

Table S1 The primers used for validation of *FOXJ1* variants

Primers	Sanger sequencing (5'-3')
Gly89Asp	Forward: CTCCATTCTCAACGCCAAG Reverse: ATCTTGGTGGCCTTGCTG
Arg213Trp	Forward: CAAGTGCTTCATCAAAGTGC Reverse: AAGTTGCCTTTGAGGGGTTG
p.Leu377Trpfs*76	Forward: GAACCCCTCAAAGGCAACTT Reverse: CTAGGTGGTGGGGTGTCTGT

FOXJ1_{mut} MAESWLRLSGAGPAEEAGPEGGLEEPDALDDSLTSLQWLQEF SILNAKAPALPPGGTDPH 60
 Fojj1_{mut} MAESWLRLCGAGPGEEAGPEGGMEEPDALDDSLTSLQWLQEF SILNAKAPTLPPGGTDPH 60
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FOXJ1_{mut} GYHQVPGSAAPGSPLAADPAACLGQPHTPGKPTSSCTSRSAAPPGLQAPPPDDVDYATNPV 120
 Fojj1_{mut} GYHQVPLVAPGSPLAADPAACLGQPHTPGKPTSSCTSRSAAPPGLQAPPPDDVDYATNPV 120
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FOXJ1_{mut} KPPYSYATLICMAMQASKATKITLSAIYKWITDNFCYFRHADPTWQNSIRHNLSLNKCFI 180
 Fojj1_{mut} KPPYSYATLICMAMQASKATKITLSAIYKWITDNFCYFRHADPTWQNSIRHNLSLNKCFI 180

FOXJ1_{mut} KVPREKDEPGKGGFWRIDPQYAERLLSGAFKKRRLPPVHIHPAFARQAAQEPSAVPRAGP 240
 Fojj1_{mut} KVPREKDEPGKGGFWRIDPQYAERLLSGAFKKRRLPPVHIHPAFARQASQEPSAAPWGGP 240
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FOXJ1_{mut} LTVNTEAQQLLREFEATGEAGWGAGEGRLGHKRKQPLPKRVAKVPRPPSTLLPTPEEQG 300
 Fojj1_{mut} LTVNREAQQLLQEFEEATGEGGWGTGEGRLGHKRKQPLPKRVAKVLRPPSTLLLQEEQG 300
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FOXJ1_{mut} ELEPLKGNFDWEAIFDAGTLGGELGALEALELSPPLSPASHVDVDTIHGRHIDCPATWG 360
 Fojj1_{mut} ELEPLKGNFDWEAIFEAGALGEESSLEGLLELSPPLSPSSHGDVDTLVHGRHINCPATWG 360
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↓L377W

FOXJ1_{mut} PSVEQAADSLDFDETFWPHSPCSTPGTRAAVAACPRSPSLRLGMPPWPPTCRTGPAWGPS 420
 Fojj1_{mut} PPAEQAADSLDFDETFWPHSPSYSIPGMRVVAAACPNPSLKQGMPPWPLTCRTGPVWVPS 420
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FOXJ1_{mut} CKRPGPAPPLDSAQVRVQNCPTQVHRHPTT----- 451
 Fojj1_{mut} CKRSGPTSPDSAQVRAQNCLPRQARGHLSTQAGTG PGL 459
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Figure S1 Amino acid sequence comparison of mutant human (FOXJ1_{mut}) and mouse (Fojj1_{mut}). Sequences were aligned using Clustal Omega, and the numbers indicate the positions of amino acid residues on the complete sequence alignment.

Table S2 Summary of previously published and this paper reported pathogenic variants in *FOXJ1*

Patient	Gender	Ethnicity	Nucleotide change	Location	Protein Change	Clinical features						Reference
						MCCD	hydrocephalus	CHD	Situs inversus	Fertility	Other phenotype	
OP-1743 II1	Male	Germany	c.901G>T	Exon3	p.Glu301*	+	+	-	-			(3)
OP-2950 II1	Female	Germany	c.868_871dup	Exon3	p.Thr291Lysfs*12	+	+	-	+	Infertility	Macrocephaly	
RBH II1	Female	Germany	c.967delG	Exon3	p.Glu323Serfs*10	+	+	-	-	Hydrosalpinx		
OP-1933 II1	Male	Germany	c.826C>T	Exon3	p.Gln276*	+	+	VSD	-			
US-1 II1	Male	USA	c.826C>T	Exon3	p.Gln276*	+	+	-	+			
US-2 II1	Male	USA	c.939delC	Exon3	p.Ile314Serfs*19	+	+	-	+	Subfertility		
KCHYD109-2 ^a	Female	European	c.287C>G	Exon2	p.Thr96Arg	-	-	-	-			(4)
KCHYD109-1 ^b	Male	European	c.287C>G	Exon2	p.Thr96Arg	-	+	-	-		Developmental delay	
KCHYD376-1	Male	European	c.826C>T	Exon3	p.Gln276*	-	+	-	-		Developmental delay	
KCHYD238-1	Female	European	c.967delG	Exon3	p.Glu323Serfs*10	-	+	-	-		Developmental delay	
UNC-1459	Female	Jewish	c.945delC	Exon3	p.Phe315Leufs*18	+	+	ASD	+			(5)
UNC-0852	Male	Irish/USA	c.929_932delACTG	Exon3	p.Asp310Glyfs*22	+	+	-	-			
II:2	Female	Chinese	c.1129delC	Exon3	p.Leu377Trp*fs76		-	-		Infertility		This paper
III:1	Male	Chinese	c.1129delC	Exon3	p.Leu377Trp*fs76	+	-	+				

