Supp. Table S1. Primers used to sequence *VAX1* and *VAX2* genes

VAX1 long isoform NM_001112704.1	**Forward primer**	**Reverse primer**
Exon 1	ggttttgtccccttttcct	gccaacaactttctcccaag
Exon 2	gactgtgtccccaaatccag	cccaagggtagttctgtcca
Exon 3 (1)	ttcctttgacgctggtcttc	ctaatgcgcgtgagtccata
Exon 3 (2A)	ttcctttgacgctggtcttc	aggctgaagaggctgtgg
Exon 3 (2B)	cgtgctacggctgctgg	cccgaatgagatgaaattgg
VAX1 short isofrom NM_199131.2		
Exon 3	tctgaagcaagcgaaaaaca	tcgagagcgaaacactcaag
VAX2 NM_012476.2		
Exon 1	cctgaggaggaggagtcgag	tgctgaatgaatgactggatg
Exon 2	gtcacaggtcatgcaaccag	gcctcatatccccaggagtt
Exon 3	ctcctttcctcctcccacct	gcctgctcagtgctgtcc
TCF7L2		
Exon 1b	gccgcaaccctctctagatc	cgtcggctggtaagtgtgg
Exon 5	gacaagccctcaaggatgcc	cgtcggctggtaagtgtgg

We used two separate sets of primers for exon 3 of the VAX1 long isoform – pair (1), in which the entire exon is amplified and pair (2A) and (2B) in which the exon is amplified in two fragments.

Supp. Table S2. Conservation of Arginine residue 152 amongst different species for VAX1 isoform 1

Homo sapiens	S	E	T	Q	V	K	V	W	F	Q	N	R	**R**	T	K	Q	K	K	D	Q	G	K	D	S	E
Mus musculus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rattus norvegicus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pan troglodytes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bos taurus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Canis lupus familiaris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Monodelphis domestica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gallus gallus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Danio rerio	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xenopus laevis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Taeniopygia guttata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Caenorhabditis elegans	D	.	Y	.	.	.	I	.	.	.	.	.	.	I	R	M	R	R	E	A	N	K	-	-	-
Nematostella vectensis	.	.	R	.	.	.	.	.	.	.	.	.	.	M	K	Y	K	R	E	R	-	-	-	-	-

The arginine residue at position 152 is in bold and is conserved in all species listed. Identical amino acids that are conserved are represented with a “.”

Supp. Table S3. Phenotype of Anophthalmia/Microphthalmia (A/M) Patients Selected for Cleft Lip +/- Palate and Agenesis of the Corpus Callosum

Phenotype	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Ocular A/M Coloboma Other	 +; B	 +; B	 +; B	 +; L +; R	 +; B +; B	 +; R +; R eyelid	 +; B +; R Anisocoria	 +; R	 +; B	 +; B +; R/?L
Orofacial clefting Cleft palate Cleft lip	 +; B +; B	 +; B +; B	 +; B. +; B	 - -	 +; R +; R	 +; R +; R	 +; B +; R	 +; B +; B	 	 +; submuc.
Central nervous system Agenesis corpus callosum Developmental delay Seizures NTD/Encephalocele Hydrocephalus	 +	 	 	 +; compl. +	 +; partial	 	 	 +	 +; compl. +	 +; ?partial
Other Craniofacial dysmorphism Cardiac anomalies Genitourinary anomalies Skeletal abnormalities	 	 ASD Renal hyp. SBO	 + HLH	 + Renal dup.	 +	 + VSD	 + PDA Hypogonad	 +; Amp.	 + +	 + VSD; ASD +
Clinical diagnosis					CHARGE syndrome	Goldenhar/ Treacher-Collins		Amniotic bands syndrome		
VAX1 sequence alteration	c.945C>T	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen) for both genes. The initiation codon is codon 1.

A/M = anophthalmia/microphthalmia; B = bilateral; L = left; R = right; Submuc. = submucous; compl. = complete; NTD = neural tube defect; ASD = atrial septal defect; HLH = hypoplastic left heart; VSD = ventricular septal defect; PDA = patent ductus arteriosus; Renal hyp. = renal hypoplasia (unilateral); Renal dup. = duplicated kidney; Hypogonad. = hypogondasism; SBO = spina bifida occulta; Amp. = amputation defect.