American Journal of Human Genetics, Volume *92*

Supplemental Information

Splice-Site Mutations in the Axonemal Outer Dynein Arm Docking Complex Gene *CCDC114* Cause Primary Ciliary Dyskinesia

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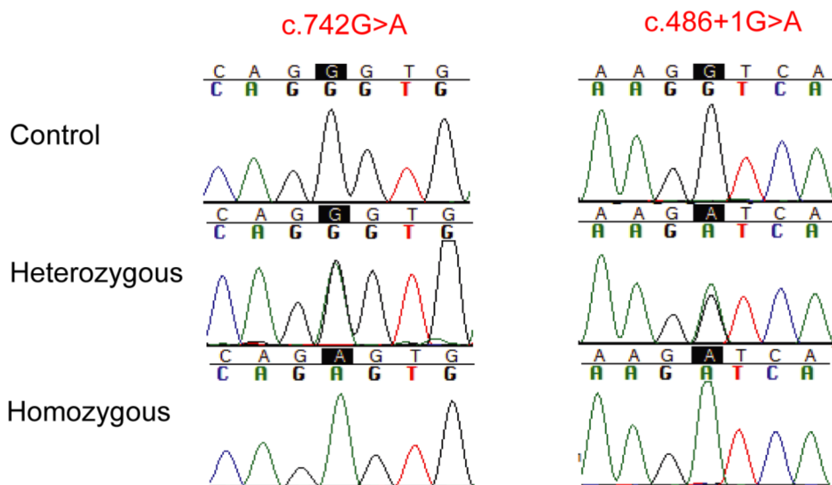


Figure S1. Sequence chromatograms of *CCDC114* mutations
Left panel shows the Volendam mutation and right panel the UK mutation. In both cases a control is shown (upper panels) compared to sequence from a heterozygous (middle panels) and homozygous mutation carrier (lower panels).

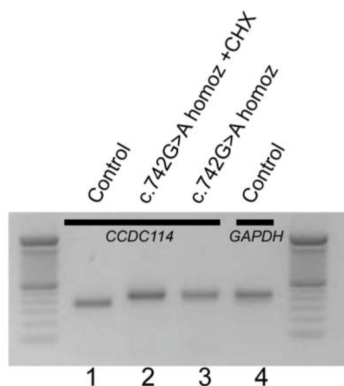
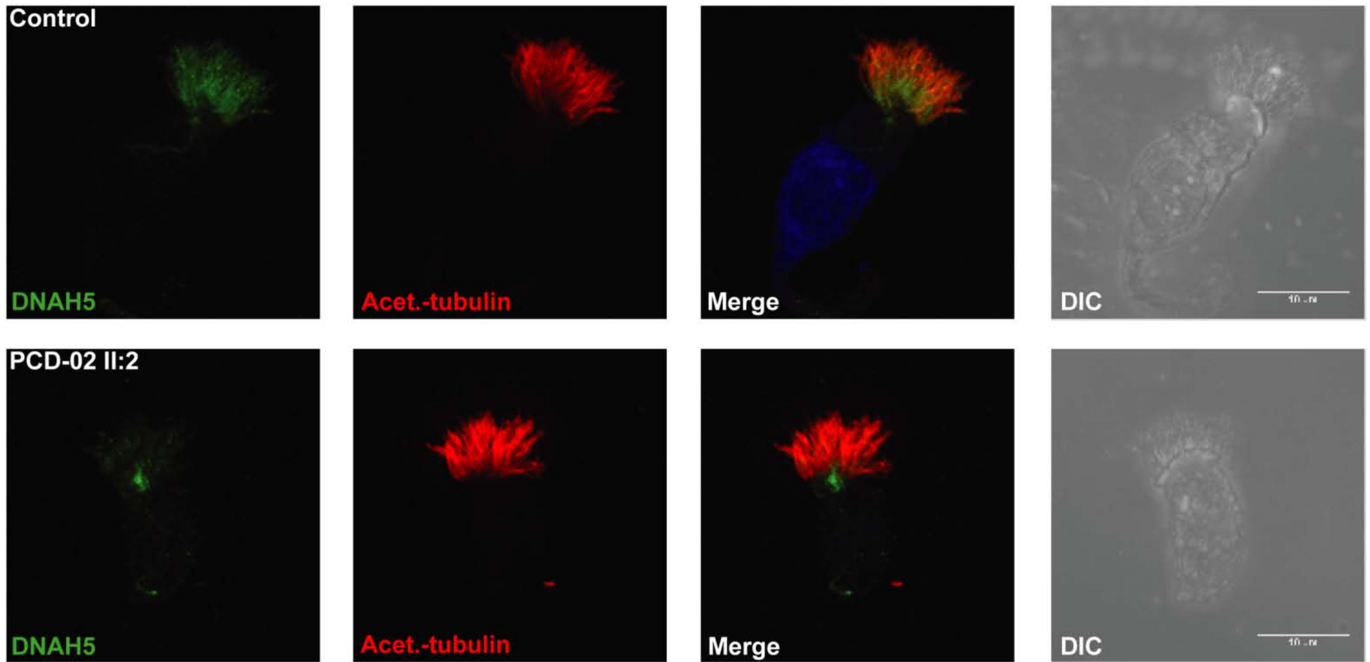


