ORIGINAL ARTICLE

Mutations of *DNAH11* in patients with primary ciliary dyskinesia with normal ciliary ultrastructure

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ABSTRACT

Rationale Primary ciliary dyskinesia (PCD) is an autosomal recessive, genetically heterogeneous disorder characterised by oto-sino-pulmonary disease and situs abnormalities (Kartagener syndrome) due to abnormal structure and/or function of cilia. Most patients currently recognised to have PCD have ultrastructural defects of cilia; however, some patients have clinical manifestations of PCD and low levels of nasal nitric oxide, but normal ultrastructure, including a few patients with biallelic mutations in dynein axonemal heavy chain 11 (*DNAH11*).

Objectives To test further for mutant *DNAH11* as a cause of PCD, *DNAH11* was sequenced in patients with a PCD clinical phenotype, but no known genetic aetiology.

Methods 82 exons and intron/exon junctions in *DNAH11* were sequenced in 163 unrelated patients with a clinical phenotype of PCD, including those with normal ciliary ultrastructure (n=58), defects in outer and/or inner dynein arms (n=76), radial spoke/central pair defects (n=6), and 23 without definitive ultrastructural results, but who had situs inversus (n=17), or bronchiectasis and/or low nasal nitric oxide (n=6). Additionally, *DNAH11* was sequenced in 13 subjects with isolated situs abnormalities to see if mutant *DNAH11* could cause situs defects without respiratory disease.

Results Of the 58 unrelated patients with PCD with normal ultrastructure, 13 (22%) had two (biallelic) mutations in *DNAH11*; and two patients without ultrastructural analysis had biallelic mutations. All mutations were novel and private. None of the patients with dynein arm or radial spoke/central pair defects, or isolated situs abnormalities, had mutations in *DNAH11*. Of the 35 identified mutant alleles, 24 (69%) were nonsense, insertion/deletion or loss-of-function splice-site mutations.

Conclusions Mutations in *DNAH11* are a common cause of PCD in patients without ciliary ultrastructural defects; thus, genetic analysis can be used to ascertain the diagnosis of PCD in this challenging group of patients.

Key messages

What is the key question?

- Dynein axonemal heavy chain 11 (*DNAH11*) is known to be a primary ciliary dyskinesia (PCD)-causing gene in very few families, but data regarding the mutation prevalence are lacking.
- This study details the mutation profiling of *DNAH11* coding region and splice junctions in a large cohort of 163 families with PCD.

What is the bottom line?

- Analysis of this large cohort shows that *DNAH11* mutations are exclusively seen in patients with PCD with normal ciliary ultrastructure.

Why read on?

- Diagnosis of PCD is challenging in patients with normal ciliary ultrastructure.
- This large-scale study reports that approximately 22% of all patients with PCD with normal ciliary ultrastructure harbour mutations in *DNAH11*.
- Taken together, this study provides compelling confirmation that there is a genetic basis for PCD in many patients with a presumptive diagnosis of PCD based on clinical features and measurements of nasal nitric oxide, even in the presence of normal ciliary ultrastructure.
- Additionally, since *DNAH11* encodes an outer dynein arm protein, the number of patients with outer dyenin arms defects were evaluated for mutations in this gene and were negative, showing that despite mutations in *DNAH11*, outer dynein arms are not affected structurally.
- Further, this provides a strong rationale for additional studies to discover other genetic mutations that can cause PCD in patients with normal ciliary ultrastructure.

INTRODUCTION

Primary ciliary dyskinesia (PCD) [OMIM# 244400 (http://www.ncbi.nlm.nih.gov/Omim/)] is a rare, genetically heterogeneous disorder. Defective ciliary and/or flagellar function underlies the clinical manifestations, which include chronic oto-sino-pulmonary disease. Situs inversus totalis occurs in around 50% of patients (Kartagener syndrome) and situs ambiguus occurs in at least 6%.[1–4]

The diagnosis of PCD is important for the initiation of clinical care. The diagnosis largely relies on

demonstration of ciliary ultrastructural defects by transmission electron microscopy (EM), but this test fails to support the diagnosis of PCD in patients with normal ultrastructure. Genetic testing holds promise as a diagnostic approach in patients with a clinical phenotype compatible with PCD, as approximately 50% of PCD can be accounted for by biallelic mutations in 12 genes.[5–23] Mutations in two genes; dynein axonemal intermediate chain 1 (*DNAI1*), and dynein axonemal heavy chain 5 (*DNAH5*), that code for ciliary outer dynein arm (ODA) proteins are the most common genetic causes of PCD (18–30% of PCD),[9 10 13 14] and mutations in the remaining genes are relatively uncommon.

DNAH11 encodes a ciliary ODA protein. Mutations in *DNAH11* were originally described in a patient with a genetic diagnosis of cystic fibrosis, but who also had features of PCD, but normal ciliary ultrastructure.[19] Subsequent reports conclusively demonstrated that mutant *DNAH11* causes PCD in patients with normal ultrastructure.[19] *DNAH11*-mutant cilia have a reduced waveform amplitude and hyperkinetic beating pattern.[20 21] Based on these findings, a European consensus conference modified the diagnostic algorithm for PCD, and highlighted the importance of high-speed videomicroscopy analysis to evaluate ciliary beat pattern.[24]

To estimate the mutation frequency in *DNAH11* in PCD, we undertook a large study of 163 unrelated patients with PCD displaying a variety of ciliary EM findings, including patients with a compatible PCD phenotype, but without ciliary ultrastructural defects.

