Mutations of *DNAI1* in Primary Ciliary Dyskinesia
Evidence of Founder Effect in a Common Mutation

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Rationale: **Primary ciliary dyskinesia (PCD) is a rare, usually autosomal recessive, genetic disorder characterized by ciliary dysfunction, sino-pulmonary disease, and situs inversus. Disease-causing mutations have been reported in *DNAI1* and *DNAH5* encoding outer dynein arm (ODA) proteins of cilia.**

Objectives: **We analyzed *DNAI1* to identify disease-causing mutations in PCD and to determine if the previously reported IVS1+2_3insT (219+3insT) mutation represents a "founder" or "hot spot" mutation.**

Methods: **Patients with PCD from 179 unrelated families were studied. Exclusion mapping showed no linkage to *DNAI1* for 13 families; the entire coding region was sequenced in a patient from the remaining 166 families. Reverse transcriptase–polymerase chain reaction (RT-PCR) was performed on nasal epithelial RNA in 14 families.**

Results: **Mutations in *DNAI1* including 12 novel mutations were identified in 16 of 179 (9%) families; 14 harbored biallelic mutations. Deep intronic splice mutations were not identified by reverse transcriptase–polymerase chain reaction. The prevalence of mutations in families with defined ODA defect was 13%; no mutations were found in patients without a defined ODA defect. The previously reported IVS1+2_3insT mutation accounted for 57% (17/30) of mutant alleles, and marker analysis indicates a common founder for this mutation. Seven mutations occurred in three exons (13, 16, and 17); taken together with previous reports, these three exons are emerging as mutation clusters harboring 29% (12/42) of mutant alleles.**

Conclusions: **A total of 10% of patients with PCD are estimated to harbor mutations in *DNAI1*; most occur as a common founder IVS1+2_3insT or in exons 13, 16, and 17. This information is useful for establishing a clinical molecular genetic test for PCD.**

Keywords: cilia; dynein; dextrocardia; Kartagener syndrome; mutation

Primary ciliary dyskinesia (PCD; online Mendelian inheritance in man [OMIM] 242650) is a heterogeneous genetic disorder associated with impairment of flagellar and ciliary structure and function (1–3). PCD has been reported in all major ethnic groups worldwide (1) and is usually inherited as an autosomal recessive trait, although other modes of inheritance have been reported (4, 5). The incidence of PCD is estimated to be 1 in 16,000 individuals (6, 7).

Defective ciliary function in the respiratory tract leads to impaired mucociliary clearance, resulting in chronic oto-sinupulmonary disease leading to bronchiectasis. Defective nodal cilial function during embryogenesis leads to situs inversus in 50% of patients with PCD (1, 8–10) and is termed "Kartagener syndrome" (OMIM 244400) (1). The diagnosis of PCD is important especially because of implications for the management of respiratory tract disease (11); however, establishing the diagnosis may be difficult. The present "gold standard" diagnostic test is ciliary biopsy with documentation of abnormal ciliary ultrastructure by electron microscopy (EM) (3, 11–13). This test is not easily accessible and requires specialized expertise.

The respiratory cilium is a highly conserved, complex structure consisting of a 9+2 arrangement of microtubules and approximately 250 proteins (14, 15). The force for ciliary beating is provided by outer dynein arms (ODA) and inner dynein arms (IDA), which are recognizable structures on transmission EM images of ciliary cross section (1, 16). The ODA is comprised of several heavy-, intermediate-, and light-chain dyneins (17, 18). The composition of IDA is highly complex and varies along the length of the axoneme (13, 19, 20). Mutations in any protein or structural subunit of cilia could lead to PCD; however, most patients ($\sim$ 60–70%) have defective ODAs (1, 3, 21). The recently developed immunofluorescent assay may hold promise for identifying the ODA defect (22).

No disease-causing gene has been defined from a genome-wide linkage analysis using multiple families (23). Mutations have been identified in a small number of patients, in *DNAI1* (OMIM 604366) and *DNAH5* (OMIM 603335), which encode an intermediate and heavy chain of ODA, respectively (24–28). *DNAI1* and *DNAH5* were identified by candidate gene approach (24) and "homozygosity mapping," respectively (27, 29). In rare instances, mutations in *RPGR* (involved in X-linked retinitis pigmentosa) have been identified in male patients with retinal degeneration cosegregating with PCD (5, 30–33). The purpose of the current study was to perform a large-scale genetic analysis for *DNAI1* to identify mutations in well characterized, geographically diverse patients with PCD. This information will be useful for the development of a clinical molecular genetic test for PCD. In addition, we analyzed whether the recurring 219+3insT (IVS1+2_3insT) mutation (24–26) represents a mutational hot

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spot or a common founder. Parts of the current study have been previously reported as abstracts (34–36).

