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Supplemental Data

Mutations in *DNAJB13*, Encoding an HSP40 Family Member, Cause Primary Ciliary Dyskinesia and Male Infertility

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Figure S1

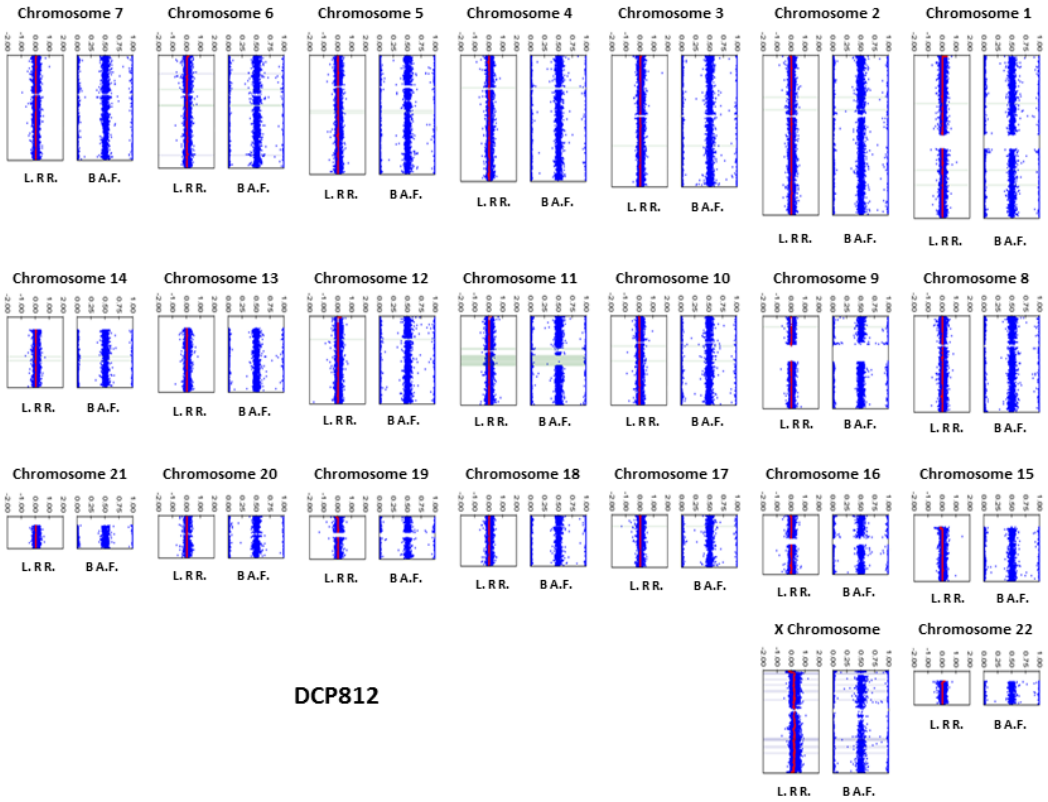

Figure S1. Whole-Genome SNP Genotyping in Individual DCP812

DCP812 was genotyped with the HumanCytoSNP-12 chip from Illumina and the data were analyzed with the Genome Studio and CNV partition 3.1.6 softwares (Illumina). Each blue dot represents one individual single-nucleotide polymorphism (SNP). For each chromosome, the right panel shows the B allele frequency (B A. F.). For each SNP, a low B allele frequency indicates that the individual is homozygous for the A allele; intermediate values mean that they are heterozygous, and a high B allele frequency means that they are homozygous for the B allele. Regions of homozygosity larger than 100 kb are shaded in green. The *DNAJB13* mutation of individual DCP812 is localized in a large region of homozygosity of chromosomal region 11q13.4 (16.4 Mb). For each chromosome, the left panel represents the log R ratio (L. R R.), which is the log ratio of observed probe density to expected probe density. A null L. R R. indicates the absence of loss or gain of material.

Figure S2

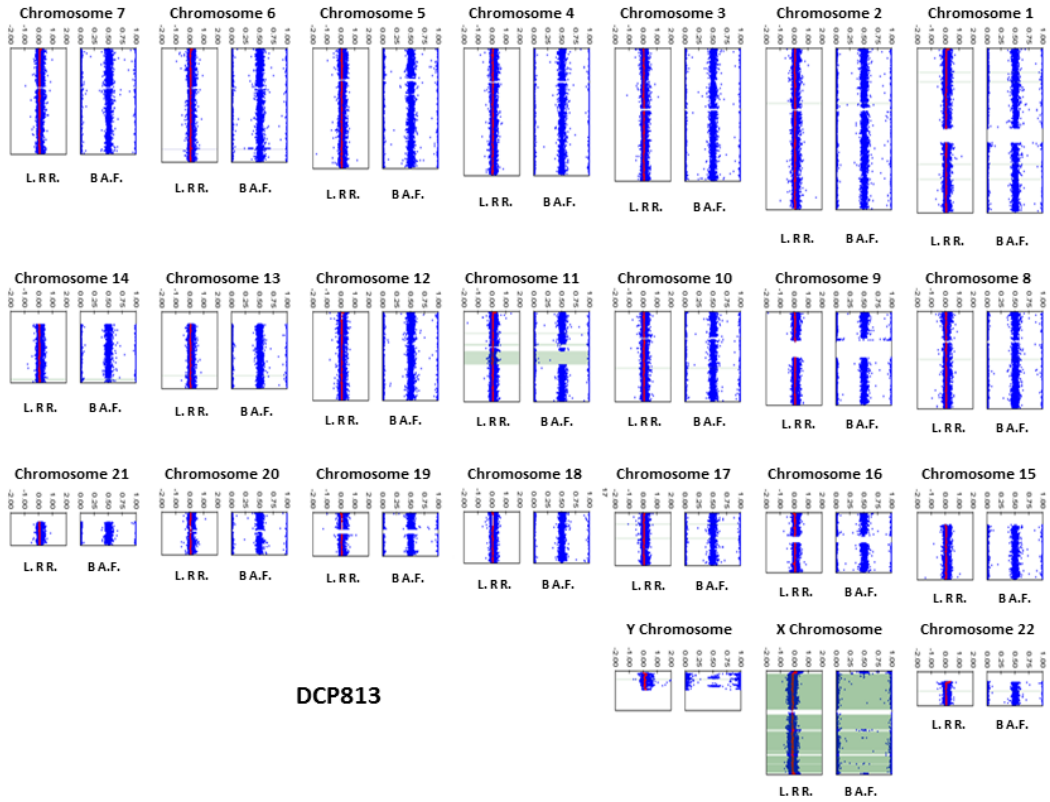

Figure S2. Whole-Genome SNP Genotyping in Individual DCP813

DCP813 was genotyped with the HumanCytoSNP-12 chip from Illumina and the data were analyzed with the Genome Studio and CNV partition 3.1.6 softwares (Illumina). Each blue dot represents one individual single-nucleotide polymorphism (SNP). For each chromosome, the right panel shows the B allele frequency (B A. F.). For each SNP, a low B allele frequency indicates that the individual is homozygous for the A allele; intermediate values mean that they are heterozygous, and a high B allele frequency means that they are homozygous for the B allele. Regions of homozygosity larger than 100 kb are shaded in green. The *DNAJB13* mutation of individual DCP813 is localized in a large region of homozygosity of chromosomal region 11q13.4 (20.7 Mb). For each chromosome, the left panel represents the log R ratio (L. R R.), which is the log ratio of observed probe density to expected probe density. A null L. R R. indicates the absence of loss or gain of material.