MATERIALS AND METHODS

The study included 195 patients with PCD from 163 unrelated families of which 137 were simplex families with only one member affected, 25 were multiplex families with two or more affected siblings and a family with three affected members from an isolated population, and 13 unrelated subjects with isolated situs abnormalities (see online supplement, table E1). The majority of patients were evaluated at the University of North Carolina (UNC) (n=98) or University Hospital, Freiburg (n=38). The remaining patients were evaluated at sites in the Genetic Disorders of Mucociliary Clearance Consortium and other specialised PCD centres in Europe, Australia and Israel (see online supplement). Evaluations included medical and family history, physical examination, spirometry, sputum microbiology, chest radiograph and/or CT scan, and nasal nitric oxide (nNO) measurement in most patients, as described.[8 25] The diagnosis of PCD in patients with a compatible phenotype was assessed by ciliary ultrastructure (see below). When ciliary ultrastructure by EM analysis or immunofluorescence was normal, a presumptive diagnosis was made by adjunct tests (ciliary waveform analysis, and/or nNO measurements; see online supplement).[11–13 25 26] Subjects with isolated situs abnormalities (n=13) had normal ciliary ultrastructure and nNO, and no clinical features of PCD (online supplement, figure E1). This study was approved by the Committee for the Protection of the Rights of Human Subjects at participating institutions, and written consent was obtained.

Ciliary ultrastructural and waveform analysis

Epithelial cells were obtained by nasal curettage from the inferior turbinate, processed for EM, and ≥20 cilia with adequate images were interpreted at UNC by three blinded observers (JLC, MRK, MWL and/or SLM), as described.[8 25 27 28] Videomicroscopy was performed as previously described[20 29 30] (details given in online supplement).

Mutation profiling

DNA was extracted from blood, buccal swabs or lymphoblastoid cell lines from proband and available relatives, as described (details given in online supplement).[8 25 31] For the evaluation of mutation frequency among unrelated families, one patient with PCD per family was used for the full *DNAH11* sequencing and analysis. The majority of sequencing of 82 exons and splice junctions was performed by NHLBI Genotyping and Resequencing Services in Seattle (http://rsng.nhlbi.nih.gov/scripts/index.cfm) using Sanger sequencing. The remainder of the sequencing was performed by Sanger sequencing at UNC (see details and primer sequences in the online supplement, methods and table E2). Estimates of allele frequencies for missense variants were obtained using direct sequencing or restriction endonuclease digestion (online supplement, methods) in at least 104 chromosomes from anonymised patients without PCD (patients with haemophilia) of Caucasian ethnicity. Additionally, 1000 Genomes (http://www.1000genomes.org/) and dbSNP public databases (http://www.ncbi.nlm.nih.gov/projects/SNP/) were searched.

cDNA analysis

To determine the effect of splice-site variants on transcripts, reverse transcriptase PCR was employed, using RNA from nasal epithelial cells or transformed lymphoblastoid cell lines, as described.[25 27] (See details and primer sequences in online supplement, methods and table E3.)

RESULTS

Clinical phenotype of study subjects

Patients with PCD

There were 195 patients (163 families) with PCD (or presumed PCD), including 90 men (46%) and 105 women (54%) between the ages of 2 months and 75 years. Parental consanguinity was present in 21 (13%) families. The majority of families were of Caucasian origin (79%), and the remaining families represented a broad mixture of ethnicities (online supplement, table E1). Situs inversus and situs ambiguus were present in 80 (41%) and 15 (8%) patients, respectively. Most patients had neonatal respiratory distress (70%), recurrent otitis media (82%), sinusitis (95%), and bronchiectasis (70%) by chest CT scan (online supplement, table E1). Of the 101 patients who had nNO measured, the values were low (24.6±22.6 nl/min; mean±SD) compared with values (376±124 nl/min) reported in healthy controls.[24] Other details of the clinical features and nNO levels are available (online supplement, table E1). Patients with normal ciliary ultrastructure, according to EM (online supplement, figure E1) or immunofluorescence staining techniques, were considered to have a presumptive diagnosis of PCD. This was based on a compatible clinical phenotype (including bronchiectasis in most patients) and/or situs abnormalities, as well as low levels of nNO and dyskinetic/hyperkinetic waveform and/or increased beat frequency in videomicroscopy studies, consistent with previous reports.[20]

Subjects with isolated situs abnormalities

There were 13 unrelated subjects with situs abnormalities but no clinical features of PCD, and all subjects who were tested (n=10) had normal nNO levels. Thus, these 13 subjects were considered to have isolated situs abnormalities unrelated to PCD (online supplement, table E1). These subjects were included because mouse models of *DNAH11* orthologue[32–34] were originally reported to have isolated situs abnormalities without the respiratory phenotype.

Mutation profiling

There were 58 unrelated patients from mutation profiling who had a clinical phenotype, nNO levels, and/or ciliary waveform or situs abnormalities compatible with PCD, but the diagnosis could not be confirmed in the patients or their affected siblings by demonstration of a defect in ciliary ultrastructure. Of these 58 unrelated patients with a presumptive diagnosis of PCD, 20 had at least one mutation in *DNAH11*, and the clinical demographics, nNO levels, situs status, ciliary phenotype and mutations are summarised in tables 1 and 2.[25 30 35] Of these 20 patients, 15 had two (biallelic) mutations, including three homozygotes, and 12 compound heterozygotes (table 1). Seven of the 15 patients with biallelic mutations had an affected sibling with identical mutations (table 2). Most of the 15 families with biallelic mutations had a patient with PCD and situs abnormalities (13 of 15) (table 2), which probably represents an ascertainment bias. As with patients with PCD and ultrastructural defects, there was an age-related distribution of bronchiectasis in patients with biallelic mutations. Three of the six patients without bronchiectasis were ≤8 years old (table 2).