METHODS

Subjects: Clinical Evaluation

We studied 218 patients with PCD (179 unrelated families) from the University of North Carolina (UNC) and other specialized PCD centers. Over half of the patients with PCD (n = 112 from 89 families) were thoroughly evaluated at UNC; the evaluation included medical and family history, physical examination, sinus and/or chest radiographs, spirometry, and sputum microbiology (3). Ciliary ultrastructural analysis was carried out on nasal epithelial cells obtained by noninvasive nasal curettage (3, 37) and processed as described previously (3, 26, 37). Nasal nitric oxide (NO) was measured as described previously (3, 26). Blood or buccal cells were obtained from the proband (and relatives when possible) for DNA extraction (3, 26, 38). Genomic DNA was obtained from 106 patients with PCD (90 families) from diverse geographical locations, including Germany (n = 28), France (n = 23), United Kingdom (n = 18), Australia (n = 11), Italy (n = 6), Israel (n = 2), Pakistan (n = 1), and the United States (n = 1). Ciliary ultrastructural analysis and compatible clinical phenotype were used for the diagnosis of PCD. In the absence of ultrastructural analysis, adjunct tests such as immunofluorescent antibody staining of axonemal proteins, ciliary beat frequency, or nasal NO measurement were used. This study was approved by the committee for the protection of the rights of human subjects at the UNC-Chapel Hill and collaborating institutions, and written consent was obtained (*see* online supplement).

Mutation Profiling

All 20 coding exons and intron/exon junctions of *DNAI1* were amplified from gDNA (primers sequences available on request). Polymerase chain reaction (PCR) amplifications used AmpliTaq polymerase, and direct DNA sequencing was performed using ABI Prism BigDye primer Cycle Sequencing Ready Reaction kit on ABI310 or ABI3100 automated DNA sequencer (Applied Biosystem, Foster City, CA). High throughput sequencing was carried out at the Genome Sequencing Center at Washington University (St. Louis, MO). Exclusion mapping was carried out for 44 families using intragenic polymorphisms of *DNAI1* (*see* online supplement).

Population Studies

To estimate the allele frequencies in the general population, 10 genetic variants or mutations (IVS1+2_3insT, 526_528delGAA, 1177G>A, 1212T>G, 1222G>A, 1307G>A, 1490G>A, 1612G>A, 1644G>A, and IVS19+1G>A) were analyzed in at least 100 chromosomes from anonymized non-PCD subjects (hemophilia patients) of Caucasian ethnicity. Methods used were by restriction digestion or sequencing (*see* online supplement).

cDNA Analysis

Total RNA on epithelial cells obtained from the inferior turbinate using noninvasive nasal curettage (37) was isolated by the TRIzol method or an RNeasy mini kit (Qiagen, Valencia, CA). First-strand cDNA synthesis was performed with a SuperScript II RNase kit (Invitrogen, Carlsbad, CA) for reverse transcriptase–PCR (RT-PCR). RT-PCR followed by sequence analysis on DNAI1 mRNA was carried out in 19 patients with PCD from 14 unrelated families using overlapping primer sets (*see* online supplement). If multiple fragments were observed on the agarose gel, the PCR products were cloned into pCR2.1-TOPO vector (Invitrogen) to separate the alleles for subsequent sequencing. To determine the effect of the IVS19+1G>A mutation on splicing, *in vitro* assay was carried out (*see* online supplement).

Genotyping of Microsatellite *D9S1805* Close to *DNAI1*

To test for the existence of a possible founder effect associated with the IVS1+2_3insT mutation, the genotype of a microsatellite marker located 0.26 Mb upstream from *DNAI1* was determined in eight independent patients and in 60 control subjects of Caucasian origin. This analysis was performed by PCR amplification with the use of a D4-PA marked primer (available on request), and the fragment size was analyzed on a CEQ8000 automated sequencer (Beckman Coulter, Fullerton, CA).

