,21	NM_004272.3	missense_variant	267	I/L	tolerated(0.84)	benign(0)		0	0	100	5906
II.6	ITPKB	G>G/A	226923779	1	Snv	PASS	899.77	Father	56,40	NM_002221.3	missense_variant	461	P/S	deleterious(0.03)	benign(0.065)	rs35823273	0.18	0.38	35	6501
II.6	NBPF1	T>T/C	16909129	1	Snv	PASS	46.78	Father	1,2	NM_017940.3	missense_variant	406	K/E			rs199798572	0	0	0	0
II.6	NFATC1	G>G/A	77170979	18	Snv	PASS	7,785.77	Father	75,257	NM_172387.1	missense_variant	222	R/Q	deleterious(0.01)	probably_damaging(0.953)		0	0	20	6479
II.6	OBSCN	G>G/A	228462101	1	Snv	PASS	3,090.77	Father	116,114	NM_001098623.1	missense_variant	1,880	C/Y	deleterious(0.02)	probably_damaging(1)		0	0	45	6449
II.6	PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8	G>G/C	140222138	5	Snv	PASS	1,215.77	Father	84,37	NM_018910.2	intron_variant	0				rs144906391	0	0	119	6492

(Continued)

TABLE 4 | Continued

Sample	Gene	Variant	Coordinate	Chr	Type	Filters	Quality	Inherited from	Allelic depths	Transcript	Consequence	dbSNP ID	Protein position	Amino Acids	Sift	PolyPhen	Allele Freq Global Minor	Allele Freq EVS	EVS Coverage	EVS Samples
II.6	PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8	C>C/CAACTGATCTGTATATATTGTATAGTTTAATA	140222139	5	Insertion	PASS	1,216.77	Father	83,35	NM_018910.2	intron_variant, feature_elongation		0				0	0	0	0
II.6	PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8	C>C/CTT	140222141	5	Insertion	PASS	1,166.77	Father	83,6	NM_018910.2	intron_variant, feature_elongation		0				0	0	0	0
II.6	PTCD2	C>C/G	71618013	5	Snv	PASS	630.77	Father	29,22	NM_024754.3	missense_variant		48	L/V	deleterious(0.01)	probably_damaging(0.998)	0	0	117	5865
II.6	USH2A	C>C/T	215901623	1	Snv	PASS	445.77	Father	25,17	NM_206933.2	missense_variant	rs146264950	3,939	E/K	tolerated(0.24)	possibly_damaging(0.588)	0.09	0.04	102	6503

EVS, Exome databases.

linked to severe functional alterations of either *FOXC1* and/or *PITX2* (28–30). The mutations associated with the ARS phenotype can range from frameshift mutations resulting in premature termination of translation in the forkhead domain or homeodomain, missense mutations reducing transactivation and protein interactions, and nonsense mutations causing haploinsufficiency of the gene product. Most of these mutations will hamper the stability of either one of these transcription factors protein complex over the DNA, and thus have a deleterious effect on transcriptional regulation of target genes (26, 31). Most of the mutations in *FOXC1* appear to be linked to isolated ocular defects or ocular, combined with cardiac, skeletal, and auditory defects. In this study, two of the children who suffered from glaucoma also suffered from CHD. Their mother however, had the same *FOXC1* variant, but did not present with glaucoma or CHD, though she could have defects pertaining to anterior segment dysgenesis. This clinical heterogeneity suggested an important role for modifier factors (genetic, environmental, and/or stochastic) on the phenotypic outcomes. We are thus proposing a digenic model to account for some of the phenotypes in this particular family as deduced from a thorough analysis using WES.

Ocular Phenotype: Does FOXC1 Need DPT for Glaucoma?