We identified 35 mutant alleles, not previously observed.[19–21] These included nonsense mutations (n=11), small insertions-deletions (n=6), splice-site mutations (n=7), and missense mutations (n=11). Except for three patients with homozygous mutations, each mutation appeared only once, which demonstrates extensive allelic heterogeneity (see all 32 unique mutant alleles and their corresponding protein domain in figure 1 and online supplement, table E4). Carrier studies in families showed that mutations were inherited *in trans*, and segregation analysis was consistent with an autosomal recessive trait. Selected pedigrees illustrate the segregation analysis (figure 2), and additional families in which segregation analysis was possible with either biallelic mutations (online supplement, figure E2) or with only monoallelic mutation (online supplement, figure E3) are presented in the online supplement.

cDNA analysis of splice-site mutations

RNA was available for transcript studies for six of the seven splice-site mutations. Three of these splice mutations (c.2275-1G→C; c.4254+5G→T; c.7266+1G→A) caused in-frame deletions of exon 14 (131 amino acids), exon 23 (53 amino acids), and exon 44 (44 amino acids), respectively (table 3, figure 3). Additionally, three mutations (c.4726-1G→A; c.5778+1G→A; c.7914G→C) caused out-of-frame deletions of exon 27, exons 32–35 and exon 48, respectively, leading to premature stop signals (table 3, figure 3).

Correlation of genotype with ultrastructure and ciliary waveform

The genetics of PCD involves locus, allelic and ultrastructural heterogeneity; thus, we studied patients with different ciliary EM findings, including patients with normal ultrastructure, but compatible clinical phenotype. Mutations in *DNAH11* were exclusively seen in patients with a clinical phenotype of PCD and normal ciliary ultrastructure. Each of the 14 patients (11 families) with biallelic mutations in *DNAH11* who were tested by video-microscopy had the characteristic hyperkinetic beating pattern and reduced waveform amplitude, as previously reported (see table 2 and online supplement, movies E1 and E2).[20] None of the other groups carried mutations, including patients with isolated situs abnormalities. In total, we identified biallelic *DNAH11* mutations

Table 1 Details of *DNAH11* mutations in 20 unrelated patients with primary ciliary dyskinesia (PCD)

					Allele 1				Allele 2			
Patient number	Family number	Sex	Situs status	Ciliary EM	Ex/Int	Base change (cDNA)	Amino acid change	Seg*	Ex/Int	Base change	Amino acid change	Seg*
Homozygous mutations												
PCD623†	UNC101	F	SS	Normal	Ex 25	4438C→T	R1480X	Pat	Ex 25	4438C→T	R1480X	Mat
PCD1022†	UNC177	M	SS	Normal	Ex 24	4333C→T	R1445X	Pat	Ex 24	4333C→T	R1445X	Mat
OP20-II:1‡	OP20	M	SI	na	Ex 71	11663G→A	R3888H	na	Ex 71	11663G→A	R3888H	na
Compound heterozygous mutations												
PCD108†	UNC14	M	SI	Normal	Ex 26	4516_4517delCT	L1506SfsX10	Mat	Int 44	7266+1G→A§	T2379_Q2422del	Pat
PCD157	UNC21	F	SI	Normal	Ex 37	6244C→T	R2082X	Mat	Ex 73	11929G→T	E3977X	Pat
PCD761	UNC126	F	SI	Normal	Int 13	2275-1G→C§	Y759_E889del	Mat	Ex 81	13213dC	R4405AfsX1	Pat
PCD919†	UNC147	M	SA	Normal	Ex 80	13065_67delCCT	4356delL	Mat	Ex 80	13075C→T	R4359X	Pat
OP98-II:1†	OP98	M	SI	Normal	Ex 48	7914G→C§	W2604X	Pat	Ex 82	13333_34insACCA	I4445NfsX3	Mat
OP406-II:1†	OP406	M	SI	Normal	Int 23	4254+5G→T§	E1366_G1418del	Mat	Int 26	4726-1G→A§	E1576AfsX4	Pat
PCD565	UNC90	M	SI	Normal	Int 33	5778+1G→A§	V1821TfsX7	Pat	Ex 80	13061T→A	L4354H	Mat
PCD1077	UNC199	F	SI	Normal	Ex 21	3901G→T	E1301X	Pat	Ex 72	11804C→T	P3935L	Mat
PCD1126	UNC222	F	SS	Normal	Ex 74	12064G→C	A4022P	na	Ex 82	13504_05insGAAGA	T4502RfsX14	na
OP235-II:2†	OP235	F	SI	Normal	Ex 77	12697C→T	Q4233X	Pat	Ex 79	12980T→C	L4327S	Mat
OP41-II:1	OP41	M	SI	Normal	Ex 1	350A→T§	E117V	na	Ex 44	7148T→C	L2383P	na
PCD812	UNC128	M	SI	na	Ex 34	5815G→A	G1939R	Pat	Ex 82	13373C→T	P4458L	Mat
Heterozygous mutations												
PCD998	UNC174	M	SS	Normal	Ex 56	9113_16delAAGA	K3038TfsX13	Pat	–	Unknown	Unknown	–
PCD1033	UNC179	F	SA	Normal	Ex 63	10324C→T	Q3442X	Pat	–	Unknown	Unknown	–
PCD1174	UNC256	F	SS	na	Ex 14	2569C→T	R857X	Mat	–	Unknown	Unknown	–
PCD974	UNC162	F	SS	Normal	Ex 60	9764T→C	L3255S	Mat	–	Unknown	Unknown	–
PCD545	UNC-O	M	SS	Normal	Ex 33	5643A→T	Q1881H	na	–	Unknown	Unknown	–

Additional demographic information is given in online supplement table 2.
*Mutant allele shown to segregate from either the father's (paternal) or mother's (maternal) side of the family.
†Patients have affected siblings who also carry the same biallelic familial mutations.
‡Consanguineous family.
§Splice site mutations, see details in table 3.
DA, dynein arms; *DNAH11*, dynein axonemal heavy chain 11; EM, electron microscopy; Ex/Int, exon/intron; F, female; M, male; Mat, maternal; na, not available; Pat, Paternal; SA, situs ambiguus; SI, situs inversus; SS, situs solitus.