RESULTS

Clinical Phenotype of Patients with PCD

We identified 218 subjects with documented PCD from 179 unrelated families. The majority of the families were white (87%). There was a broad mixture of ethnicity in the remaining families (*see* online supplement). Parental consanguinity was noted in 21 (12%) families (as shown in Table E1 in the online supplement). The clinical phenotypes are representative for that previously reported in PCD (Table E1): the patients ranged from 0 to 73 yr of age, there was an equal distribution of male and female patients, and 52% had dextrocardia (Kartagener syndrome). Most of these patients also had neonatal respiratory distress (72%), recurrent otitis media (91%), sinusitis (97%), and bronchiectasis by radiographic imaging (68%; Table E1). Nasal NO measurements were available for 100 patients with PCD, and the mean ± SD was 21.7 ± 17.4 nl/min. Values of < 100 nl/min are consistent with PCD, whereas mean values for normal control subjects were 376 ± 124 nl/min (mean ± SD) (3).

Exclusion Mapping

Exclusion mapping using intragenic single nucleotide polymorphisms for *DNAI1* was carried out in 44 families, when DNA was available from the affected and/or unaffected siblings and parents. The proband and the family members were sequenced for four intragenic polymorphisms, and 13 families were excluded from linkage to *DNAI1* based on defined criteria (listed in supplements for METHODS and Table E2).

Mutation Profiling

We identified 16 unrelated patients with PCD carrying mutations in *DNAI1*. Of these 16 subjects, 14 had biallelic, and two had only one mutation; thus, there were 30 mutant alleles. For each of the patients carrying mutations, key clinical demographics, ciliary ultrastructure, and mutations are depicted in Table 1 (*see* Table E3 for additional clinical details). For the 14 patients carrying biallelic mutations, five were homozygous, and nine were compound heterozygous (Table 1). Of the 30 mutant alleles, there were 13 different mutations (Figure 1). One previously reported mutation (IVS1+2_3insT) (24–26) was present on 17 alleles from 12 unrelated patients (Table 1). All the other mutations were novel: four nonsense, two missense, four splice defects, one insertion, and one deletion mutation. All the mutations were found only once, except for A538T, which was present in two unrelated patients (Table 1, Figure 1). Segregation analyses of the mutations were carried out wherever possible, and biallelic mutations were always found in trans (Table 1). Segregation analysis in the first-, second-, or third-degree relatives was carried out in three families, and the pedigrees were compatible with a recessive mode of inheritance (Figure 2). Wherever possible, RT-PCR was carried out in patients carrying a monoallelic mutation (data given below).

Frequency of *DNAI1* Mutations

The current data (n = 179 families) combined with previously published reports (n = 47 families) (24–26) were used to calculate mutation prevalence. From the total of 226 unrelated patients with PCD, 22 (10%) harbored mutations, of which 20 had two mutations (biallelic), giving 42 known mutant alleles. Of these 42 mutant alleles, 23 (55%) were IVS1+2_3insT mutation; 82% of all unrelated patients with mutations carried at least one IVS1+2_3insT mutant allele. The high prevalence of this IVS1+2_3insT mutation suggested a mutational hot spot or a

