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

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

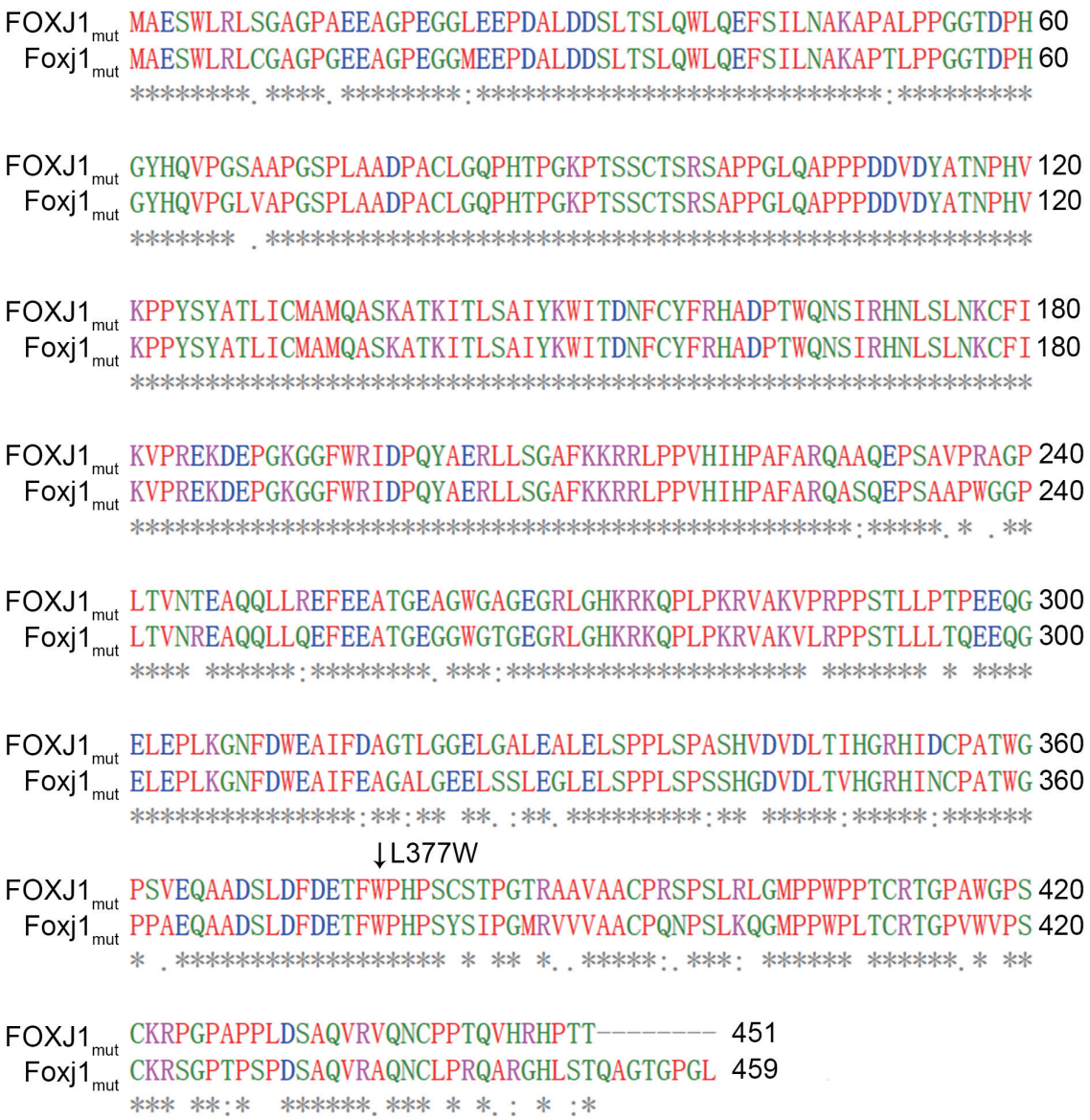

Figure S1 Amino acid sequence comparison of mutant human ($FOXJ1_{mut}$) and mouse ($Foxj1_{mut}$). Sequences were aligned using Clustal Omega, and the numbers indicate the positions of amino acid residues on the complete sequence alignment.

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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	*FOXJ1*	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	*FOXJ1*	ENSG00000129654	ENST00000322957	ENSP00000323880	Q92949	0	D	1	D	1	D	1	D	27	.	.	.	1.65E-05	4.27E-06	6.57E-06

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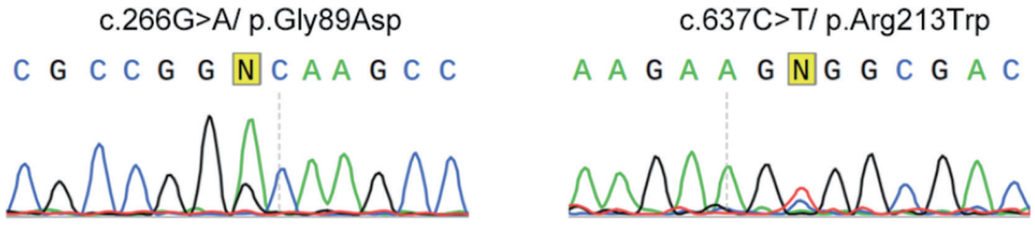

Figure S2 DNA sequence chromatographs confirming the heterozygous alteration in *FOXJ1*.

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	*GUCY1A2*, *CHRM3*, *CALML6*, *ATP2B2*	17	92	8164	20.8797954	0.06673378	0.069040701	0.069040701
KEGG_PATHWAY	hsa04020: calcium signaling pathway	4	5.79710145	0.01058194	*CHRM3*, *CALML6*, *CACNA1A*, *ATP2B2*	17	240	8164	8.003921569	0.65117839	0.523806164	0.523806164
KEGG_PATHWAY	hsa04261: adrenergic signaling in cardiomyocytes	3	4.34782609	0.03399868	*CALML6*, *ATP2B2*, *CACNA2D4*	17	150	8164	9.604705882	0.96743177	0.819035304	0.819035304
KEGG_PATHWAY	hsa04921: oxytocin signaling pathway	3	4.34782609	0.0356801	*GUCY1A2*, *CALML6*, *CACNA2D4*	17	154	8164	9.355233002	0.97259109	0.819035304	0.819035304
KEGG_PATHWAY	hsa04022: cGMP-PKG signaling pathway	3	4.34782609	0.04136542	*GUCY1A2*, *CALML6*, *ATP2B2*	17	167	8164	8.626981331	0.98473643	0.819035304	0.819035304

FDR, false discovery rate.

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