Table 2 Clinical, demographic and ciliary features of 20 unrelated families carrying *DNAH11* mutations

PCD patient no.	Family no.	Sex	Age in yrs	Ethnicity	nNO nl/min*	Situs status	Ciliary videomicroscopy wave form§	CBF (Hz)¶	Neo RDS	Otitis media	Bxsis	Sinusitis
Homozygous mutations												
PCD623	UNC101	F	24	Caucasian	9.7	SS	Dyskinetic/hyperkinetic	—	Yes	Yes	Yes	Yes
PCD627†		F	26		24.1	SS	—	—	Yes	Yes	No	Yes
PCD1022	UNC177	M	4	Caucasian	12.5	SS	—	—	Yes	Yes	No	Yes
PCD1023†		M	7.5		12.6	SI	—	—	Yes	Yes	No	Yes
OP20-II:1‡	OP-20	M	12	Turkish	na	SI	—	—	No	Yes	Yes	No
Compound heterozygous mutations												
PCD106†	UNC14	M	29	Caucasian	14	SS	—	—	No	Yes	No	Yes
PCD108		M	24		20	SI	Dyskinetic/hyperkinetic	—	Yes	Yes	No	Yes
PCD157	UNC21	F	12	Caucasian	2.1	SI	Dyskinetic/hyperkinetic	—	Yes	Yes	Yes	Yes
PCD761	UNC126	F	30	Caucasian	24.5	SI	Dyskinetic/hyperkinetic	15.2	Yes	Yes	Yes	Yes
PCD918†	UNC147	F	10	Asian	19.4	SS	—	—	Yes	Yes	Yes	Yes
PCD919		M	8		25.5	SA	Dyskinetic/hyperkinetic	7.9	Yes	Yes	Yes	Yes
OP98-II:1	OP98	M	20	Caucasian	na	SI	Dyskinetic/hyperkinetic	—	No	Yes	Yes	Yes
OP98-II:2†		M	15		na	SS	Dyskinetic/hyperkinetic	—	na	Yes	Yes	Yes
OP406-II:1	OP406	M	1	Caucasian	na	SI	Dyskinetic/hyperkinetic	—	na	na	na	na
OP406- II:2†		F	7		na	SS	Dyskinetic/hyperkinetic	—	Yes	na	na	yes
PCD565	UNC90	M	7	Caucasian	23.5	SI	Dyskinetic/hyperkinetic	10.2	Yes	Yes	Yes	Yes
PCD1077	UNC199	F	2	Caucasian	16.9	SI	—	—	Yes	Yes	na	Yes
PCD1126	UNC222	F	42	Asian	16.2	SS	Dyskinetic/hyperkinetic	13.7	No	No	Yes	Yes
OP235-II:1†	OP235	F	24	Caucasian	na	SS	Dyskinetic/hyperkinetic	—	No	Yes	Yes	Yes
OP235-II:2		F	21		na	SI	Dyskinetic/hyperkinetic	—	Yes	Yes	Yes	Yes
OP41-II:1	OP41	M	13	Caucasian	na	SI	Dyskinetic/hyperkinetic	—	Yes	Yes	na	Yes
PCD812	UNC128	M	8	Caucasian	9	SI	—	—	Yes	Yes	No	Yes
Heterozygous mutations												
PCD998	UNC174	M	29	Caucasian	70.4	SS	Dyskinetic/hyperkinetic	7.1	No	Yes	Yes	Yes
PCD1033	UNC179	F	10	Caucasian	34.8	SA	Dyskinetic/hyperkinetic	10.5	Yes	Yes	No	Yes
PCD1174	UNC256	F	35	Caucasian	32.1	SS	Dyskinetic/hyperkinetic	6.9	Yes	Yes	Yes	Yes
PCD974	UNC162	F	12	Caucasian	40	SS	Dyskinetic/hyperkinetic	14.0	No	Yes	Yes	Yes
PCD545	UNC-O	M	25	Lebanese	na	SS	—	—	No	No	Yes	Yes

*Normal nNO levels were 376±124 nl/min (mean±SD), calculated from 27 healthy subjects.[25]
†Affected sibling (only tested for targeted mutation).
‡Consanguineous family (parents of the patients were related).
§Dyskinetic/hyperkinetic: dyskinetic means non-flexible beating pattern with reduced range of motion, especially at mid-shaft of the cilia; hyperkinetic means many fields with increased ciliary activity, particularly in the distal third of the ciliary shaft.
¶25°C; normal CBF 7.28±1.5 Hz (mean±SD), and ~7.2±1.0 Hz.[30 35]
Bxsis, bronchiectasis; CBF, ciliary beat frequency; *DNAH11*, dynein axonemal heavy chain 11; F, female; M, male; na, not available; Neo RDS, neonatal respiratory distress in full-term birth; nNO, nasal nitric oxide; PCD, primary ciliary dyskinesia; SA, situs ambiguus; SI, situs inversus; SS, situs solitus.

in 13 (22%) of the 58 unrelated families with compatible clinical phenotype, low nNO and confirmed normal ciliary ultrastructure and/or abnormal videomicroscopy. Despite full gene (coding region) sequencing, we found only one mutant allele in five patients (four with confirmed normal ultrastructure), which could reflect either a second mutation in *DNAH11* (introns or promoter

