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Exploring the molecular basis of microphthalmia and anophthalmia: Insights from an Egyptian cohort

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Purpose: The aim of this study was to gain insight into the molecular spectrum of anophthalmia and microphthalmia (A/M) in the Egyptian population.
Methods: We studied a cohort of 34 patients from 31 unrelated families affected by the A/M spectrum. All patients underwent a thorough clinical examination, ophthalmological assessment, and genetic testing including conventional karyotyping and exome sequencing (ES).
Results: Chromosomal anomalies were identified in six patients. ES was performed on the remaining cases, revealing potentially causative variants in 13 families. The implicated genes were *SOX2*, *OTX2*, *CHD7, HMX1, PRR12, ATOH7, ZBTB11, B3GALNT2, GCNT2*, *DPH1*, *GJA8, FRAS1* and *UBE3B*. Among the variants, six were classified as pathogenic, five as likely pathogenic, and two as variants of uncertain significance. Notably, a *DPH1* pathogenic variant was identified in a patient with bilateral severe microphthalmia, representing a novel phenotype. Additionally, we report the fifth family diagnosed with oculo-auricular syndrome.
Conclusions: Our findings confirm that genetic factors are a predominant cause of both syndromic and non-syndromic A/M and underscore the value of ES in uncovering the molecular basis of this spectrum. By reporting novel variants and unusual phenotypes within our cohort, we contribute to expanding both the mutational landscape and the phenotypic spectrum of A/M associated syndromes.

Anophthalmia and microphthalmia (A/M) are severe congenital eye defects that fall within a spectrum of ocular anomalies, which may also include coloboma. Several developmental and regulatory genes play critical roles in eye formation during embryogenesis, and disruptions in these genes can result in either the complete absence of the eye (anophthalmia) or the formation of a small, underdeveloped eye (microphthalmia) [1-3]. A/M can occur as an isolated anomaly (non-syndromic) or as part of a broader phenotype involving extraocular anomalies (syndromic). Syndromic A/M represents the majority of cases and tend to have a higher rate of identifiable genetic causes and diagnostic yield [4-6].

The genetic basis of A/M has been extensively studied, revealing a complex interplay of single-gene mutations and chromosomal abnormalities. Nearly 100 genes have been implicated in the pathogenesis of A/M [7]. Additionally, conventional cytogenetic analysis can detect significant chromosomal aberrations in up to 15% of affected individuals [5,8,9]. The considerable genetic heterogeneity of A/M, which is further complicated by variable expressivity, incomplete penetrance, and overlapping phenotypes, poses significant challenges for diagnosis and genetic counseling.

Understanding the specific genetic landscape of A/M in any given population is essential for improving diagnostic accuracy and guiding patient care. The paucity of genetic studies on A/M makes it impossible to accomplish these objectives, underscoring the necessity for more research. Thus, the present study aimed to investigate the molecular characteristics of A/M and evaluate the role of ES in diagnosing a cohort of Egyptian patients affected by this spectrum of ocular anomalies.

METHODS

Patient recruitment: Ethical approval was obtained from the Ethics Committees of both Medical Research Institute and the Faculty of Medicine, Alexandria University. The legal guardians of all participants provided written informed consent,

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aligned with the principles outlined in the Declaration of Helsinki. In this cross-sectional cohort study, 34 patients with A/M spectrum disorders were recruited between June 2021 and December 2023.

The inclusion criteria encompassed individuals of any age and gender who had been clinically diagnosed with A/M. Patients with a documented history of teratogenic exposure were excluded from the study.

After obtaining detailed medical and genetic history, a comprehensive clinical evaluation was performed. Whole blood samples were collected from the probands and their parents, and whenever possible from their healthy (unaffected) siblings.

Cytogenetic analysis: Karyotyping was performed for all the enrolled cases using the G-banding technique, following the method described previously [10]. Cases with an abnormal karyotype were excluded from further analysis.

Exome sequencing (ES): Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Exome capture was performed using the xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, IA) and sequencing was performed on the NovaSeq 6000 (Illumina, San Diego, CA). Sequencing reads were aligned to the Genome Reference Consortium Human Build 37 (GRCh37) and the Revised Cambridge Reference Sequence (rCRS) for the mitochondrial genome. This process generated a mean depth of coverage of 146.08x across the 34,366,188 bases of the captured region, covering approximately 99.3% of the RefSeq protein- coding regions. Additionally, 98.90% of the targeted bases were covered to a depth of ≥20×.

Sequencing data analysis and variant interpretation was performed using EVIDENCE v4.3 (Seo et al., 2020). Variant Effect Predictor (VEP, v104.2) was used for variant annotation. Variants were prioritized based on the guideline recommended by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology [11].

A variant was considered potentially damaging if it was predicted to alter the protein structure or function by in silico tools such as SIFT, Mutation Taster2, PolyPhen-2, and Align GV-GD, or if it resulted in stop-gain variants, frameshift insertions/deletions, or alterations at canonical splice sites. Additionally, variant rarity was assessed using public databases including 1000 Genome Project and gnomAD (v4.1.0), with a threshold allele frequency of ≤1%. Sanger sequencing test was used to validate candidate variants and confirm segregation on DNA from family members.

RESULTS

The study cohort comprised 34 patients with A/M, from 31 unrelated Egyptian families. Their ages ranged from 1 day to 18 years. Parental consanguinity was found in 13 families (41.9%) while a positive history of familial recurrence was documented in 7 families (22.6%).

Clinical findings: Regarding the ocular phenotype, 52.9% (18/34) of the patients had bilateral microphthalmia, 23.5% (8/34) had unilateral microphthalmia, 14.7% (5/34) presented with bilateral anophthalmia, and 8.8% (3/34) had mixed forms of A/M. In 21 patients (61.7%), the A/M phenotype was complex and was accompanied by additional ocular findings (Table 1). Within the cohort, 19 patients (55.9%) exhibited extraocular manifestations and were classified as having syndromic A/M (P22-P34 in Table 1, in addition to P1-P6 in Appendix 1), whereas the remaining 15 cases (44.1%) had isolated A/M (P7-P21 in Table 1).

Cytogenetic findings: Conventional karyotyping identified chromosomal abnormalities in six patients; four cases of trisomy 13 (Appendix 2), one with trisomy 18, and one with a reciprocal translocation t(3;11; q27;p11.2). The demographic, clinical and cytogenetic data of these patients are summarized in Appendix 1.

