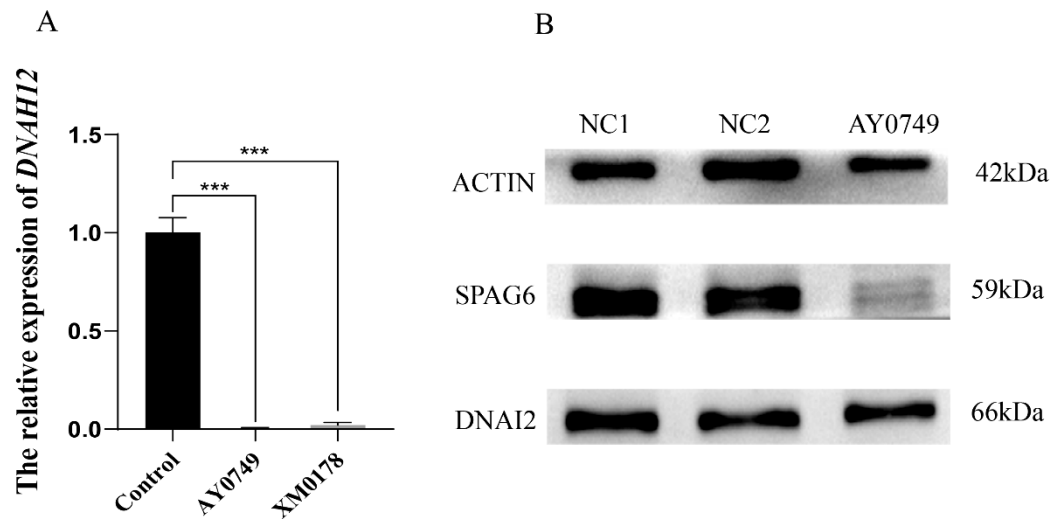


Supplemental information

**Further evidence from *DNAH12* supports favorable
fertility outcomes of infertile males with dynein
axonemal heavy chain gene family variants**

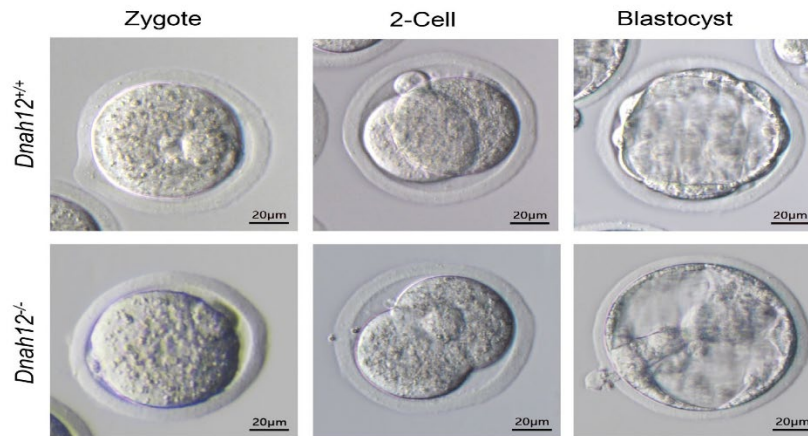
Hao Geng, Kai Wang, Dan Liang, Xiaoqing Ni, Hui Yu, Dongdong Tang, Mingrong Lv, Huan Wu, Kuokuo Li, Qunshan Shen, Yang Gao, Chuan Xu, Ping Zhou, Zhaolian Wei, Yunxia Cao, Yanwei Sha, Xiaoyu Yang, and Xiaojin He

Figure S1. Expression levels of mRNA and proteins in spermatozoa from NC and patients harboring biallelic variants of *DNAH12*, related to Figure 3.



(A) Relative expression of *DNAH12* mRNA in spermatozoa from NC and proband AY0749 and XM0178. The significance was determined via one-way ANOVA followed by Dunnett's test, $***p < 0.001$. (B) Expression of SPAG6 and DNAI2 in spermatozoa from NC and proband AY0749.

Figure S2. The blastocyst was successfully generated following intracytoplasmic sperm injection (ICSI) with spermatozoa both from *Dnah12*^{-/-} and WT male mice, related to Table 1.



	<i>Dnah12</i> ^{-/-}	WT
No. of oocytes injected	8	15
Fertilization rate (%)	75.0 (6/8)	93.3 (14/15)
Cleavage rate (%)	67.5 (5/8)	86.7 (13/15)
8-Cell formation rate (%)	37.5 (3/8)	66.7 (10/15)
Blastocyst formation rate (%)	12.5 (1/8)	40.0 (6/15)

Figure S3. Flowchart of variants filtration and selection strategy, related to STAR

Methods.