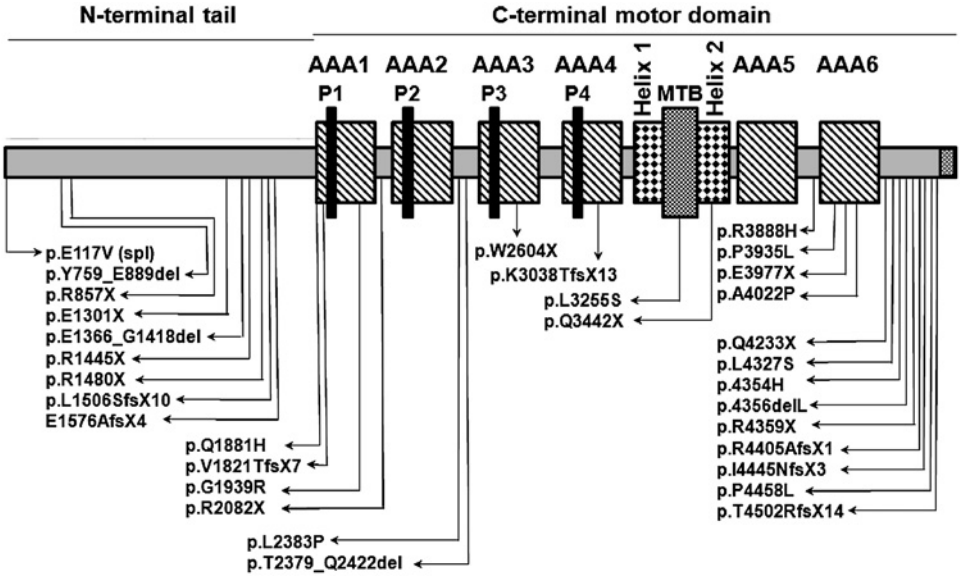

Figure 1 Schematic representation of dynein axonemal heavy chain 11 (*DNAH11*) (not to scale) showing AAA1–6 domains, four P-loops, the microtubule binding domain (MTB) and helix 1 and 2. The positions of all the mutations are shown.

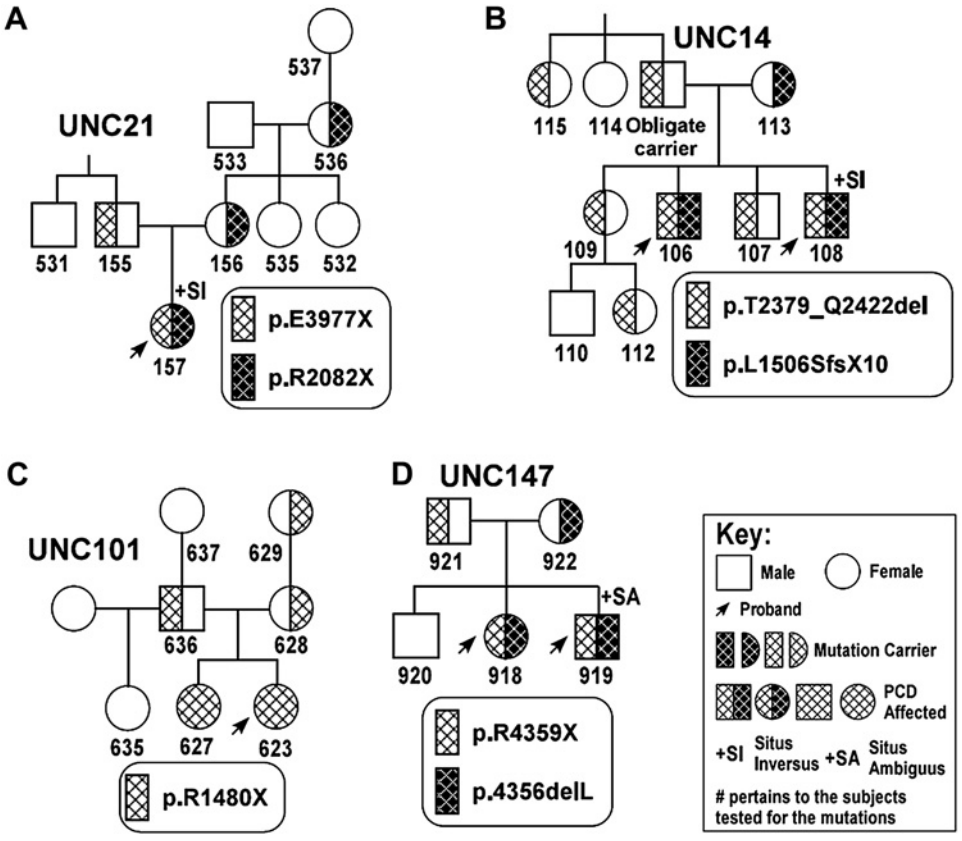

Figure 2 Representative pedigrees showing autosomal recessive mode of inheritance for dynein axonemal heavy chain 11 (*DNAH11*) mutations. Segregation analysis from the parents, siblings and the extended family members demonstrates that mutations were inherited *in trans* (A–D), and there was no bias for gender or situs status. Additional pedigrees are presented in the online supplement figures E2, E3.

regions, or large indels), or a heterozygous mutation in a different ciliary gene (which would represent a digenic mode of inheritance), or biallelic mutations in a PCD gene other than *DNAH11*.

Population studies

There were 10 unique missense variants, one possible single nucleotide polymorphism (SNP), two splice mutations and one amino acid deletion that were studied to examine their role as pathogenic or benign. Due to the nature of the sequence-based assay, certain amplicons (exons 33, 44 and 80) harboured splice and nonsense mutations in addition to variants of interest, and they were examined as well. Each of these variants was identified in only one of the 163 unrelated patients with PCD who were tested, and never identified in 13 with isolated situs abnormalities. Additionally, these missense variants were not observed in at least 104 alleles tested in subjects without PCD, ethnically matched when possible (ethnically matched controls were not available for three subjects). In addition, these variants were predicted to be deleterious based on in silico program 'Mutation Taster' (http://neurocore.charite.de/MutationTaster/). Furthermore, none of these missense variants or loss-of-function or splice mutations were seen in 1000 Genomes (http://www.1000genomes.org/) or dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) databases, except for having been listed from this study in dbSNP. Taken together, these data suggest that these variants are not benign polymorphisms (online supplement, table E5).