TABLE 1. DETAILS OF *DNAI1* MUTATIONS IN 16 UNRELATED PATIENTS WITH PRIMARY CILIARY DYSKINESIA

					Allele 1				Allele 2			
Subject	Sex	Age at Diagnosis (*yr*)	KS†	Dynein Arm Defect	Base Change	Exon/Intron	Amino Acid Substitution	Segregation	Base Change	Exon/Intron	Amino Acid Substitution	Segregation
Homozygous												
PCD552	F	13	No	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	Paternal	IVS1+2_3insT	Int 1	Splice/trunc	Maternal
PCD218	M	70	Yes	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	IVS1+2_3insT	Int 1	Splice/trunc	nd
PCD223	M	14	Yes	Not available	IVS1+2_3insT	Int 1	Splice/trunc	nd	IVS1+2_3insT	Int 1	Splice/trunc	nd
OP121 II:1	F	15	Yes	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	IVS1+2_3insT	Int 1	Splice/trunc	nd
UCL64 II2	F	4	Yes	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	IVS1+2_3insT	Int 1	Splice/trunc	nd
Compound heterozygous												
PCD455	F	40	Yes	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	IVS7–2A>G	Int 7	Splice/trunc	nd
OP-71 II:1	F	20	No	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	874C>T	Ex 10	Q292X	nd
D78X1	M	40	No	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	IVS10–4_7del	Int 10	Splice	nd
PCD930	F	10	No	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	Maternal	1490G>A	Ex 16	R468_K523del	Paternal
UCL3 II:2	F	5	No	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	1612G>A	Ex 17	A538T	nd
PCD749*	M	8	No	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	Paternal	1926_1927insCC	Ex 19	I643PfsX48	Maternal
PCD630	F	1.5	Yes	ODA defect	1212T>G	Ex 13	Y404X	Maternal	1222G>A	Ex 13	V408M	Paternal
D32X1	M	10	No	ODA defect	1307G>A	Ex 13	W436X	Maternal	IVS19+1G>A	Int 19	A607_K667del	Paternal
PCD327	M	2	Yes	ODA defect	1612G>A	Ex 17	A538T	Maternal	1644 G>A	Ex 17	W548X	Paternal
Heterozygous												
PCD480	F	37	Yes	ODA defect	IVS1+2_3insT	Int 1	Splice/trunc	nd	WT	none	WT	None
F-725 II:3	F	44	No	ODA defect	463delA	Ex 6	T155LfsX18	Paternal	WT	none	WT	None

Definition of abbreviations: Ex = exon; Int = intron; KS = Kartagener syndrome; nd = not determined; ODA = outer dynein arms; splice/trunc = splice mutation leading to insertion of intron 1 sequences in the message, thus causing truncated protein.

* Patient was half-Caucasian/half-Hispanic ethnicity; all the remaining patients were of Caucasian ethnicity.

† If dextrocardia was present, patients were termed as having Kartagener syndrome. There was no known parental consanguinity in any of these families.

founder effect (see specifics below). A cluster of mutations was noted in exons 13, 16, and 17, where 12 of 42 mutant alleles resided (Figure 1); 46% of the families carried at least one mutant allele in these three exons. Indeed, 21 (96%) of the 22 unrelated patients carrying mutations would have been detected by analysis restricted to exons 13, 16, and 17 and IVS1+2_3insT mutations. From these 21 patients, biallelic mutations would have been picked up in 15 (68%) unrelated patients.

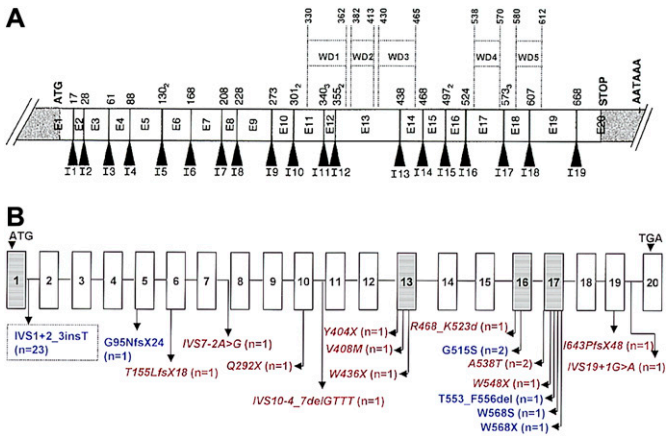

Figure 1. Distribution of novel and published *DNAI1* mutations. (*A*) Genomic organization of human *DNAI1* as shown previously (24). The 20 exons are indicated by the *boxes* containing exon numbers (E#). *Shaded boxes* represent untranslated regions. The locations of the start and stop codons and polyadenlylation signal are shown. The number of the first codon in each exon is indicated; exons beginning with the second or third base of a codon are indicated by subscript 2 or 3, respectively. The exons are drawn to scale, and intron–exon boundaries are denoted by *closed triangles*. Five conserved tryptophan and aspartic acid (WD) repeat regions are shown on the top of the genomic structure, and the codon number for the start and end of each WD repeat box is indicated. (*B*) Schematic drawing of *DNAI1* and the position of all identified mutations are indicated. The number of mutant alleles is indicated in the *parentheses*. Novel mutations that were identified in the current study are *italicized* and indicated in *red*. Founder mutation is in the *box*; *shaded* exons indicate a mutation cluster.

