Supplemental Data

Table S1. All antibodies used in the study

Antigen	Antibody	Host species	Source	Application
CFAP65(CCDC108)	A58454-050	Rabbit	EpiGentek	IF (1:400)
SPAG6	HPA038440	Rabbit	Sigma-Aldrich	IF (1:400)
Acetylated Tubulin	T7451	Mouse	Sigma-Aldrich	IF (1:1000)
Alexa Fluor 555	A-31572	Donkey	Invitrogen	IF (1:400)
Alexa Fluor 488	A-21121	Goat	Invitrogen	IF (1:400)

Table S2. Screening strategy to identify the causal genes by WES

Screening procedure	F1:II-2	F2:II-2	F3:II-1
Total variants	138,085	134,981	122642
Filter rare variants (MAF<5%) in public databases[1]	16631	15902	14328
Filter variants with functional impact[2]	359	331	291
Homozygous /compound heterozygous variants	18/21	17/20	11/13
Relevancy for phenotype[3]	2	2	1
Sanger sequencing/Co-segregation	1	1	1

Note: [1] represent the following three databases: dbSNP, 1000 Genomes Project, and ExAC; [2] represent variants predicted to be deleterious; [3] represent variants associated with phenotype, including expression in testis or the existence of a mouse KO model with infertility phenotype.

Table S3. The list of candidate variants relevant for the phenotype in affected individuals

Sample ID	Gene	RefSeq ID	Location	Amino Acid Alteration	Min Depth	Function	dbSNP ID	1000G	GO-ESP	ExAC	Co-segregation
F1:II-2	*CFAP65*	NM_194302	Chr2: 219868888	c.G5341T:p.E1781X	101	Stopgain	NA	NA	NA	NA	Yes
	SLAIN1	NM_001242868	Chr13: 78272267	c.219_220insGG:p.A73fs	50	Frameshift	rs2013800414	NA	NA	NA	No
F2:II-2	*CFAP65*	NM_194302	Chr2: 219890809	c.C2284T:p.R762X	107	Stopgain	NA	NA	NA	0.000016052	Yes
			Chr2: 219892637	c.1751delC:p.P584fs	225	Frameshift	NA	NA	NA	NA	Yes
	TCP11	NM_001261818	Chr6: 35086272	c.G1187A:p.R396Q	171	Missense	rs2234051	0.009984	0.008	0.008	No
			Chr6: 35086275	c.A1184G:p.Q395R	228	Missense	rs115171214	0.009984	0.008	0.0081	No
F3:II-1	*CFAP65*	NM_194302	Chr2: 219867716-219867723	c.5714_5721del:p.L1905fs	305	Frameshift	NA	NA	NA	NA	-
			Chr2: 219886611	c.C3021A:p.N1007K	323	Missense	NA	NA	NA	NA	-

Table S4. Infertility-related examination of the affected individuals

	Item[1]	F1:II-2	F2:II-2	F3:II-1	Normal Value Range
Hormonal profile	T	4.55	5.32	3.76	1.42-9.23ng/ml
	FSH	7.21	6.29	4.97	0.95-11.95mIU/ml
	LH	4.98	2.86	7.21	0.57-12.07mIU/ml
Testicular volume	Left	14	13	15	10-15ml
	Right	15	13	15	10-15ml
Semen routine Examination [2]	Progressive motility (%)	0	0	0	
	Semen volume	3-3.9	3.6-3.8	2.1-3.3	>1.5ml
	Sperm count	14.5-15.3	11.3-13.4	5.3-6.9	$>15\times10^6$ (/ml)

Note: [1]T: testosterone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; [2] Semen parameters were evaluated according to the World Health Organization (WHO, 2010) guidelines.

Table S5. Laboratory testing and outcomes of ICSI in the three couples with *CFAP65* mutations

	F1:II-2		F2:II-2		F3:II-1	
	Cycle-1	Cycle-2	Cycle-1	Cycle-2	Cycle-1	Cycle-2
Female age	25	25	24	24	37	37
No of oocytes retrieved	9	15	12	16	13	12
MII oocytes	2	3	5	6	9	5
Sperm origin	Ejaculated sperm		Ejaculated sperm		Ejaculated sperm	
Normal fertilization	2	3	4	6	9	4
Good quality embryos	0	1	2	3	5	3
Number of embryos transferred	0	1	2	2	2	1
Number of transfers	0	1	1	1	2	1
Clinical pregnancy	0	0	0	0	0	0
Ongoing pregnancy	0	0	0	0	0	0