Polymorphisms and variants of unknown significance

DNAH11 is a large gene, and we identified 310 novel and/or known polymorphisms. The polymorphisms and corresponding SNP database number (http://www.ncbi.nlm.nih.gov/SNP/) are available (see online supplement, table E6). The novel variants that are not present in the SNP database were considered benign due to the high minor allele frequency in patients with PCD (online supplement, table E6, footnotes). One rare variant (c.11059A→G; p.K3687E) was seen on only one allele of a patient with PCD and an ODA defect, and was not seen in either control or isolated situs abnormality groups. This was a non-synonymous substitution, conserved (80%) across species, and present at the third last base of exon 67 near the splice-donor site. Due to the unavailability of RNA, we could not check the effect of this variant on splicing. We classified this substitution as a variant of uncertain significance, because mutations in *DNAH11* are seen (otherwise) exclusively in patients with normal ciliary ultrastructure; plus, a second mutation was not identified, despite full gene sequencing.

Errors in published sequence of *DNAH11*

During analysis of cDNA from nasal epithelial cells and lymphoblastoid cell lines from two unrelated control subjects, we observed errors in the Ensembl database (http://uswest.ensembl.org/index.html), and published sequence of *DNAH11*.[19] The last 15 bases of exon 22 (and five amino acid residues) are not present in the *DNAH11* transcript from multiple control subjects (details in bottom panel of figure 3B and online supplement, figure E4A). These five amino acids were previously shown in the human *DNAH11*,[19] but not in other species, which is congruent with sequence error. Additionally, six bases in exon 32 of the Ensembl database (and two amino acid residues) are not present in the *DNAH11* transcript from multiple control subjects (correct cDNA sequence for exons 22 and 32, and multiple sequence alignment in online supplement, figure E4). Due to errors in the publicly available sequences, the full-length *DNAH11* will contain 4216 amino acids and the mutation nomenclature for all the previously published mutations (and variants/SNPs) will change (see online supplement, table E7 for mutation nomenclature that corresponds with the current and

Tablel 3 Effect of *DNAH11* splice mutations on cDNA transcript using reverse transcriptase PCR (RT-PCR) in patients with primary ciliary dyskinesia (PCD)

Sample no.	Intron/exon location	Genomic mutations and predicted amino acid change	cDNA transcript after RT-PCR	Comments
OP41-II:1	Exon 1	c.350A→T (p.E117V) splice defect?	r.(spl?) RNA not available	Second last base in exon 1 on conserved canonical splice donor site. Population studies: 0/216 control alleles and 1/326 PCD alleles
PCD761	Intron 13	c. IVS13-1G→C (c.2275-1G→C) splice defect	r.2275_2667del (p.Y759_E889del)	Inframe deletion of exon 14 consisting of 131 amino acid residues Wild-type amplification product: 1089 bp Mutant amplification product: 696 bp
OP406-II:2	Intron 23*	c.IVS23+5G→T (c.4254+5G→T) splice defect	r.4096_4254del (p.E1366_G1418del)	Inframe deletion of exon 23 consisting of 53 amino acid residues Wild-type amplification product: 741 bp Mutant amplification product: 582 bp
OP406-II:2	Intron 26	c.IVS26-1G→A (c.4726-1G→A) splice defect	r.4726_4817del (p.E1576AfsX4)	Out-of-frame deletion of exon 27 leading to premature translation termination signal Wild-type amplification product: 992 bp Mutant amplification product: 900 bp
PCD653†	Intron 33*	c.IVS33+1G→A (c.5778+1G→A) splice defect	r.5461_6041del (p.V1821TfsX7)	Out-of-frame deletion of exons 32–35 leading to premature translation termination signal Wild-type amplification product: 1013 bp Mutant amplification product: 432 bp
PCD108	Intron 44	c.IVS44+1G→A (c.7266+1G→A) splice defect	r.7135_7266del (p.T2379_Q2422del)	Inframe deletion of exon 44 consisting of 44 amino acid residues Wild-type amplification product: 918 bp Mutant amplification product: 786 bp
OP98-II:1	Exon 48	c.7914G→C (p.Q2638H) splice defect	r.7812_7914del (p.W2604X)	Last base in exon 48 on conserved canonical splice donor site. Out-of-frame deletion of exon 48 leading to premature translation termination signal Wild-type amplification product: 1090 bp Mutant amplification product: 987 bp

*Intron 23 and intron 33 analysis showed the absence of last 15 bases (five amino acid residues) in exon 22 and six bases of exon 32 (two amino acid residues) respectively, in multiple controls depicting error in published sequence.
†RNA from affected individual PCD565 was not available hence cDNA analysis was done on the carrier father (PCD653).
DNAH11, dynein axonemal heavy chain 11.

formerly published sequenced information). The Genbank (www.ncbi.nlm.nih.gov/genbank/) accession numbers for the updated *DNAH11* exons 22 and 32 sequences are JQ247524 and JQ247523 respectively.

DISCUSSION

It is challenging to confirm a diagnosis of PCD in patients with a compatible clinical phenotype, but who do not have hallmark defects in ciliary ultrastructure. Some specialised centres use nNO measurement as an aid to diagnosis. A few centres use videomicroscopy to evaluate ciliary waveform to confirm the diagnosis, but this assay is difficult and limited in availability.

Mutations in *DNAH11* have been reported in four families in which patients with PCD have normal ciliary ultrastructure.[19–21] However, the prevalence of *DNAH11* mutations, and genotype–ciliary phenotype correlations, are not well defined. In this study, we tested the hypothesis that mutations in *DNAH11* are a relatively common cause of PCD in patients with normal ciliary ultrastructure. We studied a large number of well characterised patients with PCD and different ciliary ultrastructural phenotypes to determine the frequency of *DNAH11* mutations in each group.[25] In patients with normal ciliary ultrastructure, the clinical phenotype was typical of PCD, including a high prevalence of respiratory distress in full-term neonates, chronic otitis media and sinusitis, productive cough, bronchiectasis, situs abnormalities and infertility (online supplement, table E1). In addition, these patients had low nNO and/or abnormal immunofluorescence with ciliary antibodies and/or abnormal ciliary waveform with limited range of motion and hyperkinesis, which are compatible with PCD (tables 1 and 2).[20]

We determined that biallelic mutations in *DNAH11* are relatively common (22%) in patients with PCD without a defined ciliary ultrastructural defect (table 1). None of the patients with PCD and ultrastructural defects had mutations in *DNAH11*. Thus, disease-causing mutations in *DNAH11* appear specific for patients with PCD and normal ciliary ultrastructure. It is difficult to determine the proportion of all patients with PCD carrying biallelic mutations in *DNAH11*, since the fraction of patients with PCD and normal ciliary ultrastructure is not known. However, several studies, and the experience of our centres, estimate that at least 30% of patients with PCD have normal axonemal ultrastructure[2]; thus, *DNAH11* mutations may occur in around 6–7% of all patients with PCD.