Correlation of Genotype with Ultrastructure

Genetic heterogeneity poses a significant challenge to large-scale genetic testing. One way to address this problem is to carefully categorize patients by the type of ciliary ultrastructural abnormality (e.g., ODA vs. IDA defects) and test for genes known to be associated with these specific dynein arm defects. Ciliary ultrastructure analysis was available from 152 unrelated patients, and we found that most (79%) of our patients with ciliary EM analysis had an ODA defect, whereas 13% had isolated IDA defect (Table E1) (EM images are not shown). Overall, 9% of our patients with PCD (n = 16 of 179) had *DNAI1* mutations, and 13% (15 of 120) patients with a defined ODA defect had *DNAI1* mutation. None of the 32 patients with PCD devoid of an ODA defect had a *DNAI1* mutation (Table 2); thus, mutations in *DNAI1* were noted exclusively in association with ODA defect (Table 2). Combining the present study and all previous reports (24–26), *DNAI1* mutations are present in 10% (22 of 226) of unrelated patients with PCD and in 14% (19 of 134) of patients with PCD with a known ODA defect (Table 2).

Population Studies

To test whether defined mutations are disease-causing for the PCD phenotype or represent benign polymorphisms, we determined the frequency of all missense and some of the splice and nonsense mutations in the general population by analyzing over 100 control alleles; none of the control chromosomes carried any of the tested mutations (Table 3). Although 100 chromosomes were analyzed earlier to define the frequency of IVS1+2_3insT mutation (24), we studied an additional 382 alleles and found that none carried this mutation.

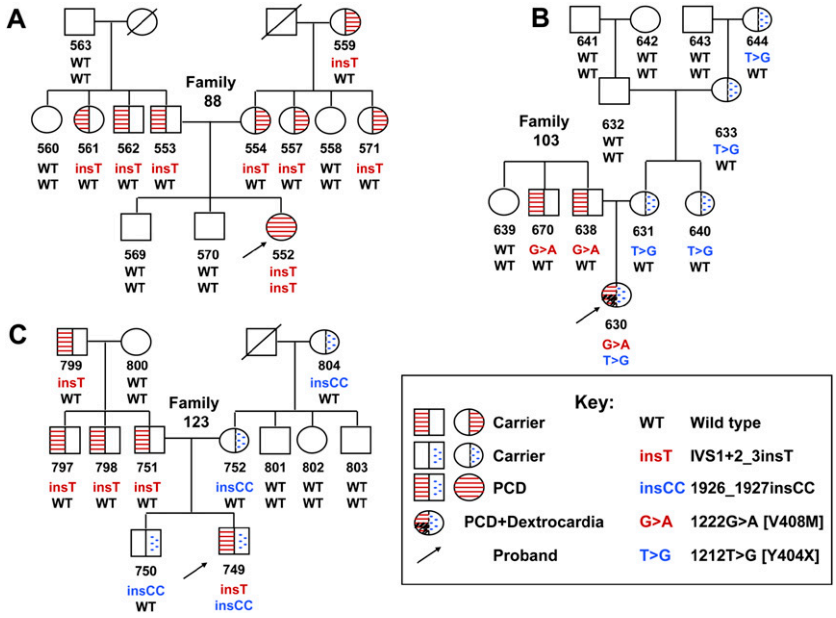

Figure 2. Segregation analysis from extended families demonstrating recessive inheritance of the *DNAI1* mutations. (*A*) Proband (PCD552) was homozygous for the previously reported IVS1+2_3insT mutation. The parents, a paternal uncle, a paternal aunt, two maternal aunts, and the maternal grandmother were found to be carriers. (*B*) Proband 630, who had primary ciliary dyskinesia with dextrocardia (Kartagener syndrome), was compound heterozygous for the novel mutations in exon 13. Segregation analysis showed that the 1212G>A (V408M) mutation was inherited from the father and that a paternal uncle was a carrier. The 1222T>G (Y404X) mutation was inherited from the mother, and a maternal aunt, the maternal grandmother, and the maternal great grandmother were carriers. (*C*) Proband 749 was compound heterozygous, carrying one novel mutation in exon 19 and a previously reported IVS1+2_3insT mutation. His unaffected sibling was a carrier of the novel exon 19 mutation; this mutation was inherited from the mother, who inherited it from her mother (maternal grandmother of the proband). The IVS1+2_3insT mutation was inherited from the father; two paternal uncles and the paternal grandfather were also carriers.