Our results do point out to the determinant role of the p.R127C variant in the ocular phenotype manifested in the three affected children, and in particular to glaucoma which was manifested at very early ages. Only those individuals with this variant do have glaucoma with the exception of the mother, who is a carrier but is glaucoma-free. In search for modifier genes using WES, we could not detect any variation in genes pertaining to the anterior segment dysgenesis phenotype in general and to glaucoma in particular (**Figure 1** and **Table 1**). In parallel, our analysis of the missense variant shows that it is novel and never reported before in the literature. However, the arginine amino acid at position 127 was linked to prior cases of ARS. In particular, a p.R127H variant was associated with an ARS case with the affected proband having severe ocular defects and glaucoma (32). As in our case, this variant was inherited from his mother who does not have glaucoma, but has typical posterior embryotoxon. More recently, a case of ARS was linked to a missense mutation at the same position leading: the p.R127L variant was found in a proband with characteristics of ARS including glaucoma and a cardiac structural defect due to a PDA (33). The patient inherited this variant from his father who was only diagnosed with glaucoma, while the patient's sister was not available for genetic testing since she passed away as a result of severe dilated cardiomyopathy. As in the published two cases, and in other cases whereby the severity of the ARS phenotype is more pronounced in the children versus their parents (32, 34), we hypothesize that a modifier variant inherited from the *FOXC1* "variant-free parent" would account for the severity of the phenotype in the children versus their parents. Our WES results identified a novel variant in the *DPT* gene inherited from the father and only present in the affected children with glaucoma but not in the healthy children. It could explain the

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Gene	Variant	Coordinate	Chr	Type	Genotype	Filters	Transcript	Consequence	Protein Position	Amino Acids	Allele Freq Global Minor	Allele Freq Evs	EVS Coverage	EVS Samples
DSG2	G>G/A	29099836	18	snv	het	PASS	NM_001943.3	stop_gained	51	W/*	0	0	72	5993

FIGURE 3 | Sequencing results for the *DSG2* variant p.W51*. **(A)** Location and description of the DSG2 variant using the Illumina Variant Studio show that the variant on chr18:290099836 G>A leads to a premature stop codon at position 51 of the transcript with an overall allele frequency of O in the Exome databases (EVS). **(B)** Integrative genomics viewer visualization of whole exome sequencing shows a novel heterozygous variant (brown for the normal "G" allele and green for the variant "A" allele) in *DSG2* in the father (I.1), the three affected children (II.3, II.4, and II.6) and in their healthy sister (II.1).

early onset of the anterior segment dysgenesis phenotype and particularly glaucoma in this case. The p.Y149C missense variant is only found in the three affected children with glaucoma: it is predicted to be deleterious and is neither found in healthy Lebanese controls, nor in the gnomAD database (www.gnomad.broadinstitute.org). Additionally, there were no previous reports on the role of *DPT* in glaucoma, and only a few publications describe its expression and role in ocular development and pathology. DPT is an extracellular matrix protein required for the organization of collagen in the skin, as depicted in the mouse knockout model (35). This latter did show also a defect in the corneal matrix organization, which coupled with the prominent expression of the protein in the optic nerve in zebrafish suggest a potential role in ocular development (36, 37). We do suggest a genetic/molecular interaction between *DPT* and *FOXC1*, which would be largely disrupted by the missense mutations detected in our case. A double-heterozygous mouse model carrying only one copy of each gene would potentially yield better insight into this proposed interaction.

Cardiac Phenotype: A FOXC1/NFATC1 Genetic Interaction

The cardiac phenotype in the indexed-family is divided into two: a mild VSD not requiring any intervention and a severe TOF-like phenotype that required major intervention (**Figure 1**). We sought that differential variants inherited from the father would contribute to this differential expressivity of the ARS phenotype within the three affected children in this family: two

with a cardiac phenotype and the third with only glaucoma. Interestingly, we unravel two novel missense mutations in *OBSCN* (p.C1880Y) and *NFATC1* (p.R222Q) that are predicted to be damaging (**Table 4**). Both variants are neither present in the Lebanese controls nor in the genome and exome databases. Given that *OBCSN* mutations have not been linked to CHD (38, 39), but could be potentially causing cardiomyopathies, we hypothesize that the *NFATC1* missense variant along the *FOXC1* variant is responsible for the cardiac phenotype. Our rationale is based on previous findings from our group that only a compound mutation in *NFATC1* could be linked to a cardiac phenotype (40), which is also mirrored in the knockout mouse model for *Nfatc1* that shows that the absence of both *Nfatc1* alleles is required to have a severe cardiac phenotype while the heterozygous mice are healthy (41, 42). Both *FOXC1* and *NFATC1* are expressed in the secondary heart field, and thus could be implicated in common transcriptional pathways that shape up the cardiac valves, and septation of the outflow tract (43, 44). This notion of digenic and/or multigenic rationale to explain differential expressivity and penetrance associated with Mendelian-inherited disease is not novel and is being considered in different forms of glaucomas. It was shown that digenic variants in *CYP1B1* and *MYOC* contribute to PCG and that variants in both *FOXC1* and *PITX2* are responsible for some cases of ARS (10, 45). This prompted us to explore the frequency of CHD in patients with ARS carrying a *Foxc1* mutation and whether or not there is a need to carry on WES to investigate the role of other variants in conjunction with *FOXC1* that would explain these cardiac defects.