Figure S3

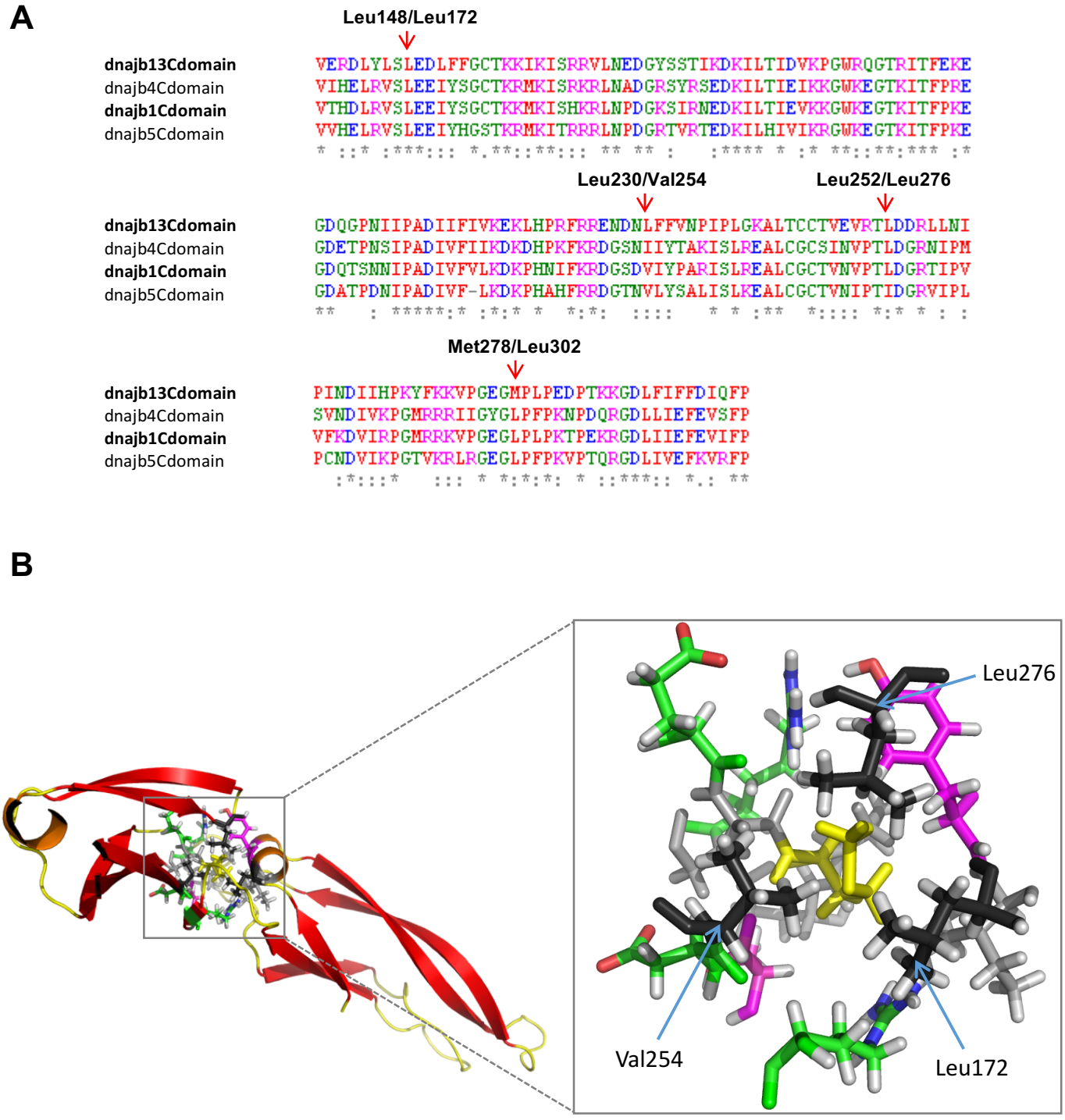
A
Leu148/Leu172
dnajb13Cdomain
dnajb4Cdomain
dnajb1Cdomain
dnajb5Cdomain
VERDLYLSLEDLFFGCTKKIKISRRVLNEDGYSSTIKDKILTIDVKPGWRQGTRITFEKE
VIHELRVSLEEIYSGCTKRMKISRKRLNADGRSYRSEDKILTIEIKKGWKEGTKITFPRE
VTHDLRVSLEEIYSGCTKKMKISHKRLNPDGKSIRNEDKILTIEVKKGWKEGTKITFPKE
VVHELRVSLEEIYHGSTKRMKITRRRLNPDGRTVRTEDKILHIVIKRGWKEGTKITFPKE
Leu230/Val254
Leu252/Leu276
GDQGPNIIPADIIFIVKEKLHPRFRRENDNLFFVNPIPLGKALTCCTVEVRTLDDRLLNI
GDETPNSIPADIVFIIKDKDHPKFKRDGSNIIYTAKISLREALCGCSINVPTLDGRNIPM
GDQTSNNIPADIVFVLKDKPHNIFKRDGSDVIYPARISLREALCGCTVNVPTLDGRTIPV
GDATPDNIPADIVF-LKDKPHAHFRRDGTNVLYSALISLKEALCGCTVNIPTIDGRVIPL
Met278/Leu302
dnajb13Cdomain
dnajb4Cdomain
dnajb1Cdomain
dnajb5Cdomain
PINDIIHPKYFKKVPGEGMPLPEDPTKKGDLFIFFDIQFP
SVNDIVKPGMRRRIIGYGLPFPKNPDQRGDLLIEFEVSFP
VFKDVIRPGMRRKVPGEGLPLPKTPEKRGDLIIEFEVIFP
PCNDVIKPGTVKRLRGEGLPFPKVPTQRGDLIVEFKVRFP
B
Leu276
Val254
Leu172

Figure S3. Protein Sequence Alignment of the DNAJ_C Domain from DNAJB13 with its Close Paralogs, and Structural Organization of the DNAJ_C Domain of DNAJB1 in which Leu302, corresponding to Met278 of DNAJB13 was replaced by a Methionine Residue, as Modeled from the Crystal Structure of the DNAJ_C Domain from DNAJB1.

(A) The multiple sequence alignment, using Clustal Omega software, demonstrates the high homology between the DNAJ_C domain of human DNAJB13 and its close paralogs DNAJB4, DNAJB1 and DNAJB5. The Met278, Leu148, Leu230 and Leu252 residues in DNAJB13 (corresponding to Leu302, Leu172, Val254 and Leu276, respectively, in DNAJB1) are indicated by a red arrow. The colors of the residues correspond to their physicochemical properties; red: small + hydrophobic (including aromatic -Y)); blue: acidic; magenta: basic – H; green: hydroxyl + sulfhydryl + amine + G. *: identical residues in all sequences; **:**: residues sharing similar properties in all sequences.