Molecular findings: After excluding the six patients with chromosomal abnormalities, ES was conducted on 28 patients from 25 unrelated families. In 13 families (13/25; 52%), potential candidate variants were identified, each in a distinct gene: *SOX2* (SRY-box transcription factor 2, Gene ID: 6657), *OTX2* (orthodenticle homeobox 2; GeneID 5015), *CHD7* (chromodomain helicase DNA binding protein 7; GeneID 55636), *HMX1* (H6 family homeobox 1; GeneID 3166), *PRR12* (proline rich 12; GeneID 57479), *ATOH7* (atonal BHLH transcription factor 7; GeneID 220202), *ZBTB11* (zinc finger and BTB domain containing 11; GeneID 27107), *B3GALNT2* (beta-1,3-N-acetylgalactosaminyltransferase 2; GeneID 148789), *GCNT2* (glucosaminyl (N-acetyl) transferase 2 (I blood group); GeneID 2651), *DPH1* (diphthamide biosynthesis 1; GeneID 1801)*, GJA8 (Gap Junction Protein Alpha 8) with human GeneID 2703), FRAS1* (Fraser extracellular matrix complex subunit 1; GeneID 80144) and *UBE3B* (ubiquitin protein ligase E3B; GeneID 89910; Table 1). Out of these variants, seven had not been previously reported and were absent from population databases. Based on ACMG guidelines, 11 variants were classified as pathogenic or likely pathogenic (P/LP), while two were considered variants of uncertain significance (VUS). In the remaining 12 families,

Table 1. Demographic, clinical, and molecular data of the A/M patients subjected to molecular studies (n=28).

	Family/ Patient ID	Age	Sex	Cons	F.H.	Ocular phenotype	Extra-ocular phenotype	Gene	Variant	Type	Zygosity	ACMG classification	gnomAD (v4.1.0)	Diag-nosis Inheri-tance
Non-syndromic (Isolated) A/M cases (n=15)	F7/P7	5y	M	-	-	Rt M – Lt A - Rt Peters anomaly	-	OTX2	NM_021728.4: c.278G>C p.Trp93Ser (Novel)	Missense	Het	LP (PM2-PM6-PP3-PM5- PP2)	Not found	Retinal dystrophy, early-onset, with or without pituitary dysfunction AD
	F8/P8	3m	M	-	-	Lt M - disc and chorioretinal coloboma	-	PRR12	NM_020719.3: c.3625C>T p.Arg1209* (Novel)	Nonsense	Het	P (PS4-PVS1-PM2-PP5)	Not found	Neuro-ocular syndrome AD
	F9/ P9&10	10y	F	+	+	BM - cataract – nystagmus - squint	-	GCNT2	NM_145649.5: c.1154G>A p.Arg385His	Missense	Hom	VUS (PM2-PP5-PS3-PP1)	1.61E-05	Cataract 13 with adult i phenotype AR
		8y	F	+	+	BM -cataract – nystagmus - squint								
	F10/ P11&12	5y	F	+	+	BM – cataract - corneal scarring -retinal detachment with subretinal hemorrhagic effusion	-	ATOH7	NM_145178: c.254C>T p.Ala85Val (Novel)	Missense	Hom	LP (PP3-PM2)	Not found	Persistent hyper-plastic primary vitreous (PHPV) AR
		3y	F	+	+									
	F11/P13	13y	F	-	+	BM – microcornea – cataract – glaucoma	-	GJA8	NM_005267.5: c.134G>C p.Trp45Ser	Missense	Het	P (PP1-PS2-PP3-PM2-PM5-PM1-PP2-PS3-PP5)	6.2E-07	Cataract 1, multiple types AD

	Family/ Patient ID	Age	Sex	Cons	F.H.	Ocular phenotype	Extra-ocular phenotype	Gene	Variant	Type	Zygosity	ACMG classification	gnomAD (v4.1.0)	Diag-nosis Inheri-tance
	F12/	7y	F	-	+	BM - Rt iris coloboma -Lt morning glory syndrome	-	Negative					-	Isolated complex A/M With unidentifiable molecular cause
	P14 &15	5y	M	-	+	Lt M - Lt iris coloboma - Rt morning glory syndrome	-						-	?
	F13/P16	1y	M	+	-	Rt M - Rt Peters anomaly	-	Negative					-	Isolated complex A/M With unidentifiable molecular cause ?
	F14/P17	3.5y	M	+	-	Rt M - Rt Peters anomaly - retroocular adhesions	-	Negative					-	Isolated complex A/M With unidentifiable molecular cause ?
	F15/P18	2.5y	F	+	-	BM – micro-cornea -cata-ract -squint	-	Negative					-	Isolated complex A/M With unidentifiable molecular cause ?
	F16/P19	3m	M	-	-	Rt M - persis-tent fetal vasculature	-	Negative					-	Isolated complex A/M With unidentifiable molecular cause ?
	F17/P20	15y	F	-	-	BA	-	Negative					-	Simple A/M ?
	F18/P21	1m	M	-	-	Lt M	-	Negative					-	Simple A/M ?
Syndromic A/M cases (n=19)	F19/P22	1d	F	-	-	BA	Esophageal atresia - Fallot tetralogy	SOX2	NM_003106.4: c.178G>C p.Ala60Pro	Missense	Het	VUS (PM2-PM1-PP3)	Not found	SOX2 Disorders AD

Family/ Patient ID	Age	Sex	Cons	F.H.	Ocular phenotype	Extra-ocular phenotype	Gene	Variant	Type	Zygosity	ACMG classification	gnomAD (v4.1.0)	Diag-nosis Inheri-tance
								(Novel)					
F20/P23	16y	F	-	-	Lt A - Rt M - iris coloboma - chorio-retinal coloboma	GR – CHD -	CHD7	NM_017780.4:	Splicing	Het	LP (PS2-PM2-PS3-PP5)	0.000 ¶	CHARGE syndrome
						primary amenorrhea - absent uterus and ovaries		c.5405-17G>A					AD
								p.?					
F21/P24	6y	F	+	-	Rt M – sclerocornea - adherent central corneal leukoma - Lt total corneal leukoma – aniridia - PHPV	Severe psycho-motor impair-ment – dysmorphic features - abnormal gait	HMX1	NM_018942.3:	Frame-shift indel	Hom	LP (PVS1-PM2)	Not found	Oculo-auricular syndrome
								c.570_571del					AR
								p. Glu191Argfs*34					
								(Novel)					
F22/P25	1m	F	+	-	BM	GR – GDD - sparse hair - hydro-cephalus	DPH1	NM_001383.6:	Missense	Hom	P (PM3-PM2-PP3-PS3-PP1-PP5)	0.000496	Developmental delay with short stature, dysmorphic facial features, and sparse hair (DEDSSH)
								c.359T>C					AR
								p.Leu120Pro					
F23/P26	7m	F	+	-	BM - cataract	CHD - plagio-cephaly	ZBTB11	NM_014415.4: c.1623+2T>G	Splicing	Hom	LP (PVS1-PM2)	6.224e-7 ¶¶	Intellectual developmental disorder, auto-somal recessive 69; MRT69
						- agenesis of corpus callosum		p.?					AR