Polymorphisms and Variants of Unknown Significance

During the current study, we found several polymorphisms that had been noted previously, and their reference sequence numbers (www.ncbi.nlm.nih.gov/SNP/) are presented in Table E4. We detected two variants of unknown significance (1177G>A [V393M] and 526_528delGAA [176delE]) on one allele of two unrelated patients with PCD. Full gene sequencing did not reveal another mutation on the trans allele (*see* online supplement for details).

cDNA Analysis

Because deep intronic mutations can be missed by genomic DNA sequencing (39), we performed RT-PCR for *DNAI1* followed by agarose gel electrophoresis and sequencing on nasal epithelial RNA from 14 patients from 12 unrelated families. No aberrant fragments on the agarose gel or additional mutations by sequence analysis were identified in any of these patients (Table 4). For one patient (PCD480) who was heterozygous for IVS1+2_3insT splice mutation, genomic DNA sequencing did not reveal any additional mutation. Further analysis of the cDNA from this patient confirmed aberrant splicing and premature termination signal due to the IVS1+2_3insT mutation, as reported previously (24), but did not reveal any additional splicing defect on the trans allele. Another patient with PCD (PCD930; Table 4) was a compound heterozygote for the IVS1+2_3insT (maternal) and 1490G>A (paternal) mutations. Because the mutation 1490G>A is located at the splice site, we checked for its effect on splicing. Analysis of maternal RNA confirmed the splicing defect due to IVS1+2_3insT mutation. Analysis of paternal RNA revealed two molecular species on agarose gel, one of the expected size of 419 bp, and a smaller 251-bp fragment, not present in the control sample. Sequencing revealed that the 419-bp fragment coincides for the wild-type sequence. Sequencing the smaller fragment revealed the aberrant splice product leading to in-frame deletion of 56 amino acids, which comprise exons 15 and 16 (R468_K523del) (Table 4, Figures 3A and 3B). The consequence of the IVS19+1G>A splice mutation (present in patient D32X1) on the processing of *DNAI* transcript was determined using an *in vitro* splicing assay. Results demonstrated that it causes aberrant splicing of *DNAI1* transcript, resulting in an in-frame deletion (61 amino acids) of exon 19 (A607_K667del; Table 4, Figure 3C).

Founder Effect

Given the relatively high frequency and wide geographic distribution of the IVS1+2_3insT mutation among patients with PCD, we wondered whether the IVS1+2_3insT allele has a common origin or reflects the existence of a mutational hotspot in the

TABLE 2. FREQUENCY OF *DNAI1* MUTATIONS OBTAINED FROM CURRENT AND PUBLISHED DATA

Study	Distribution of Families	Number of Families	Number of Families with at Least One Mutation	Families with at Least One Mutation (%)
Current	Total # of families	179	16	9
Current	Total # of families with ODA defect	120	15	13
Published*	Total # of families	47	6	13
Published*	Total # of families with ODA defect	14	4	29
Combined	Total # of families	226	22	10
Combined	Total # of families with ODA defect	134	19	14
Combined	Total # of families with defined ciliary defects other than ODA	32	0	0

Definition of abbreviation: ODA = outer dynein arms.
* From References 24–26.

TABLE 3. POPULATION FREQUENCY OF MUTATIONS/VARIANTS IN DISEASE-FREE INDIVIDUALS

Mutation	Subjects Analyzed (*n*)	Alleles Analyzed (*n*)	Wild-Type Alleles (*n*)	Hetero zygous Alleles (*n*)	Mutant Allele Frequency	Carrier Frequency
IVS1+2_3insT [splice/truncating]*	191	382	382	0	0	0
526_528delGAA [176delE]	54	108	108	0	0	0
1177G>A [V393M]	197	394	394	0	0	0
1212T>G [Y404X]	56	112	112	0	0	0
1222G>A [V408M]	56	112	112	0	0	0
1307G>A [W436X]	54	108	108	0	0	0
1490G>A [R468_K523del]	111	222	222	0	0	0
1612G>A [A538T]	113	226	226	0	0	0
1644G>A [W548X]	113	226	226	0	0	0
1657_1668del [T553_F556del]*	113	226	226	0	0	0
IVS19+1G>A [splice]	55	110	110	0	0	0

* The prevalence of this previously reported mutation by others (24, 25) was carried out in the current study.