(B) Predicted 3D structure of the DNAJ_C domain of DNAJB1 carrying a methionine residue at position 302, as modeled from the crystal structure of the DNAJ_C domain of wild-type DNAJB1 (Agx). Leu302, corresponding to Met278 in DNAJB13 (see A panel), was replaced by a methionine residue using Modeller 9.10 and PROCHECK for validation. Left panel: the secondary structure is colorized according to the following code: beta-sheets in red, alpha helices in orange, and loops in yellow. Right panel: magnification of the region of interest. Residues are represented with sticks. Oxygen, nitrogen, sulfur and hydrogen atoms are shown in red, blue, dark yellow and grey, respectively. Amino acid residues (carbon atoms of the lateral chain) are colorized according to the following code: the methionine residue of interest is in yellow; the non-polar residues in close proximity to that methionine (i.e. Leu172, Val254 and Leu276) are in black; the other non-polar residues are in grey; the neutral polar and charged polar residues are in purple and in green, respectively.

Figure S4

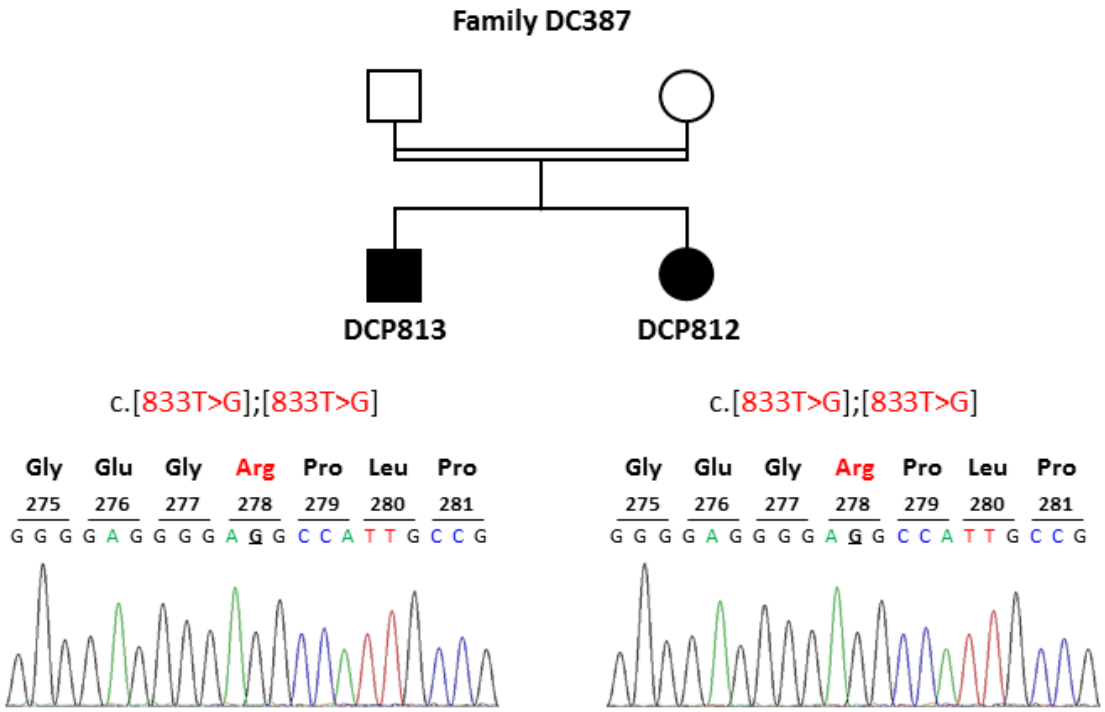

Figure S4. Sanger Sequencing Showing the *DNAJB13* c.833T>G (p.Met278Arg) Mutation Identified in Individuals DCP812 and DCP813 (Family DC387)

Direct sequencing of amplified genomic DNA was performed to validate the mutation previously identified by whole-exome sequencing. The mutation is present in the homozygous state, as deduced from homozygosity mapping data (see Figure S1).