Family/ Patient ID	Age	Sex	Cons	F.H.	Ocular phenotype	Extra-ocular phenotype	Gene	Variant	Type	Zygosity	ACMG classification	gnomAD (v4.1.0)	Diag-nosis Inheri-tance
								(Novel)					Muscular dystrophy with brain and eye anomalies, type A, 11 AR
F24/P27	7d	M	+	+	BM	Congenital hydro-cephalus -hypotonia	B3GALNT2	NM_152490.5: c.1338G>A p.Trp446* (Novel)	Nonsense	Hom	P (PVS1-PM3-PM2-PP5)	Not found	
F25/P28	6m	F	+	+	Lt A - Rt severe M	Dysmor-phic features - micro-cephaly - sparse eyebrows - contrac-tures - hypoplastic nails - inverted nipples – Lt side acces-sory nipple	UBE3B	NM_130466.4: c.1956+1G>A p.?	Splicing	Hom	P (PVS1-PM3-PM2-PP5)	1.86E-06	Kaufman oculo cerebro facial syndrome AR

Family/ Patient ID	Age	Sex	Cons	F.H.	Ocular phenotype	Extra-ocular phenotype	Gene	Variant	Type	Zygosity	ACMG classification	gnomAD (v4.1.0)	Diag-nosis Inheri-tance
F26/P29	6m	F	+	-	Lt M & cryp-tophthalmia - Rt upper lid coloboma - sclercornea	. Hypo-plastic nares - nares coloboma - bilateral stenotic auditory meatus - syndactyly - ambiguous genitalia	FRAS1	NM_025074.7: c.2376del p.Ser793Alafs*177	frame-shift indel	Hom	P (PVS1-PM3-PM2-PP5)	Not found ¶¶¶	Fraser syndrome 1 AR
F27/P30	16y	F	-	-	Lt M - Rt A	GR - facial asymmetry - short neck - Lt duplicated thumb – syndactyly -anteriorly placed anus - infantile uterus	Negative					-	CHARGE syndrome ?! ?

Family/ Patient ID	Age	Sex	Cons	F.H.	Ocular phenotype	Extra-ocular phenotype	Gene	Variant	Type	Zygosity	ACMG classification	gnomAD (v4.1.0)	Diag-nosis Inheri-tance
F28/P31	1y	M	+	-	Rt M - cataract	Right hemifacial hypoplasia - right microtia - pre-auricular skin tag - right maxil-lary and mandibular hypoplasia -right hypoplastic thumb	Negative					-	Oculo-auriculo-vertebral spectrum (OAVS) ?!
F29/P32	2m	F	-	-	BM - chorio-retinal degen-eration - optic nerve aplasia	Dysmor-phic facial features – hypotonia - agenesis of the corpus callosum	Negative					-	? Aicardi syndrome ?!
F30/P33	2.5y	M	-	-	BA	GDD – obesity -micropenis - unde-scended testicles	Negative					-	? Macro cephaly-obesity-mental disability-ocular ab normalities syndrome (MOMO) ?!

Family/ Patient ID	Age	Sex	Cons	F.H.	Ocular phenotype	Extra-ocular phenotype	Gene	Variant	Type	Zygosity	ACMG classification	gnomAD (v4.1.0)	Diag-nosis Inheri-tance
													?
F31/P34	7m	M	+	-	BM – nystagmus - Iris coloboma	Dysmor-phic features – hydro-cephalus -	Negative					-	????

F: family, P: patient, FH: family history, CL/P: cleft lip/palate, d: day, m: month, y: year, F: female, M: male, BM: bilateral microphthalmia, BA: Bilateral anophthalmia, CHD: congenital heart disease, MOI: mode of inheritance, Rt M: Right microphthalmia, Lt M: left microphthalmia Rt A: right anophthalmia, Lt A: left anophthalmia, PHPV: persistent hyperplastic primary vitreous, GR: growth retardation, GDD: global developmental delay, Het: heterozygous, Hom: homozygous, AR: Autosomal recessive, AD: Autosomal dominant, VUS: variant of uncertain significant. ¶ Reported by Legendre et al., 2018 [doi:10.1038/s41431-017-0007-0] ¶¶ Although this variant is observed at an extremely low frequency in the gnomAD v4.1.0 data set, it was absent from ClinVar and other databases analyzed and was not reported in the literature. ¶¶¶ The contrary applies to this variant which is 'NOT found' in gnomAD v4.1.0., although it is listed in ClinVar (Accession number: VCV001705727.3). The variant has been reported at least twice as pathogenic. It has been independently submitted to the database by two different clinical testing laboratories.

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no clinically significant variants could be identified (Figure 1). Notably, positive molecular findings were detected in eight out of 13 families with syndromic A/M (61.5%) and in 5 out of 12 with non-syndromic A/M (41.6%), highlighting a higher diagnostic yield in syndromic cases.

The pedigrees of the families with positive molecular findings with their associated genetic data are demonstrated in Figure 2.

Positive molecular findings:

A novel heterozygous variant in **OTX2** *(Microphthalmia, syndromic 5; OMIM* 610125*)*—A 5-year-old male patient (F7/P7), born to healthy, non-consanguineous parents, presented with left eye anophthalmia and right eye microphthalmia with Peters anomaly. Brain and orbit MRI showed left anophthalmia and optic nerve hypoplasia, while the overall brain structure and pituitary gland appeared normal. ES identified a heterozygous likely pathogenic missense variant in *OTX2*: c.278G>C, p.(Trp93Ser). This variant had not been previously