Figure S2. RT-PCR showing splice defect arising from Volendam c.742G>A mutation
Lanes 1-3, cDNA from control and patient isolated from cultured nasal epithelial cells amplified using primers in *CCDC114* cDNA exon 6 and 8. The control sample was also amplified using *GAPDH* primers as a control (lane 4). A higher molecular weight band is present in the patient cells (lanes 2, 3) indicating an aberrant insertion. Cycloheximide treatment (+CHX) of patient cells (lane 2) to block translation and prevent nonsense-mediated decay of the aberrant mRNA, showed no significant difference in cDNA levels to untreated patient cells (lane 3), indicating no significant effects of nonsense mediated decay; although the untreated band is slightly weaker than CHX-treated. 100 bp. molecular weight marker is shown each side for size reference.

A



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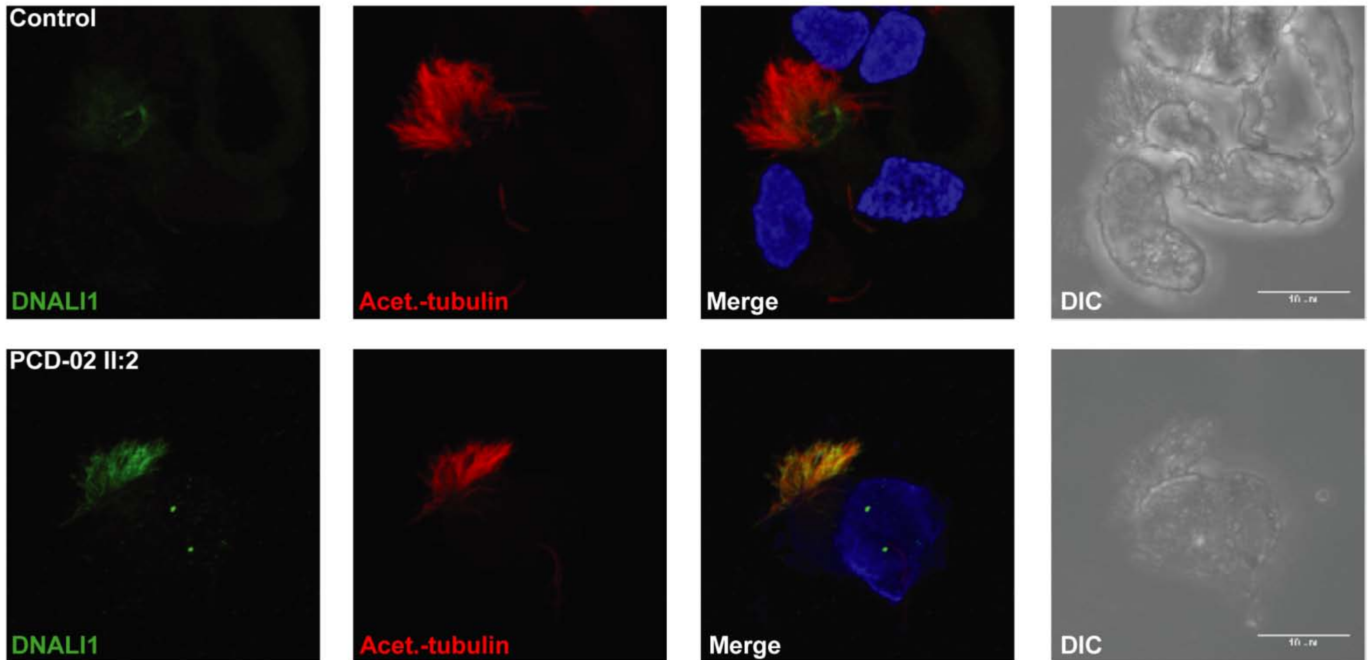
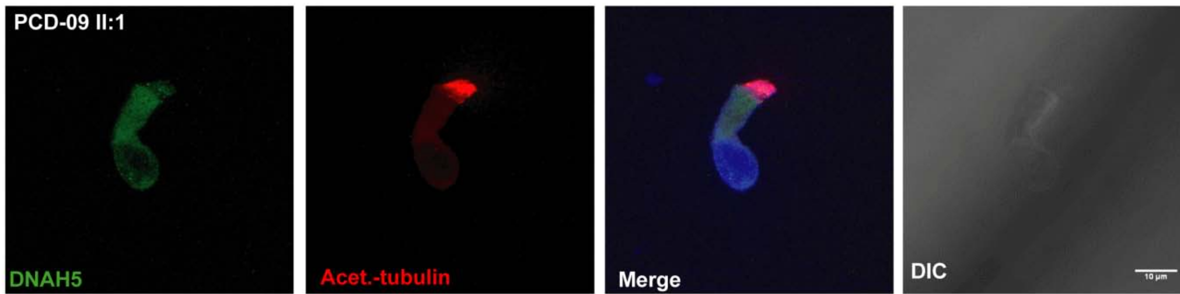