Figure S5

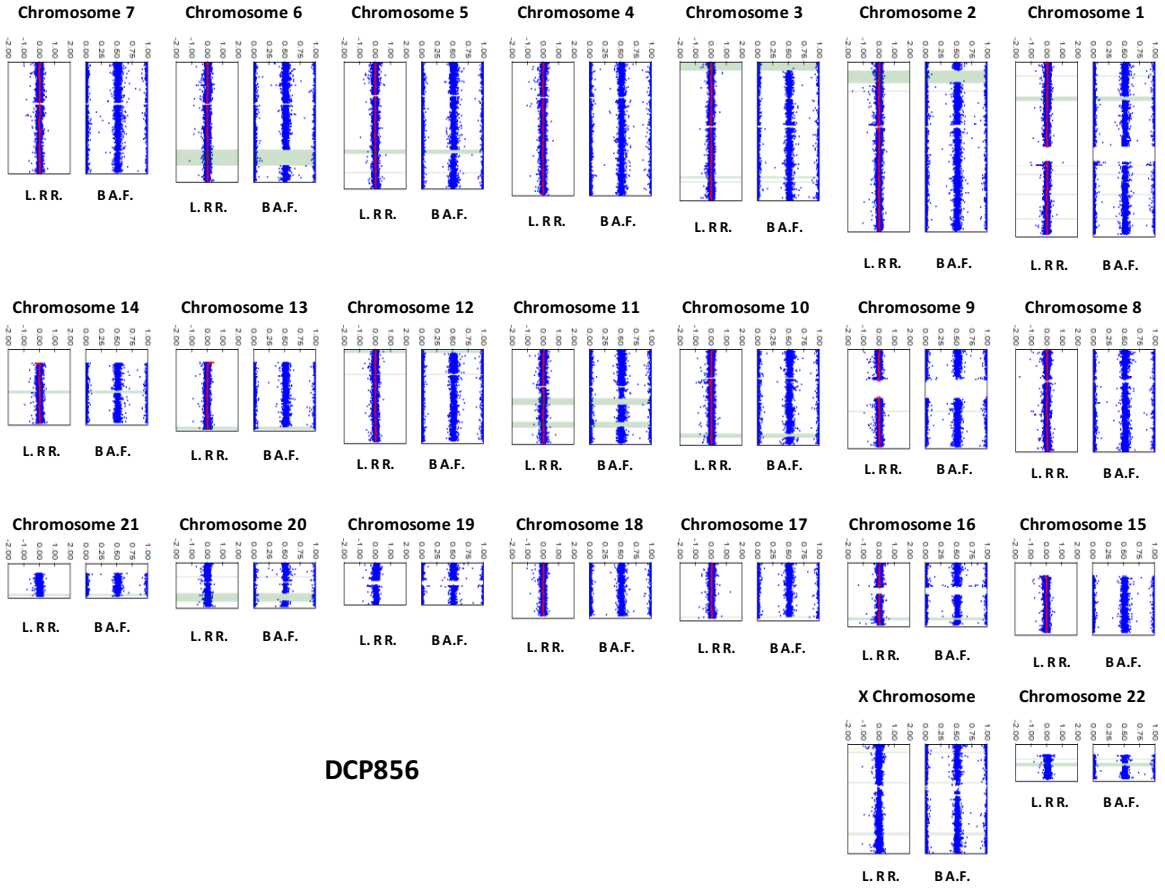

Figure S5. Whole-Genome SNP Genotyping in Individual DCP856

DCP856 was genotyped with the HumanCytoSNP-12 chip from Illumina and the data were analyzed with the Genome Studio and CNV partition 3.1.6 softwares (Illumina) designed to identify homozygous regions. Each blue dot represents one individual single-nucleotide polymorphism (SNP). For each chromosome, the right panel shows the B allele frequency (B A. F.). For each SNP, a low B allele frequency indicates that the individual is homozygous for the A allele; intermediate values mean that they are heterozygous, and a high B allele frequency means that they are homozygous for the B allele. Regions of homozygosity larger than 100 kb are shaded in green. The *DNAJB13* mutation of individual DCP856 is localized in a region of homozygosity of chromosomal region 11q13.4 (10.4 Mb). For each chromosome, the left panel represents the log R ratio (L. R R.), which is the log ratio of observed probe density to expected probe density. A null L. R R. indicates the absence of loss or gain of material.

Figure S6

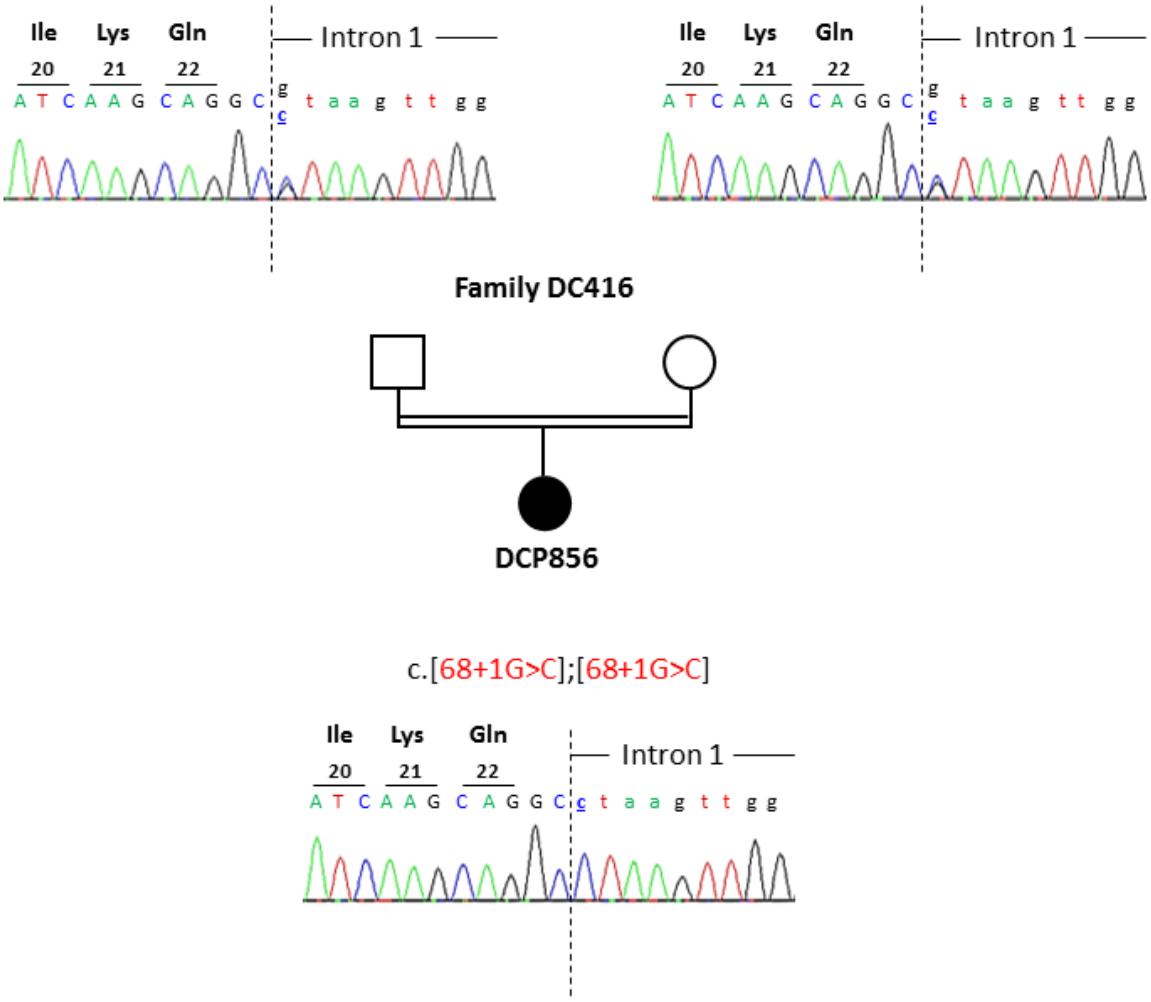

Figure S6. Sanger Sequencing Showing the *DNAJB13* c.68+1G>C Mutation Identified in Individual DCP856 (Family DC416)

Direct sequencing of amplified genomic DNA was performed to validate the mutation previously identified by whole-exome sequencing. The mutation is present in the homozygous state, both unaffected parents carrying the mutation in the heterozygous state.

