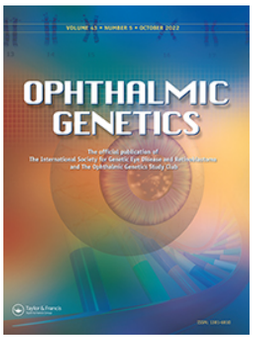

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LETTER TO THE JOURNAL

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A new nonsense mutation in *HMX1* in two siblings with oculoauricular syndrome

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A homeobox is a highly conserved DNA sequence of around 180 base pairs that encodes for a homeodomain, i.e. a portion of a protein that has DNA binding properties. Homeobox—containing genes are called homeogenes, and many developmental genes are indeed homeogenes (1). Homeogenes *HMX1*, *HMX*2, and *HMX3* belong to the homeobox family H6, are expressed during embryogenesis, and encode transcription factors that are involved in the development of sensory organs (1). A homozygous frameshift microdeletion (c.215_240del) in three affected individuals (P1, P2, and P3 in Table 1) from a Swiss consanguineous family (2,3) and the homozygous missense variant p.(Gln217Pro) in two affected cousins (P4 and P5) of a Pakistani consanguineous family (4) were previously reported in the homeogene *HMX1* (OMIM# 142992), resulting in the so—called "oculoauricular syndrome" (OAS). Another *HMX1* homozygous mutation, the nonsense variant p.(Glu163Ter), was also recently identified in an Egyptian child (P6) born to consanguineous parents and with the same clinical diagnosis (5). This syndrome was first described by Franceschetti and Valerio in 1945 (6) and includes, among other things, colobomatous microphthalmia with corneal opacities, congenital cataract, and symmetric abnormalities of the external ear. Clinically, OAS has overlapping features with other developmental syndromes characterized by eye anterior segment dysgenesis and/or microphthalmia, as well as external ear anomalies. The oculo—auriculo-vertebral spectrum (OAVS), which comprises Goldenhar syndrome, is characterized by hemifacial microsomia, abnormal development of the ear, eye, and vertebral column (7). External ear abnormalities are common and include microtia, anotia, aural atresia, preauricular tags and pits, whereas ocular features are less frequent. Limbal dermoid is the most common ocular finding, but microphthalmia, coloboma of the upper lid or of the optic disk may also be present. Moreover, heart, limb, renal, and central nervous system defects have also been observed in OAVS. Peters plus syndrome is characterized by eye anterior chamber (AC) anomalies, short limbs with broad distal extremities, variable developmental delay/intellectual disability, characteristic facial features, and cleft lip/palate (8). The most common AC anomaly is Peters' anomaly, consisting of central corneal opacification and posterior corneal defect with or without iridocorneal or lenticulocorneal adhesions. Cataract and glaucoma are common. Ear anomalies, including preauricular pits, are present in more than one third of affected individuals. CHARGE syndrome (acronym for ocular Coloboma, Heart defects, Atresia of the choanae, Retardation of growth and/or of development, Genital anomalies and Ear anomalies) is a multiple congenital anomaly condition, frequently associated with coloboma that can involve retina, optic disk, choroid or iris, as well asmicrophthalmia and outer ear anomalies (9). Another important feature of CHARGE syndrome is the abnormality of semicircular canals.

Here we report on a consanguineous family from Pakistan in which we identified a *HMX1* nonsense variant [NM_018942.3:c.457C>T,p(Arg153Ter)], present homozygously in two affected siblings and heterozygously in their parents, in agreement with a recessive pattern of heredity for OAS (Figure 1(a,c)). This new mutation was found by whole exome sequencing, followed by homozygosity mapping (10) and Sanger sequencing, to validate co—segregation of the genotypes with the phenotype.

Patient IV-2

The affected individual IV-2 is an 11-year—old boy from a consanguineous Pakistani family (P8 in Table 1, Figure 1(a)). There was a history of congenital cataract for which he had undergone cataract extraction with posterior capsulotomy followed by anterior vitrectomy (intraocular lenses were not implanted). Later on, he underwent bilateral trabeculectomy because of increased intraocular pressure. He was given anti—glaucoma medications since then, although treatment was discontinued for the last six months. On examination, best corrected