Figure S3. Axonemal immunostaining of ODA and IDA components in Volendam *CCDC114* patient
Subcellular localization of DNAH5 (panel A) and DNALI1 (panel B) in control and patient PCD-02 II:2 respiratory epithelial cells (both in green). Localisation of DNALI1 was not altered in the patient, whereas DNAH5 is markedly reduced compared to the control. Axoneme-specific staining with anti-acetylated α-tubulin antibodies (red) was used as a control, in addition to DNA (DAPI, blue).

A



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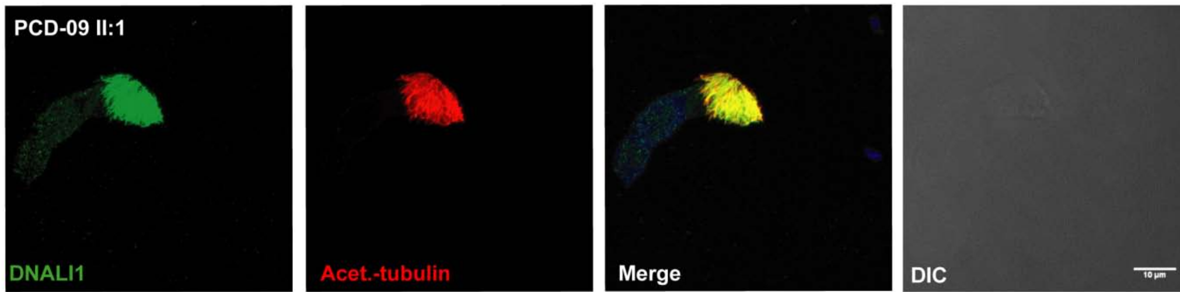

Figure S4. Axonemal immunostaining of ODA and IDA components in UK CCDC114 patient
Subcellular localization of DNAH5 (panel A) and DNALI1 (panel B) in patient PCD-09 II:1 respiratory epithelial cells (both in green). Localisation of DNALI1 is not altered in the patient, whereas DNAH5 is markedly reduced. Axoneme-specific staining with anti-acetylated α-tubulin antibodies (red) was used as a control, in addition to DNA (DAPI, blue).

Table S1. Exome sequencing coverage and mapping statistics

Exome sequenced samples	PCD-01 III:8	PCD-01 III:3
Read length	76 bp	76 bp
Total bases	6.70 Gb	6.64 Gb
Total reads	88,098,954	87,085,202
Reads mapped	86,774,682 (98.5%)	85,927,959 (98.7%)
%Target bases with coverage >=5X	91.8%	92.3%
%Target bases with coverage >=10X	86.7%	87.4%
%Target bases with coverage >=20X	77.9%	78.9%
Duplication rate	4.39%	5.85%

