Supp. Figure S1. Reads mapped to the reference human genome hg19 at the site of the c.924C>A (p.E309*) mutation. All fifteen reads are high quality and there are no wild type alleles. The image was created using IGV. NB: The CCDC151 gene (GenBank reference sequence: NM_145045.4) is on the reverse strand, therefore the sequence (shown here) must be reversed (e.g. the mutation is G>T causing GAG to become TAG, a premature stop codon). This variant status (i.e. homo/heterozygosity) was confirmed using Sanger sequencing and AvrII digestion in the proband, parents and the unaffected brother (Supp. Figure S2). Nucleotide numbering system uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon (Met) as codon 1.
Supp. Figure S2 Confirmation of variant status in proband and other family members using (a-d) *Sanger sequencing and (e-f) AvrII digestion. (a) Proband (b) Unaffected brother (c) Mother (d) Father (e) PCR amplicons before restriction enzyme digestion (f) After digestion. Ladder: 300bp (top), 200bp and 100bp (bottom). DNA Chromatogram images were created using Chromas Lite (v2.1.1). *Peak height imbalances could have been caused by low template DNA, degraded DNA and/or preferential amplification.
Supp. Figure S3A-C. Screening the Saudi population for the p.E309X variant. 96-well MADGE images reveal that none of the 238 individuals have the causal allele. Ladders last three bands are 100bp (bottom), 200bp and 300bp (top).
Supp. Table S1. Local sequence alignment containing the mutated residue from multiple alignment of the *CCDC151* gene in different organisms (relevant species shown)

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The highlighted Glutamic acid (E) residue is found to be highly conserved across many species where the gene is predicted to be a homologue of the human *CCDC151* gene. The alignment was carried out using the Uniprot website’s Blast and Align functions (http://www.uniprot.org).
Supp. Table S2. Primers used to amplify a 221bp long region containing the p.E309X mutation in the *CCDC151* gene

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<td>5'-GAACCAGCTGCAGTACCTAGAG-3'</td>
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Supp. Table S3. The AvrII enzyme will digest the PCR amplicons produced using the primers in Supp. Table S1 where it comes across the sequence CCTAGG (cutting between the two cytosine bases).

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<th>Enzyme</th>
<th>Cut site</th>
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<th>Affected</th>
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<td>221bp</td>
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Since the unaffected individuals will not have the thymine base required for digestion, the PCR amplicons will stay unaltered (i.e. 221bp long).
**Supp. Materials and Methods**

**Ethical approval and consents**

Ethical approval was obtained from the King Saud University/King Khalid Hospital, Riyadh ethical committee (approval number: E-11-448). Family and individual consent was verbal, with the recognition that positive findings would be diagnostically reconfirmed in conjunction with clinical counselling and feedback.

For the mutation screening samples (238 individuals), inclusion was voluntary, and informed written consent for anonymised genetic studies was taken in keeping with King Saud University College of Applied Medical Sciences guidelines.

