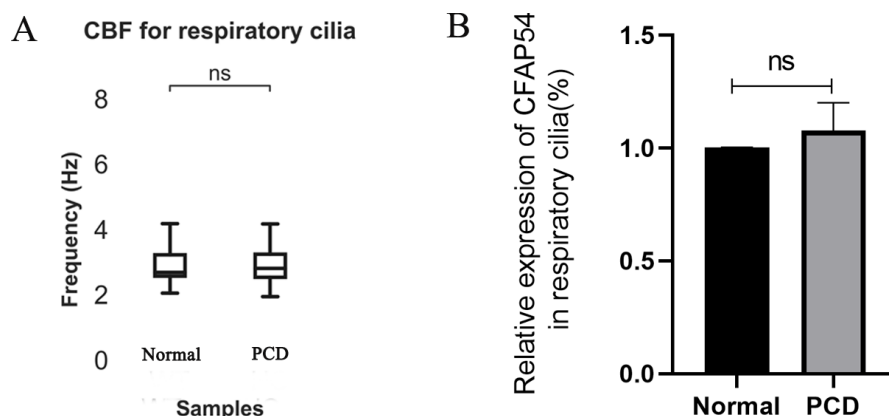
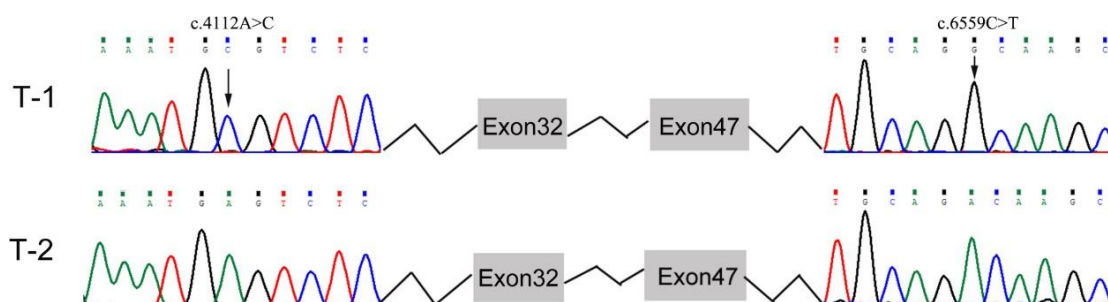


**Supplemental Figures:**



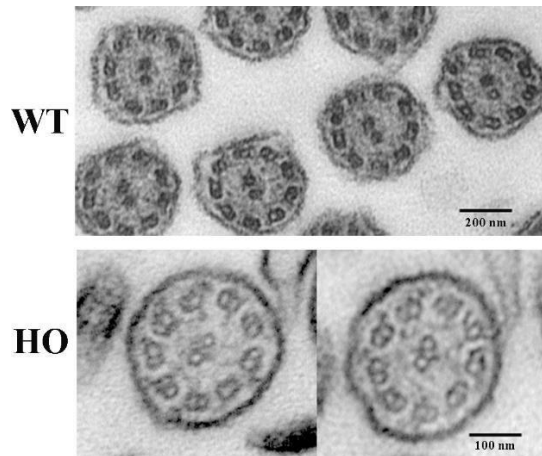
**Figure S1: Box plot of CBF and qPCR of respiratory cilia from the normal and proband. (A)**

The both value of CBF are around 3 Hz, ns means there was no difference. (B) qPCR analysis shows there was no difference in *CFAP54* mRNA expression of respiratory cilia from the normal and the proband.

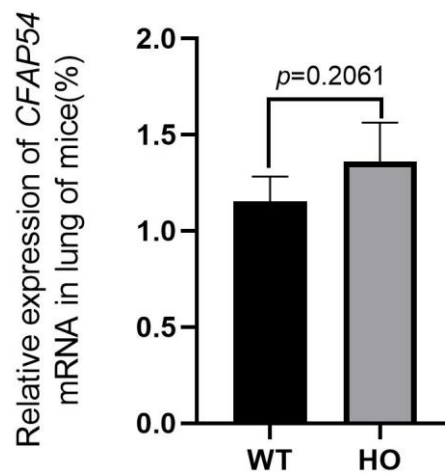


**Figure S2. The fragment of the amplification product.** The length of the product was 2784bp.

Comparison of the two mutations of c.4112A>C and c.6559C>T in the *CFAP54* mRNA from F2 cloned into the vector of pMD18-T. Two mutations were not observed in one same vector. T-1 and T-2 mean two pMD18-T vectors.



**Figure S3.** TEM of respiratory motile cilia of wild type and *Cfap54*<sup>ki/ki</sup> mouse model. No significant difference has been observed.



**Figure S4:** The expression of *Cfap54* mRNA in lung of *Cfap54*<sup>wt/wt</sup> and *Cfap54*<sup>ki/ki</sup> mice. qPCR analysis shows no statistical significance in mRNA expression of *Cfap54* using lung from wild-type and *Cfap54*<sup>ki/ki</sup> mice (n=3 WT, n=3 HO).