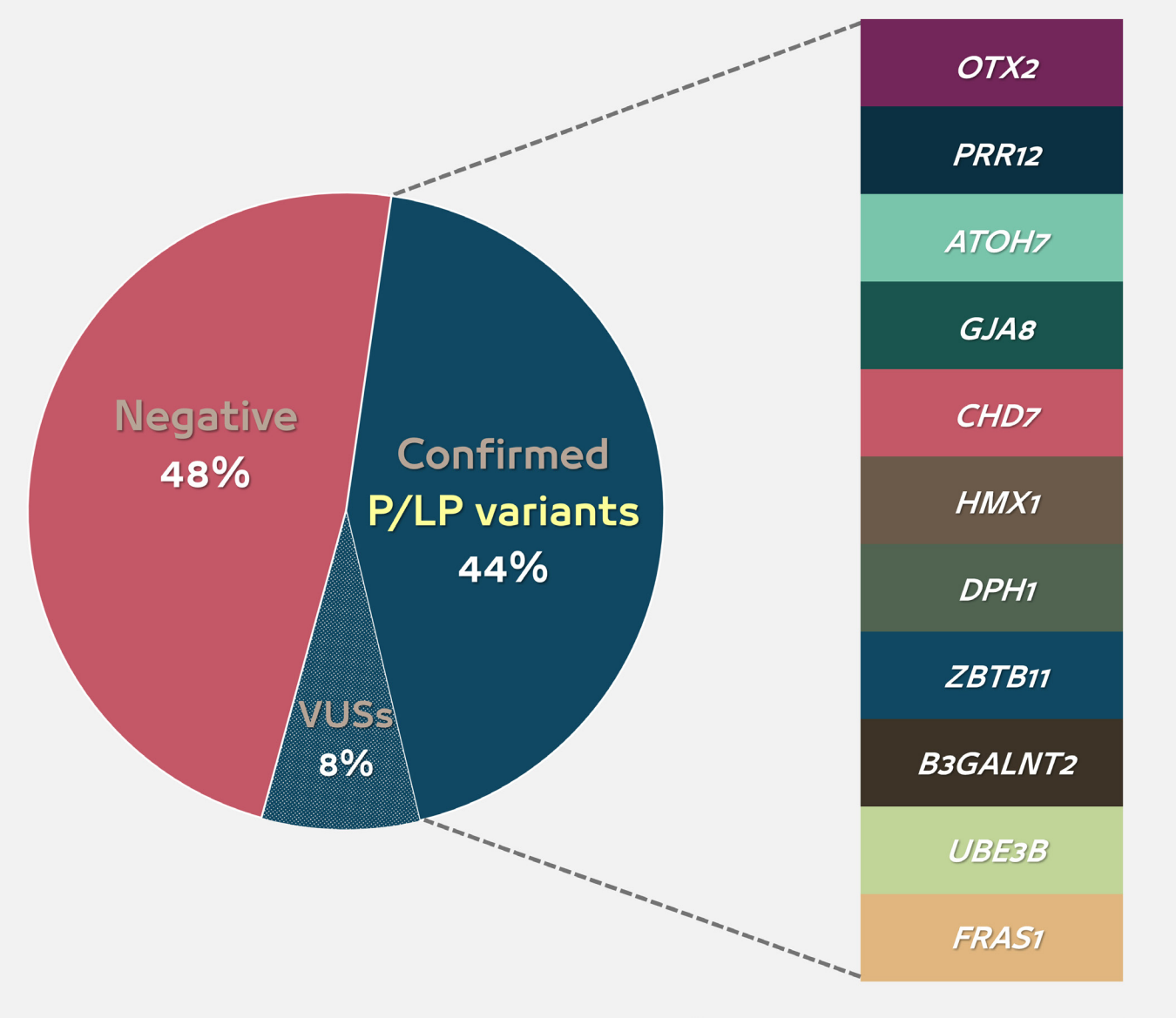

Figure 1. Molecular findings. A pie chart showing the distribution of the molecular findings in the current cohort of A/M, after the exclusion of 6 cases with chromosomal abnormalities. The spectrum of genes that we found to be involved in the etiopathogenesis of A/M (in the 11 solved families with L/LP disease-causing variants) is depicted on the right. P/LP: Pathogenic/Likely-pathogenic. VUSs: Variants of uncertain significance.

reported in the literature and was absent from the gnomAD database.

A novel heterozygous variant in **PRR12** *(Neuro-ocular syndrome; OMIM* 619539*)*—A heterozygous pathogenic nonsense variant in *PRR12*:c.3625C>T, p.(Arg1209Ter), was identified in a 3-month-old male infant (F8/P8) exhibiting unilateral microphthalmia with optic disc and chorioretinal coloboma. *PRR12* has been associated with an autosomal dominant (AD) neuro-ocular syndrome [12].

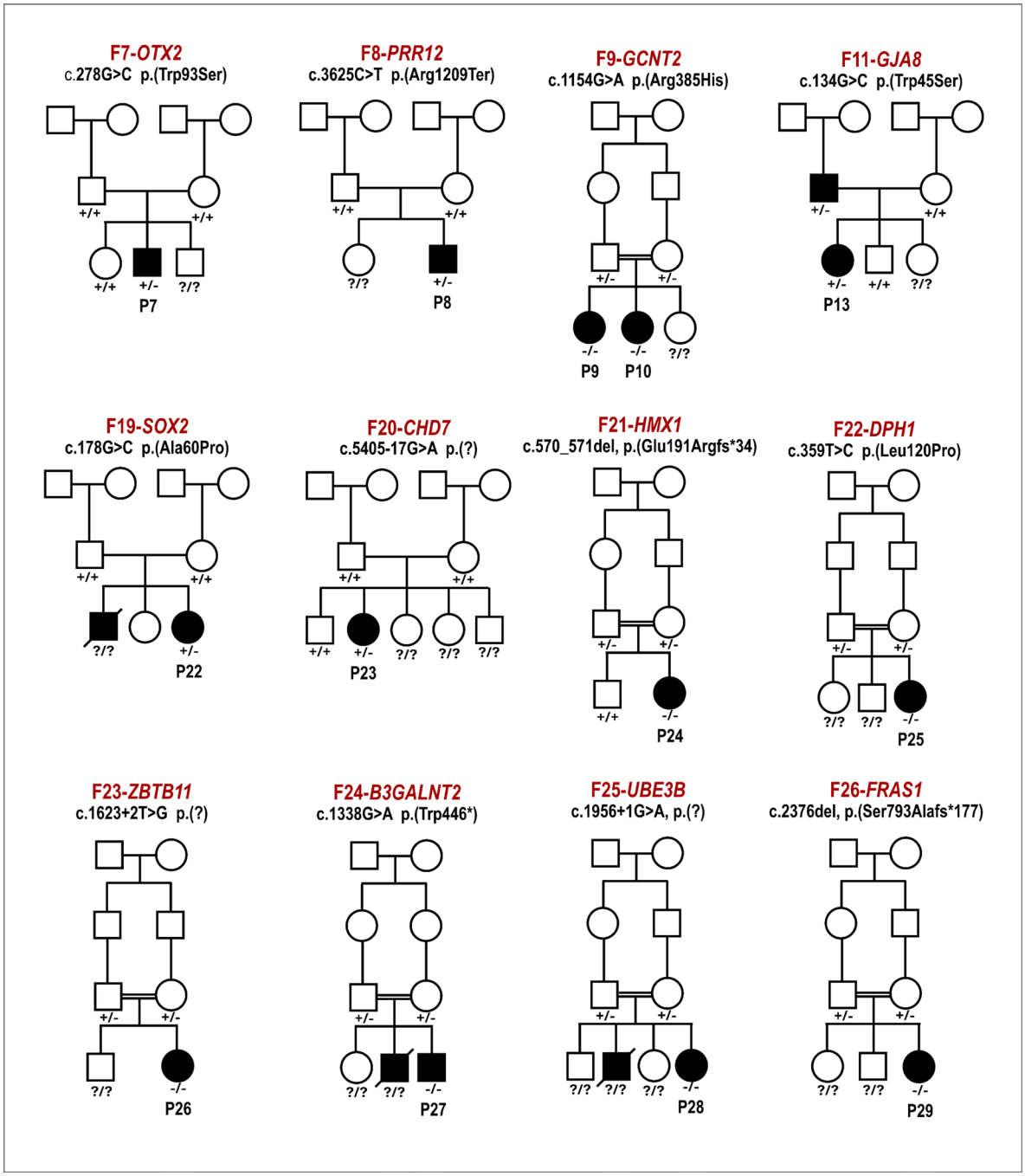

Figure 2. Pedigree figures of twelve families showing allele segregation. The squares and the circles represent males and females, respectively. The black-filled symbols indicate patients with A/M, and the diagonal line indicates a deceased family member. Above each pedigree the candidate variant is shown, while the genotype for the variant allele is marked below each of the participating individuals. "+/+" indicates homozygous wild allele, "+/–" indicates heterozygous variant, and "–/–" indicates homozygous mutant allele.

