

Supplementary data

***IFT81* as a candidate gene for non-syndromic retinal degeneration.**

Rachayata Dharmat^{1,2#}, Wei Liu^{2#}, Zhongqi Ge^{1,2#}, Zixi Sun^{3#}, Lizhu Yang³, Yumei Li^{1,2},
Keqing Wang^{1,2}, Kandace Thomas², Ruifang Sui^{3*}, Rui Chen^{1,2, 4, 5,6*}

Supplementary methods:

Statistical Analysis:

For both *in vitro* and *in vivo* cell assay, statistical analysis was performed by two-way ANOVA analysis using Graph-pad prism software. In case of *in vivo* assay, statistical analysis was performed by comparing the number of phenotypically null embryo in wild-type and mutant mRNA rescue experiments to the number of phenotypically null zebrafish from control embryos injected with PBS. In case of *in vitro* ciliogenesis assay, the statistical comparison was performed between shRNA-transfected IFT81 knockdown cells to shRNA-transfected cells rescued with wildtype and mutant vector.

Protein analysis

For determining IFT protein levels, HEK293T were lysed in RIPA buffer by sonication for 15 seconds at 30% amplitude. Western blotting performed using antibodies against c-myc (clone 9E10, 1:1000; Covance), was developed by chemiluminescence (Pierce ECL western blotting substrate-32106) and imaged using a KODAC X-OMAT 2000 processor (Fujifilm).

Immunoprecipitation

For testing interaction between IFT81 mutant allele and IFT52, plasmid constructs carrying flag-tagged *IFT81* wildtype and c.1841T>C mutant allele was co transfected with IFT52-GFP plasmid constructs into HEK-293T cells. Protein extracts were subjected to Immunoprecipitation using GFP-Nanobeads (Chromotek) and interacting proteins eluted

using TCA soft elution technique. The elutes were analyzed using anti IFT52 (Gregory Pazour) and Anti-Myc (Covance MMS-150p).

Supplementary Clinical data

Supplementary Table 1: Predicted damaging effect of missense variants observed in the proband.

Gene	NM_number	Missense variants	SI FT*	Polyphen2_HDIV*	Polyphen2_HVAR*	LR TS	Mutation-type†	Mutation-Assessment†	Allele frequency	Grant ham distance#
IFT81	NM_00143779	c.1841 T>C (p.L614P)	T	D	D	De	Di	M	0.00001849	98
IRS1	NM_005544	c.2015 C>T (p.S672F)	T	D	D	De	Di	L	0	155
IRS1	NM_005544	c.1090 C>T (p.R364W)	D	D	D	N	Di	L	0.00001809	101
FAM186A	NM_00145475	c.4191 G>A (p.M1397I)	T	B	B	.	N	N	0	60
FAM186A	NM_00145475	c.1262 G>A (p.R421Q)	T	B	B	.	N	N	0.00005096	54
PKHD1L1	NM_177531	c.3835 G>A (p.G1279R)	T	B	B	N	N	M	0.0000166	125

PKH D1L1	NM_177 531	c.8099 A>G (p.N27 00S)	T	B	B	N	N	N	0	46
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Footnotes: * - D, damaging, T, tolerant, B, benign; § - De, deleterious, N, Neutral, U, Unknown; ‡ - Di, disease-causing, N, Neutral, P, Polymorphism; † - M, medium damaging (predicted functional), L, low damaging (predicted nonfunctional). ‘.’ in all databases, Not Found; # - Grantham scores (possible range from 5 to 215) of <50 were classified as nonsynonymous conservative, 51 to 100 as moderately conservative, 101 to 150 as moderately radical, and >151 as radical changes³⁹.

Supplementary data 2: Patient's test report

Blood routine, liver function, renal function and blood fat tests were all in normal functional range suggesting lack of any syndromic defects.

Examination Information & Report:

Test: Ultrasound of the Liver, Gallbladder, Pancreas, Spleen, Kidneys

Resolutions: 1280*800, 800*1280, 1080*1440

Date of Examine: 2013/04/18

Date of Report: 2013/04/18

Region of Examine: Liver, Gallbladder, Pancreas, Spleen, Kidneys

Results: The liver edge reaches the bottom of sternum and the bottom of the right ribs. The echo from the liver is uniform. The size of gallbladder is normal, and its wall is smooth. We didn't see any gallstone or neoplasia. The diameter of the common bile duct is 0.4cm, the Portal Venous is 1.0cm. The Morphology of the pancreas is normal. The thickness of the spleen is 3.5cm, and we didn't detect it from the bottom of ribs. Two kidneys are normal in sizes, morphologies and structure. There's no dilation found in renal pelvis or renal calyx.