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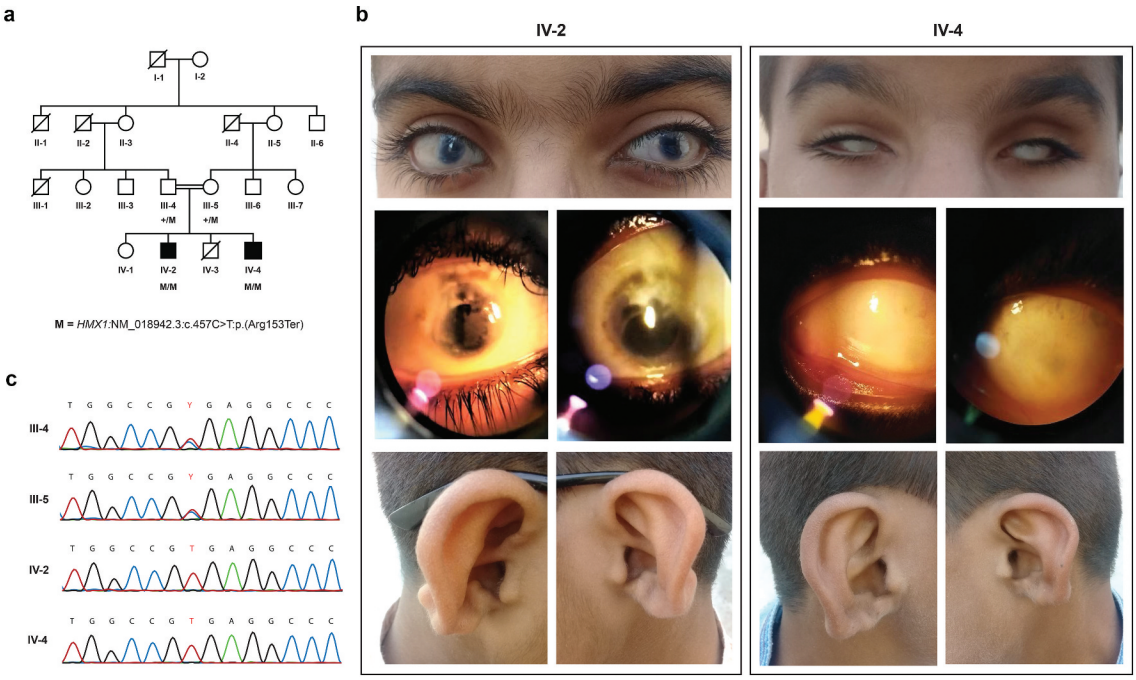

Figure 1. Family structure, clinical and molecular findings: (a) Pedigree showing segregation of the nonsense mutation (M) p(Arg153Ter) in the *HMX1* gene. White and black shapes denote healthy and affected individuals, respectively; squares, males; circles, females; symbols with a diagonal line, deceased; double horizontal lines, consanguineous union. (b) Photographs of eyes and ears of the individuals examined (IV-2 and IV-4). (c) Chromatograms showing segregation of p(Arg153Ter) within the family.

visual acuity was counting fingers in both eyes. There was a bilateral horizontal nystagmus, extraocular movements were full, and adnexa were normal (Supplemental Video 1). Sclerocornea of moderate degree with cornea plana was also present (Figure 1(b)). The AC was shallow with peripheral anterior synechiae; inferior iris coloboma was present in both eyes. The iris was also markedly atrophic and transillumination was positive in both eyes. Opacified margins of posterior capsulotomy were observed in both eyes. The vitreous was clear in both eyes. Fundus view of the right eye was hazy due to opacity, but a pale disc was nevertheless seen. The left eye also had a pale disc and an abnormal foveal reflex. No retinal pigmentary changes or gross vascular abnormalities were noted. Intraocular pressure was high digitally, for which an anti—glaucoma treatment was restarted. The patient presented with the abnormal appearance of external ears and dysplastic auricles (Figure 1(b)).

Patient IV-4

Another affected individual (IV-4, sibling of IV-2, P9 in Table 1) is a 9-year—old child, blind since birth. On examination, there was microphthalmia with pseudoenophthalmos associated with severe sclerocornea (Figure 1(b) and Supplemental Video 2). Retinal examination could not be performed due to severe sclerocornea. The external ears showed hypoplastic lobule and abnormal bridging between the crus of helix and antihelix (Figure 1(b)).

In this study, we present two patients with homozygous mutations in *HMX1* and with OAS, born to healthy parents who are heterozygous for the same mutation. The homeobox transcription factor HMX1 controls the diversification of sympathetic neurons and retinal axon guidance during development (11,12). Its role in the formation of the eye and the external ear has also been described. Expression of *HMX1* was observed in the lens of zebrafish, while knockdown animals had delayed retinal development and microphthalmia (13). In addition to the eye, it has also been shown in zebrafish that *HMX1* plays a role in the development of the craniofacial region, by controlling the expression of the *UHRF1* and *DNMT1* genes (14), while in the mouse *HMX1* mutations cause enlarged ear pinnae, microphthalmia, and minor craniofacial anomalies (15). However, the exact mechanisms by which *HMX1* mutations result in pathological phenotypes in humans is still rather unclear. It is very likely that additional factors besides *HMX1* defects are involved in the molecular pathology of the disease since variability of ocular signs can be observed across patients. This phenomenon is well illustrated by the cases presented here, for whom the same genotype results in a different extent of corneal opacity. Table 1 summarizes the phenotypic features of all OAS cases described so far.