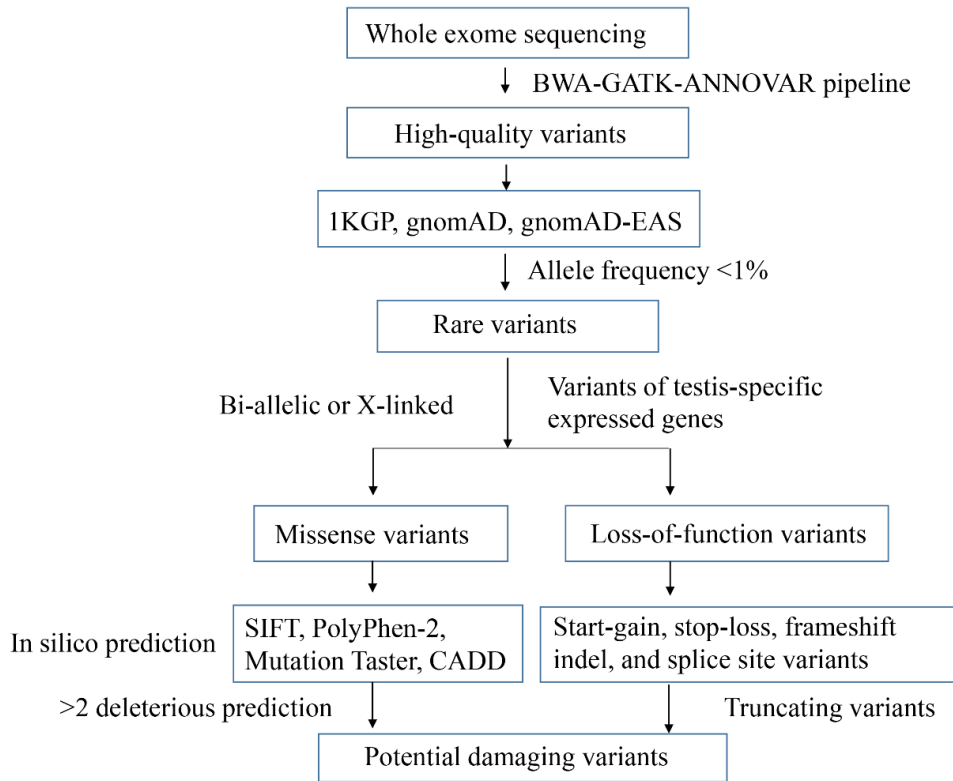


Table S1. Demographic characteristics of infertile males harboring biallelic *DNAH12* variants, related to Figure 2.

Subject	AY0749	NJ0278	XM0178
Sex	Male	Male	Male
Age(years)	31	41	33
Ancestry	East Asian	East Asian	East Asian
Ethnicity	Han Chinese	Han Chinese	Han Chinese

Table S3. Semen characteristics and sperm morphology in men harboring biallelic *DNAH12* variants, related to Figure 3.

Subject	AY0749		NJ0278		XM0178		Reference Values
Semen Parameter							
Semen volume (mL)	3.4	2.8	6.0	3.5	3.0	3.4	>1.5 ^a
Concentration (10 ⁶ /mL)	23.3	34.5	49.4	51.2	36.7	30.2	>15.0 ^a
Motility (%)	20.7	18.5	3.1	5.0	10.9	15.6	>40.0 ^a
Progressive motility (%)	2.8	3.1	0.9	2.3	2.3	6.7	>32.0 ^a
Sperm Morphology							
Normal head(%)	5		NA		3.5		>7.0 ^b
Normal flagella (%)	1.9		NA		30.4		>23.0 ^b
Absent flagella (%)	8.8		NA		0.5		<5.0 ^b
Short flagella (%)	15.2		NA		38.2		<1.0 ^b
Coiled flagella (%)	58.5		NA		3.9		<17.0 ^b
Angulation (%)	13.2		NA		15.9		<13.0 ^b
Irregular caliber (%)	2.4		NA		11.1		<2.0 ^b

^a According to WHO 5th edition standards set the reference limit.

^b Reference values shown by observation of morphologically abnormal sperm in fertile individuals.

Abbreviations: NA, not available.

Table S4. Primers used for identifying *Dnah12*^{-/-} mice, related to STAR Methods.

Primer Names	Primer Sequences (5'-3')	T_m
Dnah12-F1	AGGAGCATGACAGGAAGAGCTTG	65°C
Dnah12-R1	TGCACACAATCAAGCTCTGAGC	
Dnah12-F2	TTTAGGCGCTAGACTGAACTGC	65°C
Dnah12-R2	CTGGTCTCAAACACTACTGTGCAAC	

Table S5. Primers used for amplification and verification of *DNAH12* mutations, related to STAR Methods.

Primer Names	Primer Sequences (5'-3')	T_m
M1-DNAH12-F	GATTCAGCTACAAATGAAGAGGTCCTG	57°C
M1-DNAH12-R	AAGTTGTCTGGAGAGGATCACTGCTA	
M2-DNAH12-F	TCCAGTACTATACACATTCAACCCCAAC	57°C
M2-DNAH12-R	TCCATTCTGTGCAATCCAGGAA	
M3-DNAH12-F	GCAATGGCGTGATCTTGGCTCTT	57°C
M3-DNAH12-R	GTGTTTGTTCGCCACGGCG	
M4-DNAH12-F	GGGTCATTCTCTTCATCCTGG	57°C
M4-DNAH12-R	CTGGGTGAGGAGCAAGGACT	
M5-DNAH12-F	CGATCAATTAAAGATGTAAATGAACC	57°C
M5-DNAH12-R	TAATAAAGTGGACAAAATAGGGGA	

Table S6. Primers used for RT-qPCR assays, related to STAR Methods.

Primer Names	Primer Sequences (5'-3')	T_m
DNAH12-F	AGTCGGATGCCTATGTCTGTC	58°C
DNAH12-R	TGCCGAGTAGGTTGGTCT	
GAPDH-F	GGAGCGAGATCCCTCCAAAAT	58°C
GAPDH-R	GGCTGTTGTCATACTTCTCATGG	
Dnah12-F	TCCTGTTGACCATGCTAGCTGA	56°C
Dnah12-R	AGGCTCTTGAGTAAACGGAAG	
Gapdh-F	AGGTCGGTGTGAACGGATTTG	56°C
Gapdh-R	TGTAGACCATGTAGTTGAGGTCA	