A homozygous VUS in **GCNT2** *(Cataract 13 with adult i phenotype; OMIM 116700)*—We identified a homozygous *GCNT2* variant: c.1154G>A, p.(Arg385His), in 10- and 8-year-old female siblings (F9/P9&10), presenting with bilateral congenital cataracts and microphthalmia. The younger sister also exhibited nystagmus and strabismus. The identified VUS, was previously described in two siblings with congenital cataracts and nystagmus [13].

A novel homozygous likely pathogenic variant in **ATOH7** *(PHPV, AR; OMIM 221900)*—A homozygous *ATOH7* missense variant (c.254C>T), was identified in 5- and 3-year-old similarly affected sisters (F10/P11&12; Figure 3), who were diagnosed with persistent fetal vasculature (PFV). Ophthalmic evaluation revealed bilateral inoperable PFV associated with total retinal detachment, subretinal hemorrhagic effusion, scattered corneal scarring and bilateral congenital cataract, which was not dense enough to obscure fundus examination. Orbital MRI demonstrated bilateral microphthalmia.

A heterozygous pathogenic variant in **GJA8** *(Cataract 1, multiple types; OMIM 116200)*—A heterozygous *GJA8* missense variant (c.134G>C) was identified in a 13-year-old girl (F11/P13) with bilateral congenital cataract, glaucoma, bilateral microcornea, and microphthalmia. A similar ocular phenotype was observed in her father, indicating an AD inheritance pattern.

A heterozygous VUS in **SOX2** *(Syndromic microphthalmia-3 (OMIM 206900)*—A heterozygous VUS in *SOX2*: c.178G>C, p.(Ala60Pro) was identified in a stillborn infant

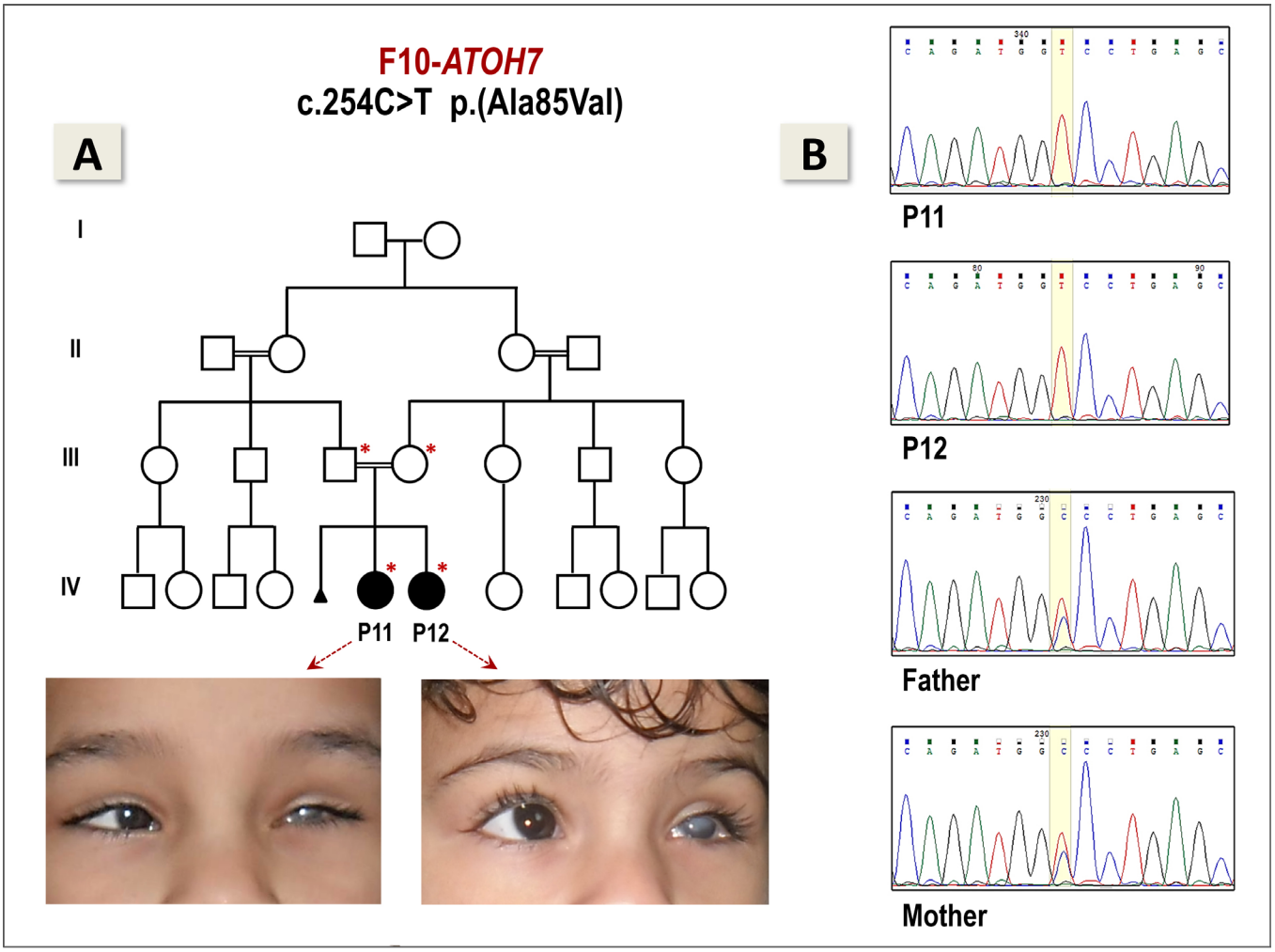

Figure 3. Identification of a novel homozygous likely pathogenic variant in *ATOH7* (c.254C>T). **A**: The family pedigree demonstrating two similarly affected sisters (F10/P11&12) born to consanguineous healthy parents. Below the pedigree clinical photos of the two patients showing microphthalmia, cataracts and corneal scarring. **B**: Partial sequence electropherograms showing the *ATOH7* missense variant (c.254C>T) in homozygous state in P11&12 and in heterozygous state in their healthy parents. The nucleotide change is shaded in yellow.

(F19/P22) displaying bilateral anophthalmia, esophageal atresia, tetralogy of Fallot and bilateral talipes equinovarus. The variant is a missense change with a high pathogenicity prediction score and was absent from the gnomAD database. The above-mentioned congenital malformations were revealed during a routine prenatal US examination and verified by fetal MRI.

A heterozygous likely pathogenic variant in **CHD7** *(CHARGE syndrome; OMIM* 214800*)*—In a 16-year-old female (F20/P23) presenting with left anophthalmia, right eye microcornea, inferior iris coloboma, inferior chorioretinal coloboma and high myopia, ES identified a heterozygous *CHD7* splicing variant in: c.5405–17G>A, p.(?). Clinical examination revealed dolichocephaly, broad forehead with receding anterior hairline, facial asymmetry, left anophthalmia, right eye coloboma with nystagmus, a bulbous nose with flattened tip, columella extending below alae nasi, wide mouth with thin upper lip and low-set malformed ears. Furthermore, abdominal and pelvic ultrasound revealed agenesis of the uterus and both ovaries.