Figure S7

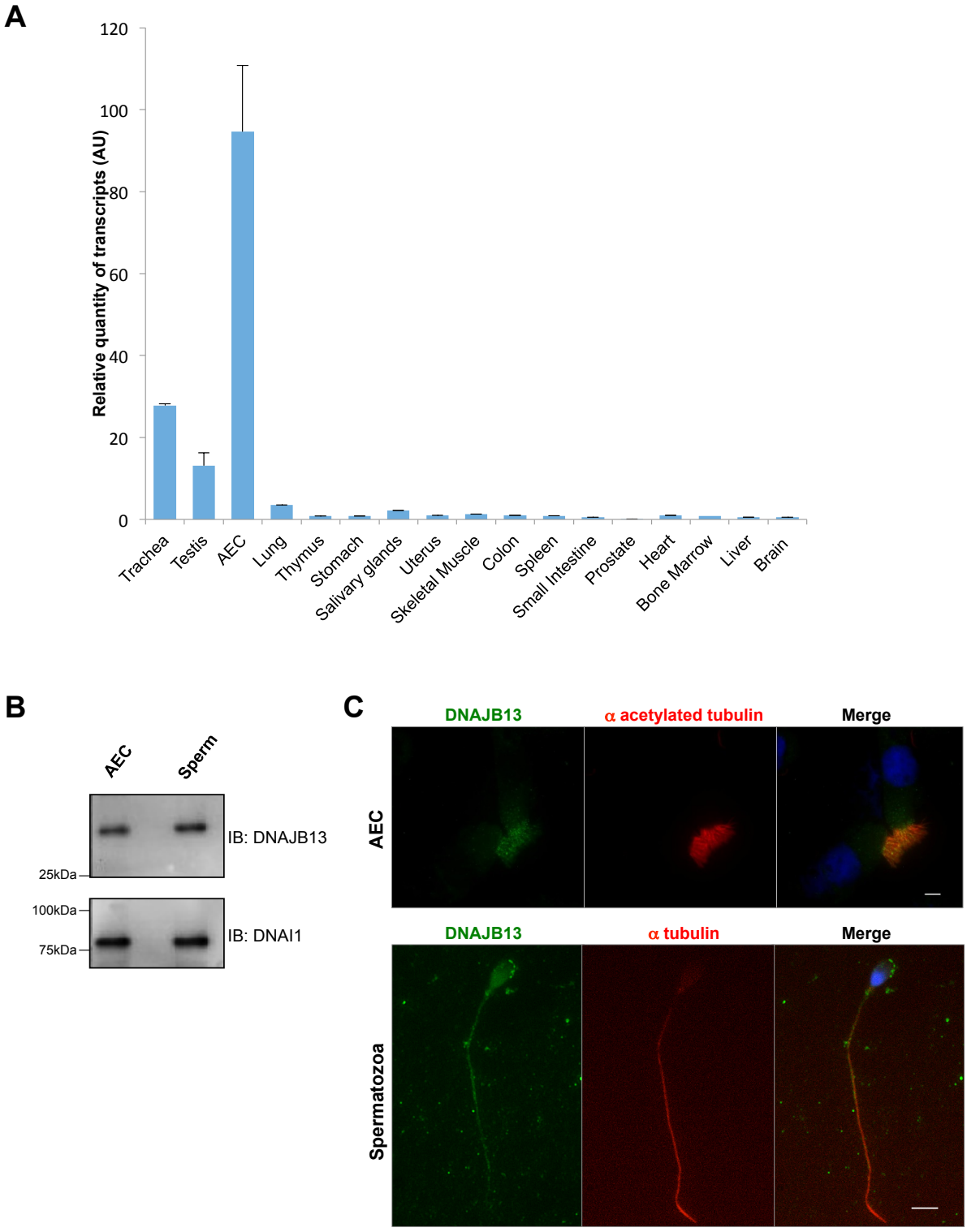
A
Relative quantity of transcripts (AU)
120
100
80
60
40
20
0
Trachea
Testis
AEC
Lung
Thymus
Stomach
Salivary glands
Uterus
Skeletal Muscle
Colon
Spleen
Small Intestine
Prostate
Heart
Bone Marrow
Liver
Brain
B
AEC
Sperm
IB: DNAJB13
25kDa
100kDa
75kDa
IB: DNAI1
C
DNAJB13
α acetylated tubulin
Merge
AEC
DNAJB13
α tubulin
Merge
Spermatozoa

Figure S7. Expression Pattern of Human *DNAJB13* and Localization of DNAJB13 protein in the Axoneme of Human Cilia and Flagella

(A) Expression analysis of *DNAJB13*, as assessed by quantitative RT-PCR. The ubiquitously expressed *ERCC3* gene was used as internal control; values are the mean ± SD of two independent experiments. *DNAJB13* transcripts are found at highest levels in the trachea, testis and airway epithelial cells (AEC). **(B)** Western blot analysis showing the detection of DNAJB13 protein in human AEC and sperm. **(C)** Immunofluorescence assays performed on human AEC and spermatozoa show DNAJB13 co-localization with α-Tubulin in cilia and flagella, respectively. Scale bars represent 5 μm.

Figure S8

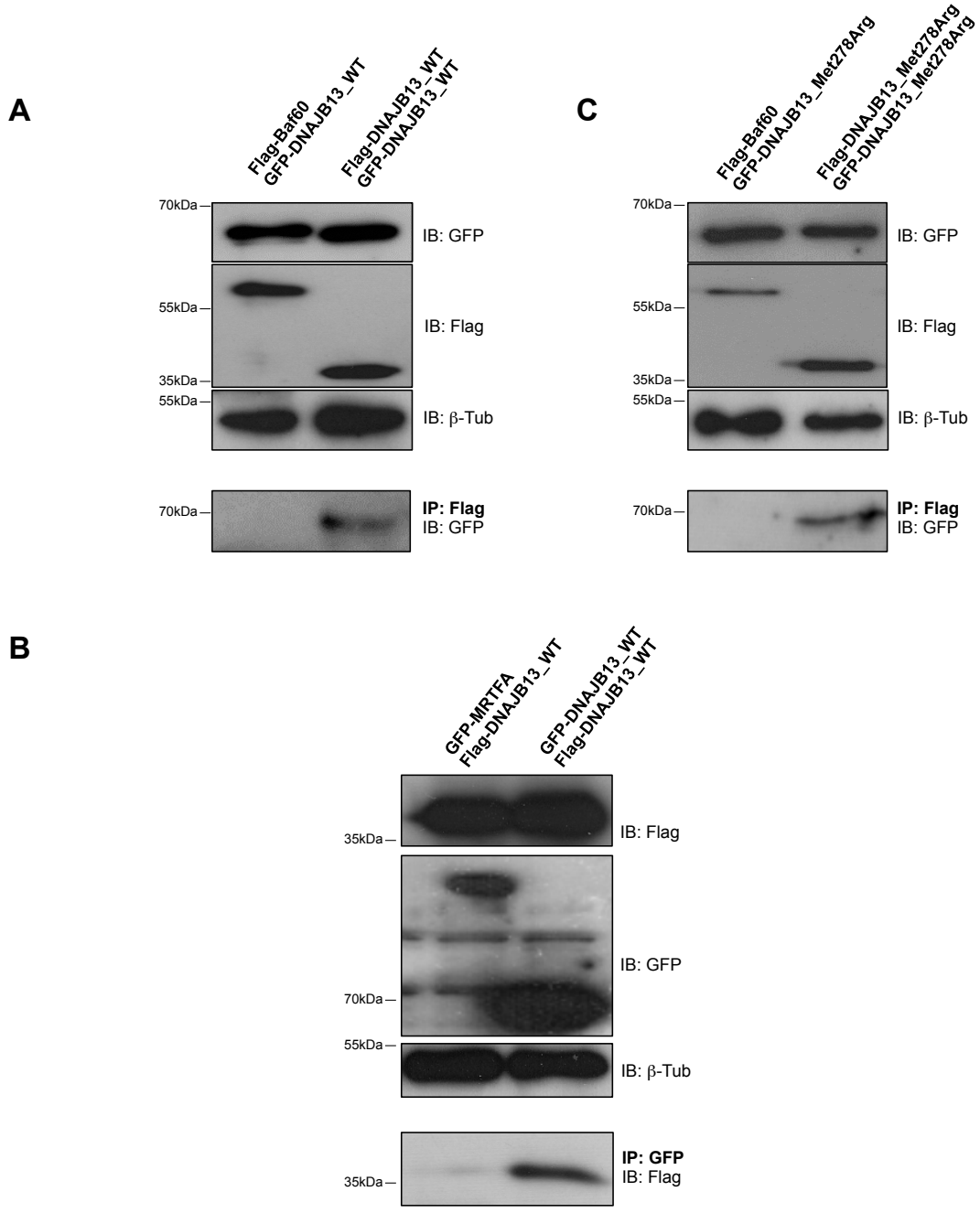
A
Flag-Baf60
GFP-DNAJB13_WT
Flag-DNAJB13_WT
GFP-DNAJB13_WT
70kDa
IB: GFP
55kDa
IB: Flag
35kDa
55kDa
IB: β-Tub
70kDa
IP: Flag
IB: GFP
C
Flag-Baf60
GFP-DNAJB13_Met278Arg
Flag-DNAJB13_Met278Arg
GFP-DNAJB13_Met278Arg
70kDa
IB: GFP
55kDa
IB: Flag
35kDa
55kDa
IB: β-Tub
70kDa
IP: Flag
IB: GFP
B
GFP-MRTFA
Flag-DNAJB13_WT
GFP-DNAJB13_WT
Flag-DNAJB13_WT
35kDa
IB: Flag
IB: GFP
70kDa
55kDa
IB: β-Tub
35kDa
IP: GFP
IB: Flag