MCCD, mucociliary clearance disorder; CHD, congenital heart disease; VSD, ventricular septal defect; ASD: atrial septal defect; a, b, mother-child relationship.

Table S3 In silico pathogenicity prediction of *FOXJ1* variants

chr	hg19_pos	ref	alt	aaref	aaalt	rs_dbSNP	aapos	genename	Ensembl_geneid	Ensembl_transcriptid	Ensembl_proteinid	Uniprot_acc	SIFT_score	SIFT_pred	Polyphen2_HDIV_score	Polyphen2_HDIV_pred	Polyphen2_HVAR_score	Polyphen2_HVAR_pred	MutationTaster_score	MutationTaster_pred	CADD_phred	h1000Gp3_AF	UK10K_AF	ESP6500_AF	ExAC_AF	gnomAD_exomes_AF	gnomAD_genomes_AF
17	74136211	C	T	G	D	rs780094426	89	<i>FOXJ1</i>	ENSG00000129654	ENST00000322957	ENSP00000323880	Q92949	0.025	D	0.999	D	0.94	D	1	D	26.4	.	.	.	2.56E-05	.	.
17	74134063	G	A	R	W	rs764249412	213	<i>FOXJ1</i>	ENSG00000129654	ENST00000322957	ENSP00000323880	Q92949	0	D	1	D	1	D	1	D	27	.	.	.	1.65E-05	4.27E-06	6.57E-06

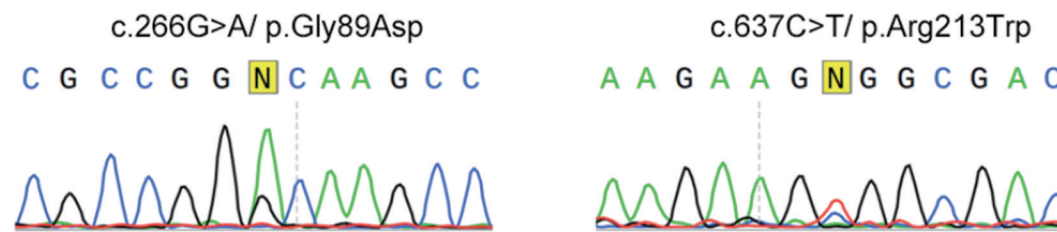


Figure S2 DNA sequence chromatographs confirming the heterozygous alteration in *FOXJ1*.

Table S4 The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway results of down-regulated genes in *FOXJ1*-c.637C>T overexpressed AC16 cells

Category	Term	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
KEGG_PATHWAY	hsa04970: salivary secretion	4	5.79710145	6.97E-04	<i>GUCY1A2, CHRM3, CALML6, ATP2B2</i>	17	92	8164	20.8797954	0.06673378	0.069040701	0.069040701
KEGG_PATHWAY	hsa04020: calcium signaling pathway	4	5.79710145	0.01058194	<i>CHRM3, CALML6, CACNA1A, ATP2B2</i>	17	240	8164	8.003921569	0.65117839	0.523806164	0.523806164
KEGG_PATHWAY	hsa04261: adrenergic signaling in cardiomyocytes	3	4.34782609	0.03399868	<i>CALML6, ATP2B2, CACNA2D4</i>	17	150	8164	9.604705882	0.96743177	0.819035304	0.819035304
KEGG_PATHWAY	hsa04921: oxytocin signaling pathway	3	4.34782609	0.0356801	<i>GUCY1A2, CALML6, CACNA2D4</i>	17	154	8164	9.355233002	0.97259109	0.819035304	0.819035304
KEGG_PATHWAY	hsa04022: cGMP-PKG signaling pathway	3	4.34782609	0.04136542	<i>GUCY1A2, CALML6, ATP2B2</i>	17	167	8164	8.626981331	0.98473643	0.819035304	0.819035304

FDR, false discovery rate.