A Novel **HMX1** *variant in the fifth family of the Schorderet-Munier-Franceschetti 'Oculoauricular syndrome' (OMIM* 612109*)*—Molecular testing revealed a novel homozygous *HMX1* frameshift variant: c.570_571del, p.(Glu191Argfs*34) in a 6-year-old female (F21/P24) presenting with bilateral microcornea and dense bilateral congenital cataract. The patient exhibited average physical growth, while both motor and intellectual development were significantly delayed. Craniofacial dysmorphism included long face, deeply set eyes, convergent strabismus, scanty eyebrows, and protruding, low-set ears with lobule aplasia and a narrow external acoustic meatus (Figure 4). The patient also displayed excessive sweating in cold weather. Ophthalmic examination, including B-scan ocular ultrasonography, revealed microphthalmia with marked microcornea, sclerocornea and adherent central corneal leukoma in the right eye and total corneal leukoma, aniridia, and PFV in the left eye. Appendix 3 summarizes the clinical and molecular findings of this patient compared to the previously reported cases of Schorderet-Munier-Franceschetti oculoauricular syndrome.

Molecular and phenotypic expansion of **ZBTB11-***related neurodevelopmental disorder (OMIM* 618383*)*—A homozygous splicing variant in *ZBTB11:*c.1623+2T>G was identified in a 7-month-old infant (F23/P26) born to consanguineous healthy parents. The patient presented with microcephaly, plagiocephaly, scanty hair, receding frontal hairline, thin lips, high arched palate, and low set, cupped ears. Ophthalmic assessment revealed bilateral lamellar cataract, attenuated retinal vessels, and bilateral microphthalmia. Neuroimaging showed bilateral dense cerebral calcifications, hydrocephalus, agenesis of corpus callosum and a hypoplastic cerebellar vermis.

A Novel homozygous pathogenic variant in **B3GALNT2** **(***muscular dystrophy-dystroglycanopathy, type A, 11; # 615,181)*—A homozygous *B3GALNT2* nonsense variant: c.1338G>A, p.(Trp446*) was identified in a 7-day-old male (F24/P27) presenting with congenital hydrocephalus and left microphthalmia. The consanguineous parents reported a similarly affected sibling. Brain MRI revealed cobblestone type II lissencephaly, marked supratentorial hydrocephalus, hypoplasia of the cerebellar hemispheres and vermis, and a z-shaped hypoplastic pons. Ophthalmic examination showed optic disc hypoplasia in the right eye, and severe microphthalmia with reduced intraocular pressure in the left eye. Electroencephalography demonstrated left temporal epileptiform discharges and creatine kinase levers were elevated.

A homozygous variant in **UBE3B** *(Kaufman oculocerebrofacial syndrome; OMIM* 244450*)*—A homozygous pathogenic variant in *UBE3B*: c.1956+1G>A, p.(?), was identified in a 6-month-old female (F25/P28) with left anophthalmia and severe right microphthalmia. She also exhibited microcephaly, frontal bossing and sparse eyebrows. Additionally, congenital contractures, hypoplastic distal phalanges of the index fingers, and hypoplastic nails of fingers and toes, inverted hypoplastic nipples and an accessory nipple were noticed. The patient was born to consanguineous parents, who reported a similarly affected child that died in early infancy. The identified *UBE3B* variant is a strong splice-alternating variant that causes exon skipping, ultimately disrupting the reading frame and impairing protein function.

A homozygous variant in **FRAS1** *(Fraser syndrome 1; OMIM* 219000*)*—In a 6-month-old female (F26/P29), presenting with syndromic microphthalmia, a homozygous *FRAS1* frameshift variant: c.2376del, (p.Ser793AlafsTer177) was detected. The patient displayed left cryptophthalmos, right upper eyelid coloboma, sclerocornea, hypoplastic nares with nares coloboma and bilateral stenotic auditory meatus. Bilateral incomplete syndactyly between the 3rd through 5th fingers, as well as complete syndactyly of all toes were also noted. The external genitalia showed hypoplastic labia majora, absent vaginal opening and clitoromegaly. Karyotyping revealed normal female karyotype. MRI of the orbit showed left microphthalmia with a cyst, decreased optic nerve diameter, and hypoplasia of the left optic chiasma. Abdominal US demonstrated agenesis of the right kidney.

The possible structural effects of the three novel missense variants identified in the cohort were predicted by SWISS MODEL and Hope [14,15], and demonstrated in Appendix 4.

DISCUSSION

Anophthalmia and microphthalmia (A/M) represent a spectrum of ocular developmental abnormalities. A/M is a highly heterogeneous condition, with nearly 100 associated genes identified to date [3]. Establishing genotype-phenotype correlations is essential for guiding patient care and genetic counseling.