Segregation analysis in families was consistent with *trans* allelic inheritance of the mutation as an autosomal recessive trait (table 2, figure 2 and online supplement, figures E2 and E3). Pedigree analysis showed horizontal transmission, and carrier analysis showed that parents carried the mutation, but were clinically unaffected; therefore, autosomal dominant inheritance was ruled out (online supplement, figure E2). In the five patients in whom a second mutation was not identified, it is likely that a second mutation in *DNAH11* is present but not discovered by sequence analysis (eg, promoter, intronic or large insertions-deletions).[15] Alternatively, a few of these patients may only be a carrier of a *DNAH11* mutation, and the actual biallelic PCD-causing mutations are present in a different gene. Finally, there might be a heterozygous mutation in another axonemal gene, and (together with the identified *DNAH11* mutation), would represent a digenic mode of inheritance; however, digenic inheritance has never been reported in PCD.

Of the 20 unrelated patients carrying mutations, there were 35 mutant alleles, including seven splice-site mutations (table 1). These splice-site mutations abrogated splicing in all six cases tested, which resulted in shorter *DNAH11* transcripts (table 3, figure 3). We also made the following conclusions: the p.E117V splice-donor site variant (when RNA was not available) and 10

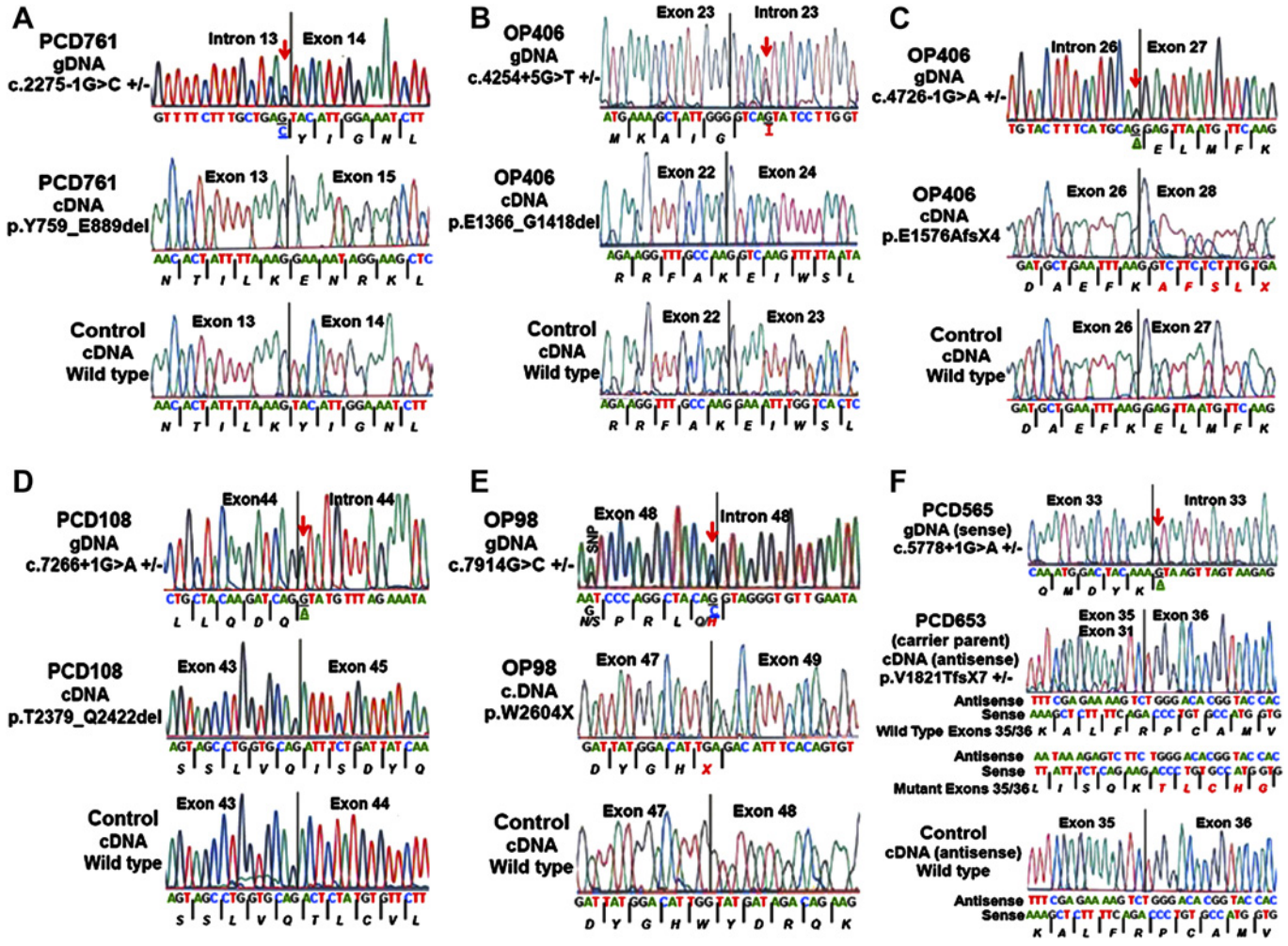

Figure 3 Effect of splice-site mutations on the dynein axonemal heavy chain 11 (*DNAH11*) transcript using reverse transcriptase PCR. (A) Splice-acceptor site mutation in intron 13 (c.2275-1G→C) in patient PCD761 led to the in-frame deletion of exon 14 that consisted of 131 amino acid residues. (B) Splice-donor site mutation in intron 23 (c.4254+5G→T) in patient OP406-II:2 led to the in-frame deletion of exon 23 that consisted of 53 amino acid residues. (C) Splice-acceptor site mutation in intron 26 (c.4726-1G→A) in patient OP406-II:2 led to out-of-frame deletion of exon 27, and resulted in a premature stop signal. (D) Splice-donor site mutation in intron 44 (c.7266+1G→A) in patient PCD108 led to the in-frame deletion of exon 44 that consisted of 44 amino acid residues. (E) Splice-donor site mutation in exon 48 (c.7914G→C) in patient OP98-II:1 led to out-of-frame deletion of exon 48, and resulted in a premature stop signal. (F) Splice-donor site mutation in intron 33 (c.5778+1G→A) in patient PCD565 led to out-of-frame deletion of exons 32—35, and resulted in a premature stop signal. The cDNA was available only from the carrier parent of patient PCD565, which was used to check the transcript. All of the six panels with three electropherograms each shows the genomic location of the mutation (top) with a red arrow and bases underlined, mutant cDNA transcript (middle) and wild-type transcript (bottom). Amino acid residues are italicised and the protein product due to the out-of frame mutation is shown in red font. The genomic base change for the mutation is underlined. A known single nucleotide polymorphism (SNP) was observed in OP98-II:1 and its location is shown. Further details on reverse transcriptase PCR are shown in table 3 (primer sequences shown in online supplement, table E3).

missense variants were likely disease causing because each variant was seen only once, and not seen in the dbSNP and 1000 Genomes databases; variants were absent in control subjects who were tested; the majority of missense mutations had a loss-of-function mutation on the *trans* allele; the amino acid affected by the missense mutations was highly conserved across species, and in silico analyses predicted it to be deleterious; and the majority of missense mutations were in a conserved AAA module or were on a microtubule binding domain (table 1 and figure 1). We also discovered some errors in the published sequence of *DNAH11*; thus, the mutation nomenclature needs to be updated based on the currently revised sequence (online supplement, table E7, figure E4).