Figure S8. Impact of the DNAJB13 p.Met278Arg Mutation on the Homodimerization state of DNAJB13

(A) CHO-K1 cells were transiently co-transfected with plasmids encoding the GFP-DNAJB13 WT and the Flag-DNAJB13 WT proteins or the Flag-Baf60 protein used as a negative control. After 24 hours, the proteins were extracted and immunoprecipitation was performed using an anti-Flag antibody. The upper panel shows protein levels of Flag and GFP tagged DNAJB13 prior to immunoprecipitation. β-tubulin was used as a loading control. The lower panel shows the detection of GFP-DNAJB13 upon immunoprecipitation (IP Flag). The results presented are representative of 3 independent experiments.

(B) The same experiment described in (A) was conducted using the DNAJB13 p.Met278Arg mutant protein.

(C) CHO-K1 cells were transiently co-transfected with plasmids encoding the Flag-DNAJB13 WT and the GFP-DNAJB13 WT proteins or the GFP-MRTFA protein used as a negative control. After 24 hours, the proteins were extracted and immunoprecipitation was performed using an anti-GFP antibody. The upper panel shows the detection of Flag and GFP tagged DNAJB13 proteins prior to immunoprecipitation. β-tubulin was used as a loading control. The lower panel shows the detection of Flag-DNAJB13 after immunoprecipitation (IP GFP).

Figure S9

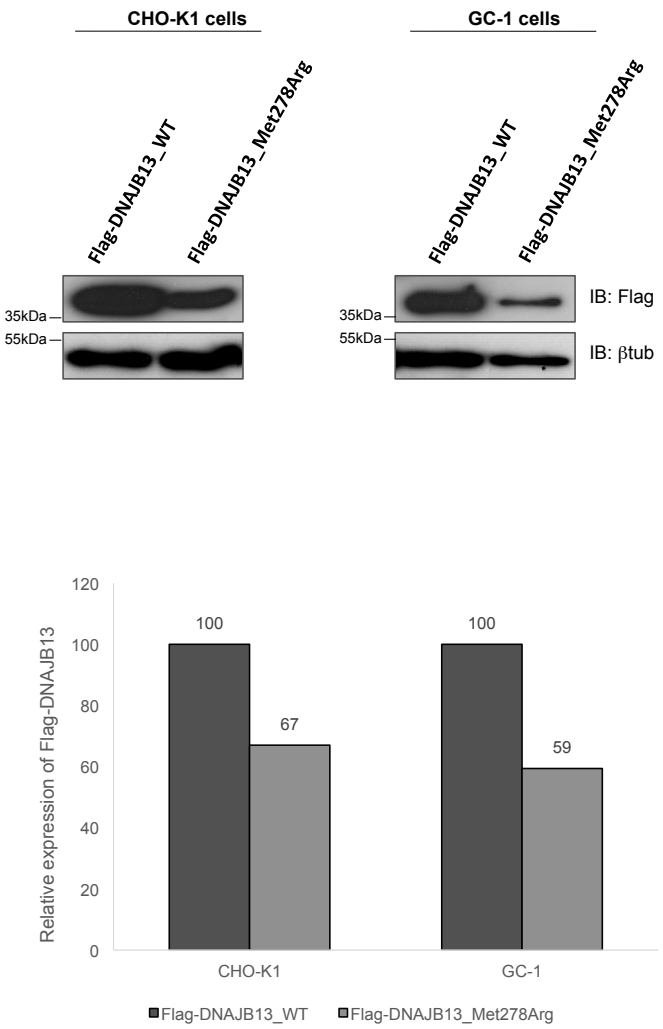

Figure S9. Impact of the DNAJB13 p.Met278Arg Mutation on DNAJB13 protein levels in CHO-K1 and GC-1 cells

Upper panel: CHO-K1 cells and GC-1 cells were transiently transfected with 0.5µg of the plasmids encoding for the Flag-DNAJB13_WT and Flag-DNAJB13_Met278Arg proteins. After 24 hours, the proteins were extracted and equal amounts of protein extracts were subjected to SDS-PAGE using 14% gels and immunoblotted with an anti-Flag antibody. β-tubulin was used as a loading control. **Bottom panel**: The Flag signal was quantified using ImageJ software and normalized to the β–tubulin levels. Results are expressed as a percentage of the wild-type condition.

Figure S10

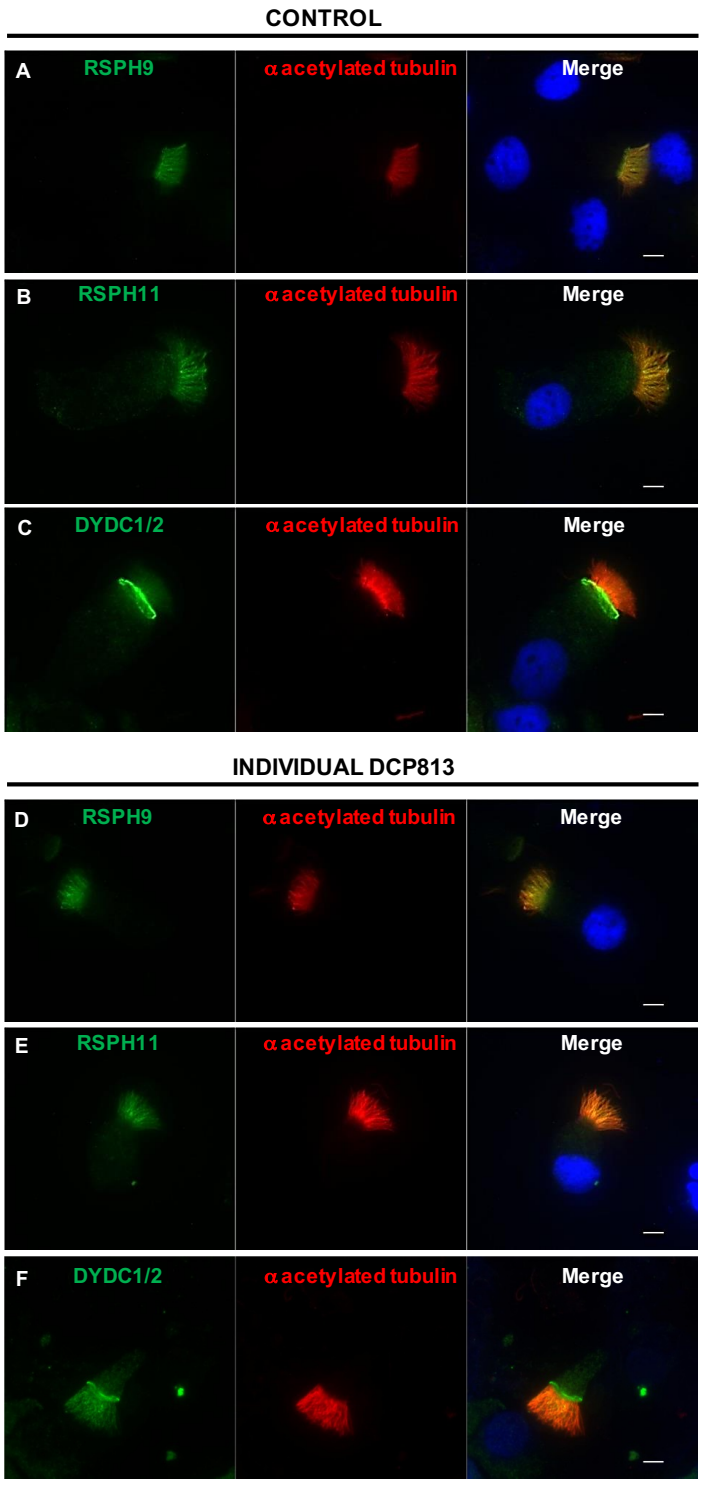
CONTROL
A
RSPH9
αacetylated tubulin
Merge
B
RSPH11
αacetylated tubulin
Merge
C
DYDC1/2
αacetylated tubulin
Merge
INDIVIDUAL DCP813
D
RSPH9
αacetylated tubulin
Merge
E
RSPH11
αacetylated tubulin
Merge
F
DYDC1/2
αacetylated tubulin
Merge