**Participants and Genetic Data Analysis**

DNA was extracted from peripheral blood using the QIAamp DNA Mini kit provided by QIAGEN (Catalogue No: 51304); and the protocol for DNA Purification from Blood or Body Fluids was followed in the QIAamp DNA Mini and Blood Mini handbook. The exome was captured using the Agilent SureSelect Human All Exon 50M exon capture kit and WES data was obtained by subsequent sequencing using the Illumina Hiseq2000 platform. The Burrows-Wheeler Aligner (BWA)\(^1\) software was used to align the reads to the latest human genome reference sequence (hg19), filtering out reads which have low base quality bases (more than half of the bases which have a base quality of ≤ 5, including no calls) and/or with a mapping score of zero. Picard (http://picard.sourceforge.net) was used to mark duplicated reads and the alignment results were generated in BAM format. Single nucleotide polymorphisms (SNPs) were called using SOAPsnp\(^2\) and small insertion/deletion events (indel) were detected by Samtools and GATK, and exported in VCF format\(^3\)\(^-\)\(^5\). VCF annotations were obtained from the Ensembl Variant Effect Predictor (VEP, for SNPs)\(^6\) and Annovar (for indels)\(^7\). Predictions for missense mutations were obtained from FATHMM\(^8\), SIFT\(^9\), Polyphen-2\(^10\) and Condel\(^11\). Long runs of homozygosity larger than 5Mb (LRoH) were detected using Plink\(^12\), and shorter ones (<5Mb) were detected manually using a custom Python script (input format: VCF) which plots homozygosity/heterozygosity state; and the resulting regions were converted to BED format (http://genome.ucsc.edu/FAQ/FAQformat.html#format1) to be viewed in IGV\(^13\). Initially we created two lists of genes to be reviewed as ‘prime candidates’ in the proband. The first one (hereafter called list 1, see PCD List 1) had all the known human PCD genes aforementioned.
The second (hereafter list 2, see PCD List 2) had all the genes (except known ones) in the Ciliome database (last updated: 24th Dec 2007) plus a few additional genes which matched the keywords ‘dynein’, ‘radial spoke’, ‘nexin link’ and/or ‘cilia’ in the GeneCards website (www.genecards.org, v3.11). A separate analysis was done on all mutations with all genes included to ensure a non-biased analysis. Using the statistical software Stata (v13.1), VEP/Annovar annotations and VCF files were merged using the rsID as the key variable (a custom Python script was produced to assign an rsID which matches VEP output in the VCF files if missing). This enabled reviewing all ‘predicted high impact’ (PHI, hereafter Φ) mutations (i.e. very rare stop gains/losses, start losses, splice-site acceptor/donor variants, missense mutations, indels – both non-frameshifting and frameshifting) simultaneously which are homozygous and either are absent or very rare (<0.1%) in The Single Nucleotide Polymorphism Database (dbSNP) database. The remaining mutations were then compared against our internal non-PCD patient database (previously whole-exome sequenced 13 individuals of Arabic ancestry), Exome Variant Server (EVS) and 1000 Genomes Project (1000GP) for their presence and minor allele frequency (MAF). The STRING software was used to predict the interactome of remaining candidate genes. Additionally, a CNV analysis was carried out on the WES data using Control-FREEC.

**Screening for c.924C>A in Saudi Arabian sample**

DNA was extracted as abovementioned. PCR was used to amplify a region 221bp long (containing the stop gain loci) in these individuals (Supp. Table S1); and these fragments were digested using the AvrII enzyme (following manufacturer New England Biolabs’ protocol, catalogue no: R0174L) and viewed using 96-well microplate array diagonal gel electrophoresis (MADGE) to check for the presence of the p.E309* mutation in the Saudi population. Nucleotide numbering system uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon (Met) as codon 1.

**Electron Microscopy**

Endoscopic nasal biopsy was taken from the posterior portion of the inferior turbinate. A piece of tissue measuring 2mm in (maximum) diameter was obtained and fixed in 2% buffered glutaraldehyde. Following fixation, the tissue was post-fixed in a buffered solution of osmium tetroxide in order to enhance the contrast.
For ultrastructural examination, the tissue was subsequently plastic embedded and ultrathin sections are cut using a diamond knife. The ultra-thin sections were mounted on a grid and sequentially stained by immersing the grid in solutions of lead citrate and urinyl acetate. Semi-thin sections were cut at a thickness of 0.5-1 µm and stained with toluidine blue. The semi-thin sections were used to guide the selection of the area to be viewed in ultra-thin sections. The ultra-thin sections were then examined using the JEOL transmission Electron Microscope (model: JEM-1400).

**Supp. Results**

**Whole-exome sequencing of proband**

Total captured region was 118,507,605 base pairs (50,620,566 bases on target and 67,887,039 bases near target, the latter being flankings region within 200bp of exons). Coverage of target (i.e. exons) and flanking regions (e.g. introns, splice sites) was 98.2% and 92.7% respectively. The average sequencing depth on target was 61.49 and the fraction of target covered with at least 20 and 10 reads was 78.5% and 88.2% respectively (and >4 read depth = 94.4%). There were a total of 51,751,389 (high quality) reads with a mapping rate of 99.21%. All known PCD genes had similar figures and no idiosyncrasies were observed.

**Motility of cilia**

Although we cannot provide a video of cilia beating, our technicians have noted that >80% of the cilia were immotile in the tissue analysed.

**Supp. References**


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