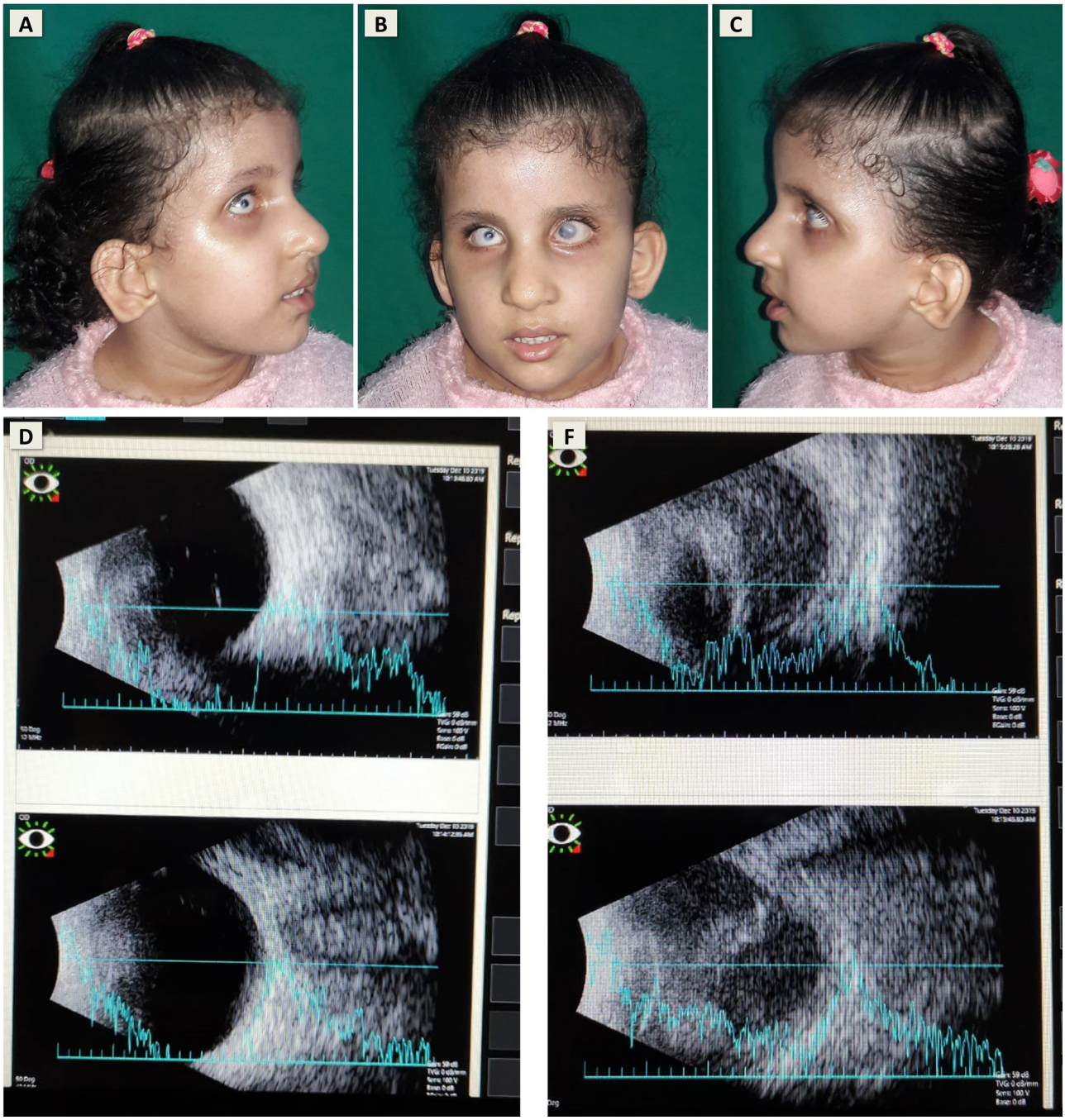

Figure 4. Clinical and ultrasonographic features of patient P24 with 'oculoauricular syndrome' Upper panel **A-C**: Clinical photos demonstrating excessive sweating in cold weather, a long face and protruded low-set ears with lobule aplasia, deeply set eyes, convergent squint, scanty eyebrows, microphthalmia with adherent central corneal leukoma in right eye and total corneal leukoma in left eye. Lower panel **D**, **E**: B-scan ultrasonography revealing retina in place with sonoluscent vitreous cavity in right eye and closed funnel retinal detachment in the left eye.

Among the 28 patients (from 25 unrelated families) subjected to ES in the current study, causative variants were identified in 13 families (13/25; 52%). This relatively high diagnostic yield may be attributed, in part, to the high rate of parental consanguinity in our cohort, which increases the likelihood of AR inheritance. Our findings are comparable to those of the limited number of studies reporting the diagnostic yield of ES in ocular development disorders [7,16-19]. The higher diagnostic rate reported by Matias-Perez et al. [20], however, may be due to the predominance of bilateral and severe A/M phenotypes in their cohort.

This study revealed several noteworthy findings, including: the identification of novel variants, the association of A/M with genes not previously linked to ocular anomalies, and the diagnosis of patients with ultra-rare genetic disorders. Notably, seven out of the variants identified in our cohort were novel; all absent from gnomAD. Furthermore, we observed instances of phenotypic expansion with A/M emerging as a newly recognized clinical feature in patients carrying *DPH1* and *GCNT2* variants (Appendix 5). We also report bilateral microphthalmia and congenital heart disease as novel features in a patient with bi-allelic *ZBTB11* variants, thus broadening the phenotype of this ultra-rare neurodevelopmental disorder.

Among this cohort, a novel heterozygous LP variant in *OTX2* (c.278G>C, p.Trp93Ser) was identified in a patient with A/M and Peters anomaly (F7/P7). The transcription factor OTX2 is known to play a crucial role in ocular, craniofacial, and pituitary development. Pathogenic variants in *OTX2* have been associated with diverse ocular phenotypes [21-24]. Extraocular anomalies - including variable congenital hypopituitarism and ID, growth retardation and deafness - have occasionally been reported [25-27]. In contrast to these reports, the case described here exhibited normal development and growth, with no apparent extraocular anomalies. These inconsistencies may be attributed to the variable expressivity of *OTX2* mutations or to the possibility of late-onset pituitary dysfunction.

Another novel genetic defect – a heterozygous nonsense variant in *PRR12* (c.3625C>T, p.Arg1209*) - was identified in an infant (F8/P8) presenting with unilateral microphthalmia accompanied by disc and chorioretinal coloboma. *PRR12* is predicted to be highly intolerant to loss-of-function (LOF) variants [28], and its haploinsufficiency has been linked to a neuro-ocular syndrome, which encompasses a wide range of clinical features, including consistent neurodevelopmental impairment in all reported cases and variable ocular anomalies [12]. The isolated complex microphthalmia observed in this study is consistent with previous reports [29]. However, intellectual capacity could not be evaluated due to the patient's young age. As such, ongoing follow-up and reassessment are necessary.

Moreover, a homozygous VUS in *GCNT2* was identified in two similarly affected sibs (F9/P9&10) with bilateral microphthalmia and cataract. Biallelic variants in *GCNT2* have been associated with cataract 13 with adult i phenotype. To the best of our knowledge, this is the first report of a homozygous *GCNT2* variant associated with A/M. One possible explanation for this novel phenotype could be the limited number of reported *GCNT2* variants until now. Therefore, additional cases and functional studies are needed to provide further evidence for reclassifying this variant and to confirm the potential association of *GCNT2* and A/M.

Although heterozygous *GJA8* mutations are known to be associated with isolated cataracts 1, multiple types, we identified a heterozygous *GJA8* pathogenic variant (c.134G>C) in a patient with congenital cataract, glaucoma and microphthalmia (F11/P13). Ma et al. (2016) also reported A/M anomalies in patients carrying *GJA8* variants [30]. More recently, *GJA8* variants have been implicated in a rare condition not currently listed in the OMIM database, termed 'familial acorea-microphthalmia-cataract syndrome' [31]. The case described in our cohort further supports the notion that the phenotypic spectrum associated with *GJA8* mutations extends beyond isolated cataract.

In the current study, a novel *SOX2* heterozygous VUS (c.178G>C) was identified in a stillborn fetus (F12/P14) presenting with syndromic A/M. The recurrence of the condition in the family, despite both parents being clinically unaffected, raises the possibility of germline mosaicism. This phenomenon has previously been reported in association with *SOX2* anophthalmia syndrome in four families where maternal mosaicism was identified [32-35]. To look for a potential mosaicism, we recommend that parents should undergo molecular testing of different tissues. Identifying mosaicism is important for accurately determining the recurrence risk and guiding genetic counseling.