In conclusion, although the number of cases reported to date does not allow for a precise genotype/phenotype association, the identification of a fourth mutation in *HMX1* strongly corroborates the hypothesis that this gene is directly involved in OAS.

Table 1. Phenotypic features of OAS patients.

	P1	P2	P3	P4	P5	P6	P7*	P8	P9
Reference(s)	(2,3)	(2,6)	(2,3,6)	(4)	(4)	(5)	(5)	present study	present study
Age at first reported examination	2 months	3 y	6 y	3 days	at birth	40 days	45 days	11 y	9 y
Country of origin	Switzerland	Switzerland	Switzerland	Pakistan	Pakistan	Egypt	Egypt	Pakistan	Pakistan
EYE									
Visual acuity	OD 0.05; OS 0.16 (aged 7 y); OD 0.02; OS 0.1 (aged 10 y)	Light perception	0.016 in both eyes, maintained until the age of 65 years	OD 0.125; OS 0.2 (unclear age)	OD 0.16; OS 0.20 (aged 4 y); both eyes 0.025 (aged 9 y); OD light perception; OS 0.016 (aged 14 y)	Decreased	NA	Counting fingers	No light perception
Globe	Microphthalmia	Microphthalmia	Microphthalmia	Microphthalmia	Microphthalmia	Microphthalmia	Microphthalmia	Microphthalmia	Microphthalmia
Axial length	OD 15.5 mm; OS 16.5 mm (unclear age)	NA	NA	OD 13.9 mm; OS 14.9 mm (aged 6 weeks)	OD 16.9 mm; OS 17 mm (unclear age)	OD 19.8 mm; OS 17.9 mm (aged 7 y)	OD 15 mm; OS 16 mm	NA	NA
Cornea	Microcornea with diffuse cornea guttata. Thickness, OD 770 µm; OS 710 µm (aged 7 y)	Sclerocornea	Microcornea	Posterior embryotoxon, localized sclerocornea, microcornea, thickness OD 729 µm; OS 649 µm (aged 6 weeks)	Posterior embryotoxon, sclerocornea, microcornea, progressive corneal opacification (aged 12 y)	Microcornea	Microcornea	Sclerocornea, cornea plana	Severe sclerocornea
Lens	Rapidly progressive congenital cataract	NA	Cataract, microphakia	Dense congenital cataract	Dense congenital cataract	Congenital cataract	Congenital cataract	Congenital cataract	NA
Iris	Posterior synechiae, incomplete coloboma, stromal cyst, corneal adherences	NA	Inferior coloboma	Inferior coloboma	Inferior coloboma, anterior synechiae	Coloboma	Coloboma	Anterior synechiae, inferior coloboma, iris atrophy	NA
Optic disk	Dysplastic, large (Morning Glory-like)	NA	NA	Dysplastic, small	Dysplastic, small	Coloboma, persistent hyaloid artery	Cupping	Pallor	NA
Retina	Macular hypoplasia, peripheral infero-nasal chorioretinal coloboma, progressive rod-cone dystrophy (aged 7 y)	Total retinal detachment (unclear age)	Inferior chorioretinal atrophy, diffuse pigment deposits (aged 65 y)	Chorioretinal coloboma, rod dysfunction (aged 2 y)	Progressive generalized photoreceptor dysfunction (aged 14 y)	Moderate photoreceptor dysfunction (aged 7 y)	NA	Abnormal foveal reflex	NA
Movements	Nystagmus, esotropia (aged 4 y)	Nystagmus	Nystagmus	Nystagmus, exotropia (aged 6 weeks)	Nystagmus (aged 12 y)	Nystagmus	Nystagmus	Nystagmus	Roving
Intraocular pressure	Normal	High	Normal	Normal	Normal	NA	NA	High	NA
EAR									
External ear	Lobule aplasia, narrow intertragic incisure, abnormal bridging between the crus of helix and antihelix, narrow external acoustic meatus	Abnormal pinna, lobule aplasia	Abnormal pinna, lobule aplasia	Low-set pinna with crumpled helix, narrow external acoustic meatus, lobule aplasia	Low-set pinna with crumpled helix, narrow external acoustic meatus, lobule aplasia	Large, protruded, and low-set pinna, prominent tragus, antitragus and antihelix, hypoplastic lobule	Large pinna, lobule aplasia	Hypoplastic lobule, abnormal bridging between the crus of helix and antihelix,	Hypoplastic lobule, abnormal bridging between the crus of helix and antihelix,
Hearing	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Abbreviations: P = patient; NA = not available; y = years
* = genotype not available, sister of P6
Visual acuity is expressed in decimals

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