Figure S10. Localization of Ciliary Proteins in Airway Epithelial Cells from a Healthy Control Individual and Individual DCP813

RSPH9 (A,D), RSPH11 (B,E) and DYDC1/2 (C,F) proteins were detected by immunofluorescence assays in airway epithelial cells from both control individual and individual DCP813. Scale bars represent 5 μm

Figure S11

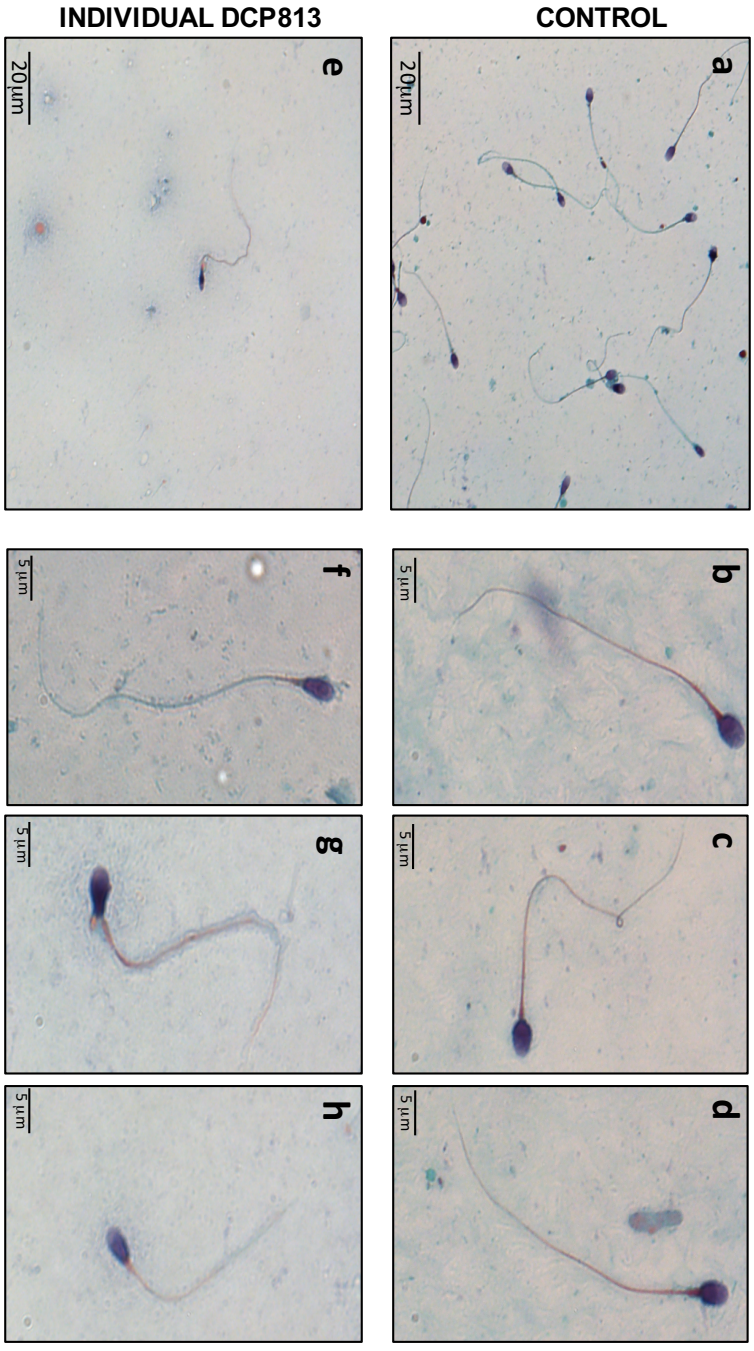
INDIVIDUAL DCP813
CONTROL
a
20µm
b
5µm
c
5µm
d
5µm
e
20µm
f
5µm
g
5µm
h
5µm

Figure S11. Morphology of Sperm Cells from Individual DCP813

Semen sample of individual DCP813 was obtained by masturbation and sperm morphology was assessed by Giemsa staining according to the guidelines of the World Health Organization (WHO). In contrast to control individual, spermatozoa from individual DCP813 displayed irregular length and caliber of the flagellum and abnormal head morphology. Scale bars represent 5 μm.

Table S1. Genomic Coordinates of the Homozygous Regions Shared by Individuals DCP812 and DCP813

#Chromosome	Start	-	End	Size (bp)
1	95324989	-	96056161	731172
1	175824793	-	176837770	1012977
1	187832345	-	188751379	919034
1	198066364	-	199585917	1519553
2	55719226	-	56198400	479174
2	79225689	-	79561647	335958
2	102294192	-	102698603	404411
2	129899375	-	130405841	506466
2	213597626	-	214135709	538083
5	20947355	-	21896196	948841
5	23722769	-	24137389	414620
5	41560078	-	42530101	970023
5	112312527	-	112947049	634522
6	44980340	-	45368796	388456
6	151264777	-	151443654	178877
7	68963428	-	69699073	735645
9	72992109	-	73406550	414441
11	48238178	-	49609842	1371664
11	49811224	-	51392715	1581491
11	**59543331**	**-**	**76011095**	**16467764**
14	66632180	-	67258961	626781
14	102451982	-	103232424	780442
15	74551602	-	75142760	591158
15	80719357	-	81079332	359975
16	31151520	-	31664746	513226
17	15314879	-	16322363	1007484
17	46543805	-	47047561	503756
18	11192144	-	11700533	508389
19	11284538	-	11988970	704432
19	23446278	-	23774941	328663
21	31416459	-	31859573	443114
22	20040601	-	20059762	19161

Genomic coordinates are based on GRCh37.