## Supplemental Materials and Methods

### *In vitro* minigene assay of the frameshift variant in *CFAP54*

An *in vitro* minigene assay was conducted as previously described(30). In general, exon 20 and the flanking intronic region of *CFAP54* with or without the frameshift variant were cloned into the intron of the two-exon (Exon A and Exon B) of *pCAS2* vector, including 525 nucleotides (nt) at the 3' end

of intron 19 and 2448 nt at the 5' end of intron 21, using the In-Fusion HD Cloning Kit (Takara, Japan). The wild-type and mutant plasmids were then transfected into HEK293T cells, followed by RNA extraction and reverse transcription using the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time; Takara, Japan). cDNA was subjected to quantitative PCR (qPCR) using Hieff qPCR SYBR Green Master Mix (YEASEN) with the internal control primer pair neo-F (5'-TTGTCAAGACCGACCTGTCC-3') and neo-R (5'-CCAATAGCAGCCAGTCCCTT-3'), amplifying the neo gene in the *pCAS2* plasmid and the target sequence primer pair exon 54-B-F (5'-TGACTCAAACCTGCTCCA-3') and exon 54-B-R (5'-AACTGTTGTTGCGACCTTCC-3'), which spans exon 20 of *CFAP54* and exon B of the *pCAS2* plasmid. The minigene assay was replicated twice, and qPCR was repeated three times for each experiment. A two-tailed simple *t*-test was performed to analyze the difference in RNA expression between the wild-type and mutant plasmids.

### **MRI of the mouse model**

MRI was performed on wild-type (n=3) and *Cfap54*<sup>ki/ki</sup> (n=3) mice older than 8 weeks to observe mucus accumulation in nasal sinuses and hydrocephalus in lateral ventricles. Mice were anesthetized with inhaled 3% isoflurane/O<sub>2</sub>, and an MRI scan was performed using a 7.0T Aligent (Varian) MRI system. The images were acquired in the coronal plane with a T2-fast spin-echo multislice sequence (FSEMS) (repetition time=3500 ms, echo time=72 ms, field of view =25.5×25.5 mm, number of slices=18, slice thickness=1 mm, matrix=256×256). During the MRI scan, the mice were anesthetized with 1.5% isoflurane/O<sub>2</sub>.

### **Histological analysis**

Mice older than 8 weeks were sacrificed by cervical dislocation. Heads from wild-type and *Cfap54*<sup>ki/ki</sup> mice were isolated, fixed in 4% paraformaldehyde/PBS solution overnight, and immersed in decalcification buffer (Servicebio Inc., China) for 2 weeks. Testes were fixed in 4% paraformaldehyde/PBS solution overnight. All samples were dehydrated through a graded ethanol series, embedded in paraffin wax, and sectioned. Nasal sinus sections were stained with alcian blue and periodic acid-Schiff reagent (AB-PAS) to observe mucus accumulation. Testes sections were stained with hematoxylin and eosin (H&E) to observe morphology. Sections were observed under a Leica DM6 B upright microscope, and photographs were obtained using a Leica DMC5400 color CMOS camera. Three wild-type mice paired with three *Cfap54*<sup>ki/ki</sup> mice were involved in testes histological study. Five wild-type, four heterozygous, and seven homozygous mutant mice were

sampled in nasal sinus analysis.

### **Sperm morphological study**

Fresh epididymides were isolated from wild-type (n=3) and *Cfap54<sup>ki/ki</sup>* mice (n=3) older than 8 weeks and immersed in PBS. The epididymides were cut into small pieces using clean scissors, mixed by pipetting, and allowed to stand for five minutes. Spermatozoa were dispersed in PBS solution, and the sperm suspension was spread onto slides, dried in air, fixed in methanol. Spermatozoa from mice and patient was stained with H&E. Slides were observed under a Leica DM6 B upright microscope, and photographs were obtained using a Leica DMC5400 color CMOS camera.

### **Cfap54 mRNA expression in testes of the mouse model**

Total RNA was isolated from testes of wild-type and *Cfap54<sup>ki/ki</sup>* mice under the standard RNA extraction procedure using TRIzol (Gibco-BRL, San Francisco, CA, USA) and chloroform and then reverse transcribed into cDNA using PrimeScript RT Master Mix (Takara, Japan). *Cfap54* mRNA expression was evaluated by qPCR using Hieff qPCR SYBR Green Master Mix (Yeastar, China) with the primer pair mCFAP54-F (5'-CCTCATGTCATGCTACTG-3'); mCFAP54-R (5'-CCACTTACTGATGTCCTT-3')(13) and mGAPDH-F (5'-AGGTCGGTGTGAACGGATTTG-3'); mGAPDH-R (5'-TGTAGACCATGTAGTTGAGGTCA-3').

### **CFAP54 mRNA expression in PCD patient**

Total RNA was extracted from patient bronchial mucosa and sperms under the standard RNA extraction procedure using TRIzol (Gibco-BRL, San Francisco, CA, USA) as previously described. CFAP54 mRNA expression in patient was evaluated by qPCR using Hieff qPCR SYBR Green Master Mix (Yeastar, China) with primer pair hCFAP54-F (5'-TTCCCATCTCAACAAATTGCCAG-3'); hCFAP54-R (5'-GTCCATAACATTGAGTCACAGCTTG-3') and hGAPDH-F (5'-GGAAGGTGAAGGTCGGAGTC-3'); hGAPDH-R (5'-GTTGAGGTCAATGAAGGGGTC-3') and compared with a healthy control.