Whole Exome Sequencing

A tool to draw genotype–phenotype correlation out of the 67 *FOXC1* variants reported so far to be linked to the ARS, only nine have been shown to be linked to cardiac defects in addition to the ocular defects. A scrutinized review of the literature of these nine variants, namely p.Q70Hfs*8, p.P79T, p.S82T, p. A85P, p.L86F, p.F112S, p.R127L, p.G149D, and p.R170W, did show that the cardiac phenotype with which they are associated is not as clear as it is presumed. In some cases, the defect is not a structural one, while in most cases, it is found in only one affected child but not in the parent despite sharing the same variant (33, 46). This reinforces the notion that another variant from the healthy parent in a genetic and/or molecular pathway implicating *FOXC1* would be a potential hit to explain the cardiac-associated phenotype. In the case of p.Q70Hfs* 8 and p.P79T, only one affected patient out of two with the variant has an atrial septal defect (ASD) and PDA, respectively, while the parent carrying the mutated allele has a mild ARS phenotype and no cardiac defects (47, 48). The same applies to p.A85P and p.R127L whereby only one of the two affected individuals has either ASD and pulmonary and aortic stenosis or PDA, respectively, whereas the parent from whom the mutation is inherited does not have cardiac defects (33, 49, 50). In the p.S82T case, the initial description of the familial case did not include any structural cardiac defects (17), whereas the report by Mears et al. mentioned cardiac anomalies (14), and that by Du et al. mentioned ASD with neither description of the methods nor the number of affected individuals (33). The same confusion applies to the p.G149D missense mutation, whereby the reported mutation was linked to ASD in one patient from a family whose members were not included, and whereby the information on the cardiac defect was only listed in a table. There was no description of the phenotype in the text, nor in the methodology used to assess it (49). As for the p.F112S missense mutation, the two reports documenting this mutation point out to mitral valve regurgitation and/or congestive heart failure as phenotypes encountered at older ages in only two cases with this genotype whereas the rest did not show any cardiac anomaly (47, 51). The same applies to p.R170W whereby one patient out of five with the mutation has mitral valve regurgitation requiring its replacement, and another an ASD diagnosed in early adolescent (46). Finally, the p.L86F with only one patient having a myocardial infarct at 41 years of age (52). These facts combined with the phenotypes observed in the *Foxc1* initial knockout that did not show any cardiac defects should break down the claims that cardiac structural defects are often associated with the ARS phenotype (24). Indeed, the expression of Foxc1 is barely detected in the mouse developing heart, and only the LacZ harboring knockout construct leads to structural cardiac defects when both *Foxc1* alleles are deleted (47, 53). The deletion of only one allele of *Foxc1* has no effect on heart development in mice even in the context of a genetic model with both *Foxc2* alleles deleted (23, 44). Cardiac defects are thus seldom associated with the ARS phenotype, and we propose that other genes like *NFATC1* when mutated would lead to CHD in the presence of a mutated allele of *FOXC1*.

The limitations in interpreting the cardiac phenotypes in the anterior segment dysgenesis cases could be therefore solved by applying WES for the parents and siblings of any such indexed patient. In our case, the results even go beyond the anterior segment dysgenesis phenotype to highlight a potential life-threat to members of this family with the expression of a nonsense mutation in *DSG2* within its members that could lead to dilated cardiomyopathy and sudden cardiac death.

CONCLUSION

This is the first study on a familial case of anterior segment dysgenesis glaucoma in Lebanon, a country with still a high rate of consanguineous marriages. We unravel by WES a novel mutation in *FOXC1* behind the ocular basic phenotype, and we propose a digenic model for the glaucoma phenotype along a mutation in the *DPT* gene and another digenic model for CHD involving yet a novel mutation in *NFATC1*.

ETHICS STATEMENT

All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol—Bioch.GN.01—was approved by the Institution Review Board (IRB), at the American University of Beirut.

AUTHOR CONTRIBUTIONS

AK: did the experiments, analyzed the data, and wrote the first draft of the paper. CA-H, FB, MK, and MA: did the clinical diagnosis, analyzed the data, and participated in the writing up. HH and KS: did the recruitment and participated in the analysis of the data. GN: conceived the project, supervised the experiments, analyzed the data, participated the writing up, and obtained the funding (with MA).

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