The homozygous region containing the *DNAJB13* c.833T>G transversion is in bold characters.

Table S2. Gene Variants Identified in the Regions of Homozygosity Shared by Individuals DCP812 and DCP813

Gene	Chr	ExAC allele frequency	Variation	RefSeq	known or presumed function, or involvement in other diseases[a]
CPSF7	11	0.000008	p.Ile285Val	NM_001136040.2	mRNA cleavage factor complex
ATL3	11	0.000008	p.Gly97Asp	NM_015459.4	involved in neuropathy, hereditary sensory, 1F (OMIM: 615632)
SF3B2	11	0.006	p.Ala26Ser	NM_006842.2	mRNA splicing, via spliceosome
BBS1	11	0.0008	p.Glu79Lys	NM_024649.4	involved in Bardet-Biedl syndrome 1 (OMIM: 209900)
GPR152	11	ND	p.Arg240Lys	NM_206997.1	angiotensin receptor activity
DNAJB13	**11**	**ND**	**p.Met278Arg**	**NM_153614.3**	**radial spoke protein**
C2CD3	11	0.000016	p.Ile1463Asn	NM_001286577.1	involved in orofaciodigital syndrome 14 (OMIM: 615948)
GPR137	11	ND	p.Ala12-Ala17del	NM_020155.3	G protein-coupled receptor

ND: not described

[a]None of the corresponding genes is known as a ciliary component or as involved in ciliogenesis, except (*i*) *BBS1* whose mutations are known to be responsible for Bardet-Biedl syndrome (a ciliopathy affecting primary cilia) and (*ii*) *DNAJB13* (bold characters) that encodes a radial spoke protein, which is an axonemal component of motile cilia. In addition, except for *DNAJB13*, none of those genes has an expression pattern compatible with an involvement in PCD.

Table S3. Genomic Coordinates of Homozygous Regions in Individual DCP856

#Chromosome	Start	-	End	Size (bp)
1	11711702	-	12034087	322385
1	18532882	-	20015268	1482386
1	47870884	-	55031201	7160316
1	57042109	-	57747785	705676
1	147305744	-	147467750	162006
1	150863667	-	151455674	592007
1	159913448	-	161588657	1675209
1	188651759	-	190163376	1511617
1	215632104	-	216324363	692259
1	223649439	-	224775021	1125582
1	227157714	-	227954960	797246
2	10401064	-	29401030	19063913
2	40601509	-	41692511	1091002
2	70859897	-	71556090	696193
2	108904520	-	109254118	349598
2	127902158	-	128887775	985617
2	145320381	-	146060703	740322
2	158814505	-	159382868	568363
2	166934393	-	167329181	394788
2	200339085	-	200962201	623116
2	231632550	-	232254553	622003
3	45895	-	11811386	11765491
3	78378354	-	79273601	895247
3	152709902	-	153383552	67365
3	163486967	-	164764164	1277197
3	165054700	-	166381935	1327235
3	171339701	-	172581582	1241881
3	192818155	-	193338191	520036
5	24943196	-	25404582	461386
5	57381625	-	57793140	411515
5	125139262	-	132383046	7243784
5	157990400	-	159062800	1072400
6	63112895	-	63460098	347203
6	81072168	-	81913384	841216
6	90745318	-	91162617	417299
6	101025383	-	101812944	787561
6	125164430	-	148724116	23559692
7	6186568	-	6951596	765028

7	53317406	-	54108376	79097
7	100806741	-	101137488	330747
8	49445299	-	49985036	539737
8	90951107	-	91861528	910415
8	96255831	-	96600147	344316
8	96787742	-	97129891	342149
8	115087798	-	115927695	839897
9	72216660	-	72783686	567026
9	74087091	-	74983264	896173
9	87293981	-	88332725	1038744
10	12634442	-	13248069	613627
10	43755252	-	44130635	375383
10	46392146	-	47122505	680359
10	83168887	-	83594713	425826
10	119401787	-	126195527	6793740
11	**69406566**	**-**	**79770063**	**10394578**
11	102998306	-	111542764	8544458
11	128278623	-	128782488	503865
12	68102	-	4865481	4797379
12	25698575	-	26114012	415437
12	33536102	-	34659435	1123333
12	116049664	-	116496774	44711
13	110152789	-	114125098	3752208
14	48287010	-	48848293	561283
14	51680166	-	52064081	383915
14	53451764	-	53877291	425527
14	58200220	-	62971495	4771275
15	31473108	-	32013879	540771
15	32027625	-	32902602	874977
15	43225829	-	43761920	536091
15	68498107	-	68971549	473442
16	9746268	-	10057711	311443
16	77172009	-	77456908	284899
16	78232754	-	82587790	4355036
17	2840415	-	3344645	50423
17	13145462	-	13480289	334827
17	17662182	-	18503445	841263
17	34610560	-	35339248	728688
17	40229318	-	40812023	582448
17	76938238	-	78640854	1723922
18	48439467	-	49222971	783504
20	17814344	-	19413907	1599563
20	22440606	-	22926973	486367
20	23067766	-	23630133	562367

20	30793776	-	31164056	37028
20	42275207	-	52077175	9801975
20	52081775	-	54964194	2882419
21	14487585	-	14692803	205218
21	14821268	-	15052304	231036
21	29020037	-	29830125	810088
21	42755622	-	45583147	2876166
22	19654308	-	20786675	1132367
22	26076118	-	31092937	5016819

Genomic coordinates are based on GRCh37.

The homozygous region containing the *DNAJB13* c.68+1G>C transversion is in bold characters.

Table S4. Gene Variants Identified in the Regions of Homozygosity in Individual DCP856

Gene	Chr	ExAC allele frequency	Variation	RefSeq	known or presumed function, or involvement in other diseases[a]
DMRTA2	1	ND	p.Ala396_Ala398del	NM_032110.2	involved in dorsal telencephalon and sex differentiation
DMRTB1	1	0.0007	p.Glu339Gly	NM_033067.1	involved in sex differentiation
SLC22A5	5	ND	c.952-2A>G (splice_acceptor_variant)	NM_003060.3	involved in systemic primary carnitine deficiency (OMIM: 212140)
KRTAP5-8	11	ND	p.Ser12_Gly13insGlyCysGlyGlyCysGlySer	NM_021046.2	hair keratin associated protein
DNAJB13	**11**	**ND**	**c.68+1G>C (splice_donor_variant)**	**NM_153614.2**	**radial spoke protein**
SERPINH1	11	0.00016	p.Thr189Ala	NM_001235.3	invovled in osteogenesis imperfecta 10 (OMIM: 613848)
GDAP1L1	20	ND	p.Arg127Gln	NM_001256737.1	glutathione transferase that translocates into mitochondria
OCSTAMP	20	ND	p.Pro2Ser	NM_080721.1	involved in osteoclast bone resorption

ND: not described

[a]None of the corresponding genes is known as a ciliary component or as involved in ciliogenesis, except *DNAJB13* (bold characters) that encodes a radial spoke protein, which is an axonemal component of motile cilia. In addition, except for *DNAJB13*, none of those genes has an expression pattern compatible with an involvement in PCD.