Table S2. Summary statistics of whole exome filtering process

Filter	Total variants	Variant category
Total variants in PCD-01 III:8	123,880	
Total variants in PCD-01 III:3	123,677	
Total shared variants unfiltered	40,883	
Total shared changes filtered vs. 181 controls	59	
Subset of filtered heterozygous variants	42	
Subset of filtered homozygous variants	11	
Heterozygous variants MAF <0.005	32 (of 42)	
of which		
	1	3' UTR
	1	5' UTR
	17	intronic
	4	synonymous
	5	non synonymous
	3	splice site
	1	upstream
Homozygous variants MAF <0.005	4 (of 11)	
of which		
	1	5' UTR
	2	intronic
	1	non synonymous (*CCDC114*)

Table S3. Primer sequences used for Sanger sequencing of *CCDC114* coding exons

Oligonucleotide	**Sequence 5' to 3'**
CCDC114-EX1-F	CCAAAGGGAGCAGAATTCCTA
CCDC114-EX1-R	CAGTCTTCAGCCCCTCTGAC
CCDC114-EX2-F	GTCAGAGGGGCTGAAGACTG
CCDC114-EX2-R	GGCCTAATAGCCCCAATTTC
CCDC114-EX3-F	GCCATTCAATCTCTCCCACA
CCDC114-EX3-R	ATGACCATGCCCAGTTCTTC
CCDC114-EX4-F	GGAACCCCAAAGAACCTCTG
CCDC114-EX4-R	CCTCCATGCCTTTTGGACTA
CCDC114-EX5-F	GCTTTACTCCTTATTGGAGAAGCA
CCDC114-EX5-R	AAGGGGATATTATGGGAGAAAAA
CCDC114-EX6-F	ATCTGGGACACCAGCTGACT
CCDC114-EX6-R	AGGGGAAGAGAGAACAGCAG
CCDC114-EX7-F	CCAGGAGGTCTCTGTGTTGG
CCDC114-EX7-R	CAGCCTGCACTGGACTCAG
CCDC114-EX8-F	GCGTCCACTGGCGTCTTA
CCDC114-EX8-R	GGGTGTGGAGCTAGGAAGAA
CCDC114-EX9-F	CCTGCTTCTTCCTAGCTCCA
CCDC114-EX9-R	GTCCTTCCAAGTGGAGAAGC
CCDC114-EX10-11-F	TTGGTCTCTGAGCCTTGACC
CCDC114-EX10-11-R	CCAGCCAGTCCCCAAAAG
CCDC114-EX12-13-F	CCTTTTTGAGGGCTGAGGTC
CCDC114-EX12-13-R	AAAAAGACCCCACAGAGAGC

Table S4. Primer sequences used in RT-PCR

Oligonucleotide	Sequence 5' to 3'
CCDC114_cDNA_2F	AAGATCAGGCGAAGGATCAG
CCDC114_cDNA_5R	CGGTCGTTGTTCTTGAGCTT
CCDC114_cDNA_6F	CTCTCCTCCACCTCTGCCTACGC
CCDC114_cDNA_8R	ATGCACCAACAGGTCAGGGTCACT
GAPDH_For	ACCACCAACTGCTTAGCACC
GAPDH_Rev	CACCCTGTTGCTGTAGCCA
ACTB_For	CTGGGACGACATGGAGAAAA
ACTB_Rev	AAGGAAGGCTGGAAGAGTGC

Table S5. Primers and probes in QPCR assays

Primer	Sequence 5'-3	UPL probe	Genbank
CCDC114-c624F	TACGGGCAAGACTGGAGAGT	#24	NM_144577.3
CCDC114-c672R	CCTTCCATCACTTTGCATTG		
CCDC63-c352F	CAGCAGAAGATTGCGAGTCA	#15	NM_152591.1
CCDC63-c352F	CATGAGACTCAACAGTAGGGTGA		
DNAH5-c5602F	AAAATCATGCAGAAAACTAATCAGG	#1	NM_001369.2
DNAH5-c5648R	TCGTGGTGACGTCTATCAATG		

The Roche UPL® Universal probe library was used, with the reference gene *ACTB*. The assay was performed on a Lightcycler LC480 (Roche), with assay design via Roche Probefinder vs. 2.48. The PCR cycle was as follows: 10 min 95°C, 1 cycle; 30 sec 60°C, 1 sec 72°C + fluorescence acquisition, 55 cycles. Analysis was performed using Lightcycler software vs. 1.5, with advanced relative quantification mode.

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