Test: Liver function

Tests	Initials	Values	Units	Indicators	Reference Intervals
pH	PH	7.5			5.0-8.0
White Blood Cells	WBC	70	Cell/ul	H	<15
Nitrite	NIT	NEG			NEG
Protein	PRO	NEG	g/L		NEG

Glucose	GLU	NEG	mmol/L		NEG
Ketone	KET	NEG	mmol/L		NEG
Urobilinogen	UBG	3.2	umol/L		3-16
Bilirubin	BIL	NEG	umol/L		NEG
Red Blood Cell	BLD	NEG	Cell/ul		<25
Density/specific gravity	SG	1.025			1.005-1.030

Test: Kidney function

Tests	Initial s	Value s	Units	Indicator s	Reference Intervals
Alanine aminotransferase	ALT	16	U/L		05 -40
Total Protein	TP	75	g/L		60-85
Albumin	Ab	44	g/L		35-51
Albumin/Globulin Ratio	A/G	1.4			1.0-2.5
Total Bilirubin	Tbil	8.7	uMol/L		5.1-22.1
Direct Bilirubin	Dbil	2.7	uMol/L		0.0-8.6
Glutamyl Endopeptidase	GGT	33	U/L		10 -67
Alkaline phosphatase	ALP	56	U/L		30-120
Aspartate Aminotransferase	AST	15	U/L		05 -37
Total Bile Acid	TBA	3	uMol/L		0.1-10.0
Lactate Dehydrogenase	LD	114	U/L		97-270
Cholinesterase	ChE	9.1	kU/L		4.0-12.6
Potassium	K	4.4	mmol/L		3.5-5.5

Sodium	Na	143	mmol/L		134-145
Chlorine	Cl	108	mmol/L		96-111
Total carbo dioxide	TCO2	27.1	mmol/L		20.0-34.0
Calcium	Ca	2.4	mmol/L		2.13-2.70
Creatinine	Cr(E)	65	mmol/L		45-84
Urea	Urea	4.06	mmol/L		1.07-7.14
Glucose	Glu	4.9	mmol/L		3.6-6.1
Uric Acid	UA	371	mmol/L	H	150-357
Phosphate	P	1.33	mmol/L		0.81-1.45
Adenosine dehydrogenase	ADA	11.6	U/L		4.0-24.0
Pre Albumin	PA	238	mg/L		180-400.

Test: Blood Routine

Tests	Initials	Value s	Units	Indicator s	Reference Intervals
White Blood Cell	WBC	7.82	*1059/L		4.0-10.0
Lymphocyte Percentage	LY%	35.3	%		20-40.0
Monocyte Percentage	MONO%	4.2	%		3.0-8.0
Neutrophil Percen	NEUT%	57.2	%		50.0-75.0

Eosinophil Percen	EOS%	3.2	%		0.5-5.0
Basophil Percentage	BASO%	0.1	%		0.0-1.0
Lymphocyte Count	LY#	2.76	*1059/L		0.8-4.0
Monocyte Count	MONO#	0.33	*1059/L		0.12-0.80
Neutrophil Count	NEUT#	4.47	*1059/L		2.0-7.5
Eosinophil Count	EOS#	0.25	*1059/L		0.02-0.50
Basophil Count	BASO#	0.01	*1059/L		0.0-0.1
Red Blood Cell	RBC	4.38	*10512/L		3.5-5.0
Hemoglobin	HGB	132	g/L		110-150
Hematocrit	HCT	38.9	%		35.0-50.0
Mean Corpuscular Volume	MCV	88.8	fl		82.0-97.0
Mean Corpuscular Hemoglobin Concentration	MCHC	339	g/L		320-360
Mean Corpuscular Hemoglobin	MCHC	30.1	pg		27.0-32.0
Red Blood Cell Distribution Width	RDW-S	39.7	fl		39.0-46.0
Red Blood Cell Distribution Volume	RDW-C	12.4	%		0.0-15.0
Platelet	PLT	225	*1059/L		100-300
Platelet Hematocrit	PCT	0.26	%		0.11-0.28
Platelet Distribution Width	PDW	14.3	%		9.0-17.0
Mean Platelet Volume	MPV	11.7	fl		7.0-13.0
Large Platelet Ratio	P-LCR	39.3	%		13-43