Heterozygous pathogenic variants in *CHD7* have been well documented in individuals diagnosed with CHARGE syndrome [36-39]. Within the current cohort, two patients with syndromic A/M met the clinical criteria of CHARGE syndrome. A heterozygous *CHD7* pathogenic variant (c.5405–17G>A, p.?) was identified in one patient (F20/P23), while no causative variant was detected in the second (F27/P30). Prior studies have reported variable detection rates of *CHD7* mutations among clinically diagnosed CHARGE patients [36,40,41]. These discrepancies may be attributed to differences in patient selection criteria, the sensitivity of

molecular techniques employed, or the presence of pathogenic variants in noncoding regions of *CHD7* or other critical regulatory elements not typically covered by standard ES.

Oculoauricular syndrome is a very rare AR genetic disorder, caused by biallelic LOF mutations in *HMX1*. Since its initial description by Schorderet et al. in 2008 [42], affected individuals from four families have been documented, all presenting with ocular anomalies and auricular malformations, while displaying normal neurodevelopment [42-45]. Here, we report a novel *HMX1* variant (c.570_571delG, (p.E191Rfs*34) identified in a consanguineous Egyptian family, bringing the total number of reported families to five. Additional to the typical features of the syndrome, the patient (F21/P24) demonstrated psychomotor impairment and an abnormal gait – features not previously reported. The identification of the fifth *HMX1* variant further supports the gene's role in the pathogenesis of the syndrome and suggests a broader phenotypic variability than previously recognized.

In this study, one notable example of phenotypic expansions was the detection of a homozygous *DPH1* variant (c.359T>C, p.Leu120Pro) associated with bilateral microphthalmia (F22/P25). Biallelic *DPH1* variants have been recently linked to a rare neurodevelopmental disorder known as DEDSSH. To date, only 17 affected individuals from seven families have been reported, the majority were from Middle Eastern countries [46-51]. To the best of our knowledge, our case is the first reported from Egypt. The presence of severe microphthalmia in our patient suggests that ocular involvement may be an underrecognized feature of *DPH1*-related disorders. Further clinical, genetic, and functional studies involving larger cohorts are needed to confirm this potential association.

Moreover, this study further expands the molecular and phenotypic spectrum associated with *ZBTB11* mutations by reporting a novel splicing variant (c.1623+2T>G) in an infant (F16/P18) presenting with bilateral microphthalmia. Biallelic variants in *ZBTB11* have been linked to a neurodevelopmental condition (intellectual developmental disorder 69), characterized by ID, motor delay, and, in some cases, cataract [52]. Additional to the typical features of the syndrome, our patient exhibited bilateral microphthalmia and congenital heart disease – findings not commonly reported in association with *ZBTB11* mutations. While most previously described variants have been missense mutations, the variant identified in this study is a predicted LOF mutation, which may explain the phenotypic expansion. Yet, the potential association of *ZBTB11* mutations with ocular and cardiac anomalies warrants further investigation in larger cohorts. To the best of our knowledge, this is also the first documented case of *ZBTB11*-related disorder among Egyptians.

In the present study, we report a novel homozygous pathogenic nonsense variant in the *B3GALNT2* gene in an infant (F17/P19) presenting with microphthalmia, congenital hydrocephalus and diffuse cobblestone-type II lissencephaly. This severe phenotype is consistent with previously reported cases involving truncating *B3GALNT2* mutation, which are predicted to result in nonsense-mediated decay and are associated with severe clinical manifestations [53].

Despite the relatively high diagnostic yield, 48% of the tested families in this study remained without a definitive molecular diagnosis. This might suggest that additional genetic factors, such as mutations in yet undiscovered genes, may play a role in the etiology of A/M. It could also be attributed to the limitations inherent to the used methodology, which cannot detect non-coding, regulatory and variants in genes with corresponding pseudogenes, gene fusions, large and balanced chromosomal rearrangements, uniparental disomies, mosaicism and repeat expansions. Another consideration is that ES has a high likelihood of identifying VUSs. The identification of VUSs in two of our patients underscores the challenges in interpreting genomic data and highlights the need for complementary approaches, including functional studies and segregation analyses. Nonetheless, the main limitation was the use of proband-only ES in some cases, rather than the trio approach, due to financial constraints. For the unsolved families, we recommended further genetic testing using methylation profiling or genome sequencing (GS). Among the cohort, several families, including Family-12 with two similarly affected siblings, have already proceeded to GS, but the results are still pending.

Conclusion: The study provides a comprehensive molecular and clinical characterization of Egyptian patients with A/M. By reporting novel variants and unusual phenotypes, we contribute to expanding both the mutational landscape of A/M and the phenotypic spectrum of associated syndromes. Our results also support the genetic heterogeneity that underlies A/M etiology and reinforce the value of genetic testing for accurate diagnosis, counseling and future management. Nevertheless, further studies involving larger cohorts and a genome-wide approach are needed to uncover additional developmental genes.

APPENDIX 1. SUPPLEMENTARY TABLE 1.

To access the data, click or select the words "Appendix 1." Demographic, clinical and cytogenetic data of the A/M patients diagnosed with chromosomal abnormalities.

APPENDIX 2. SUPPLEMENTARY FIGURE 1.

To access the data, click or select the words "Appendix 2." The clinical features of two cases of Trisomy 13. Clinical photos illustrating bilateral microphthalmia (A&B&F&F), severe bilateral cleft lip (B), pre-axial polydactyly of right hand (C) and left foot (D) and post-axial polydactyly in both hands (H) and feet (G).

APPENDIX 3. SUPPLEMENTARY TABLE 2.

To access the data, click or select the words "Appendix 3." Clinical and molecular findings in the reported cases of OAS.

APPENDIX 4. SUPPLEMENTARY FIGURE 2.

To access the data, click or select the words "Appendix 4." The three-dimensional model of the protein with close-up on three missense variants. [A] OTX2 c.278G>C p.(Trp93Ser): Tryptophan is replaced by Serine at position 93. The wild-type and mutant amino acids differ in size, with the mutant residue is smaller, this might lead to loss of interactions. Moreover, the hydrophobicity of the wild-type and mutant residue differs. [B] *ATOH7* c.254C>T p.(Ala85Val): Alanine, which is only the residue type found at position 85, is mutated into Valine. First, mutation of a 100% conserved residue is usually damaging for the protein. Second, the wild-type and mutant amino acids differ in size, with the mutant residue bigger, which might lead to bumps. *[C] SOX2 c.178G>C p.(Ala60Pro)*: Alanine is replaced by Proline at position 60. First, the wild-type residue is very conserved and based on conservation scores this mutation is probably damaging to the protein. Second, the mutant residue is bigger than the wild-type residue which might lead to bumps. Third, the wild-type residue is located in a region annotated in UniProt to form an α-helix. Proline disrupts an α-helix when not located at one of the first 3 positions of that helix, and in this case can have severe effects on the structure of the protein. Figures [A] and [B] were generated using SWISS-MODEL [15], while the report on the third variant [C] was produced by HOPE [14].

APPENDIX 5. SUPPLEMENTAL TABLE 3.

To access the data, click or select the words "Appendix 5." Clinical and molecular findings in the reported cases of DEDSSH.

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