The ability to establish (or rule out) a diagnosis of PCD by a genetic test in patients with a compatible phenotype and normal ciliary ultrastructure is significant at several levels. For example, several reports suggest that the vast majority (around 90%) of patients with PCD have defined ultrastructural defects.[2 3 36 37] However, this perspective may greatly underestimate the number of patients with PCD and normal ciliary ultrastructure, particularly in patients with normal situs status. At an individual case level, the importance of being able to establish (or exclude) PCD by a genetic test is demonstrated by the situation in one of our families (UNC101; figure 2C), in which one woman (#623) had a compatible clinical phenotype and low levels of nNO consistent with PCD, but no situs abnormalities. Her sister (#627) also had some clinical features of PCD, as did an 8-year-old paternal half sister (#635). Before genetic testing was possible, we were unable to clarify the diagnosis of PCD in this family. Subsequently, we defined

Web resources

- http://www.ncbi.nlm.nih.gov/Omim/
- http://www.ncbi.nlm.nih.gov/SNP/
- http://uswest.ensembl.org/index.html
- http://rsng.nhlbi.nih.gov/scripts/index.cfm
- http://rarediseasesnetwork.epi.usf.edu/gdmcc/index.htm
- http://neurocore.charite.de/MutationTaster/
- http://www.1000genomes.org/

At a glance commentary

Primary ciliary dyskinesia (PCD) is an autosomal recessive, genetically heterogeneous disorder with oto-sino-pulmonary disease. Most patients are diagnosed on the basis of ciliary ultrastructural defects. This study identified biallelic mutations in DNAH11 in 22% of 58 unrelated patients with normal ciliary ultrastructure, which validates the concepts of ciliary dysfunction in the presence of normal ultrastructure, and the use of genetic analysis to facilitate the diagnosis of PCD.

biallelic nonsense mutations in *DNAH11* in the proband and the full sibling, but the half sibling did not carry any mutation.

There are some instructive genotype–phenotype correlations in *Chlamydomonas* and murine orthologs of mutant *DNAH11*. The *Chlamydomonas reinhardtii* orthologue of *DNAH11* is β-dynein heavy chain (β-DHC), and *Chlamydomonas* mutants of β-DHC can assemble outer arm subunits into the flagellar axoneme, but swimming velocity and/or beat frequency are reduced.[38–41] In humans, immunofluorescence studies show normal distribution of ODA proteins (DNAH9 and DNAH5) in a patient with biallelic *DNAH11* mutations.[29] Thus, mutant *DNAH11* does not cause defective ODA assembly, but causes defective ciliary function.[2 20] The mouse orthologue of *DNAH11* (*Dnahc11*) is left-right dynein (*lrd*) and *lrd* null mice have situs defects.[32 34] The spontaneously occurring mouse model of *Dnahc11* (*inversus viscerum* mutant; *iv/iv*) has situs defects and recent work shows these mice have no detectable ciliary beat frequency, and suffer otitis media and rhinitis, even though they have normal ciliary ultrastructure.[32 33 42]

In conclusion, our large-scale mutation analysis indicates that biallelic mutations in *DNAH11* occur in 22% of patients with a clinical phenotype of PCD, but normal ciliary ultrastructure, and is consistent with an autosomal recessive mode of inheritance. Transcript analysis of six splice-site mutations revealed abrogation of normal splicing. These data clearly establish that clinical disease (PCD) occurs in patients with normal ciliary ultrastructure. This study also demonstrates that genetic analysis of *DNAH11* can be useful to assist in the diagnosis of PCD, and supports the concept to search for additional genetic origins of PCD.

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