DNAI1 gene. To address this issue, we analyzed a microsatellite marker (*D9S1805*) located 0.26 Mb upstream of *DNAI1* in eight unrelated patients with PCD, two of whom were homozygous for the mutation and six of whom were heterozygous (i.e., compound heterozygotes). These unrelated patients originated from different geographic regions: four from the United States, two from France, and two from Germany. This analysis showed that these patients shared the same *D9S1805* allele, characterized by 19-CA repeats. This allele was heterozygous in all patients except for two, who were homozygous for the IVS1+2_3insT mutation and for the 19-CA allele. In addition, a similar analysis performed in a parent carrying the IVS1+2_3insT mutation on one allele showed a direct association of this mutation with the 19-CA allele at the *D9S1805* locus. Moreover, the genotype of the *D9S1805* marker on the trans allele was found to be different among six compound heterozygous patients (data not shown). To estimate the frequency of the 19-CA *D9S1805* allele in the general population, the *D9S1805* marker was genotyped in 60 independent control subjects. Nine different alleles were identified among these 120 chromosomes (Figure E1); the 19-CA allele represented 17.5% of all tested chromosomes.

DISCUSSION

PCD is a genetically heterogeneous disease associated with abnormal ciliary ultrastructure and function. Before this study was done, disease-causing mutations had been described in two genes (*DNAI1* and *DNAH5*) encoding ciliary outer dynein arms in a

TABLE 4. REVERSE TRANSCRIPTASE–POLYMERASE CHAIN REACTION AND cDNA ANALYSIS OF *DNAI1* IN PATIENTS WITH PRIMARY CILIARY DYSKINESIA

Subject Number	Family Number	Sex	Age at Diagnosis (*yr*)	KS[‖]	Dynein Arm Defect	NO (nl/min)	*DNAI1* gDNA Sequence	*DNAI1* cDNA Sequence
3	1	F	40	No	IDA	13	No mutation	WT
10	2	F	51	Yes	IDA	na	No mutation	WT
16	3[‡]	F	27	No	ODA	3	No mutation	WT
57	7*[‡]	M	9	No	ODA	2	No mutation	WT
58	7*[‡]	M	4	Yes	ODA	7	No mutation	WT
144	18[‡]	F	30	Yes	ODA	12	No mutation	WT
145	18[‡]	M	33	No	ODA	4	No mutation	WT
157	21	F	12	Yes	IDA	2	No mutation	WT
158	22	F	73	No	ODA	2	No mutation	WT
159	22	F	63	Yes	ODA	10	No mutation	WT
274	46*[†]	M	29	Yes	ODA	43	No mutation	WT
436	74	F	33	Yes	ODA	19	No mutation	WT
440	76	M	56	Yes	ODA	20	No mutation	WT
564	89	F	59	Yes	IDA	12	No mutation	WT
480	83	F	37	Yes	ODA	21	IVS1+2_3insT +/−	Aberrant
930	150	F	10	No	ODA	13	IVS1+2_3insT +/−, 1490G>A +/−	na
957[§¶]	150	F	—	—	—	—	1490G>A +/−	Aberrant
931[§¶]	150	M	—	—	—	—	IVS1+2_3insT +/−	Aberrant
D32X1	D32	M	10	No	ODA	na	IVS19+1G>A +/−	Aberrant**

Definition of abbreviations: IDA = inner dynein arms; KS = Kartegener syndrome; na = not available; NO = nasal nitric oxide; ODA = outer dynein arms; WT = wild type.

* Parental consanguinity was noted for Families 7 and 46.

[†] All the families were of Caucasian origin except Family 46.

[‡] Families published earlier and were wild type by genomic DNA sequencing (26).

[§] PCD 957 and PCD 931 were the unaffected father and mother of an affected individual (PCD930), respectively.

[‖] Patients with dextrocaridia were termed as having Kartegener syndrome.

[¶] For PCD480, PCD931, PCD957, and D32X1, mRNA analysis showed aberrant message consistent with the results obtained on genomic DNA sequencing.

** *In vitro* splicing assay was carried out (*see* RESULTS).

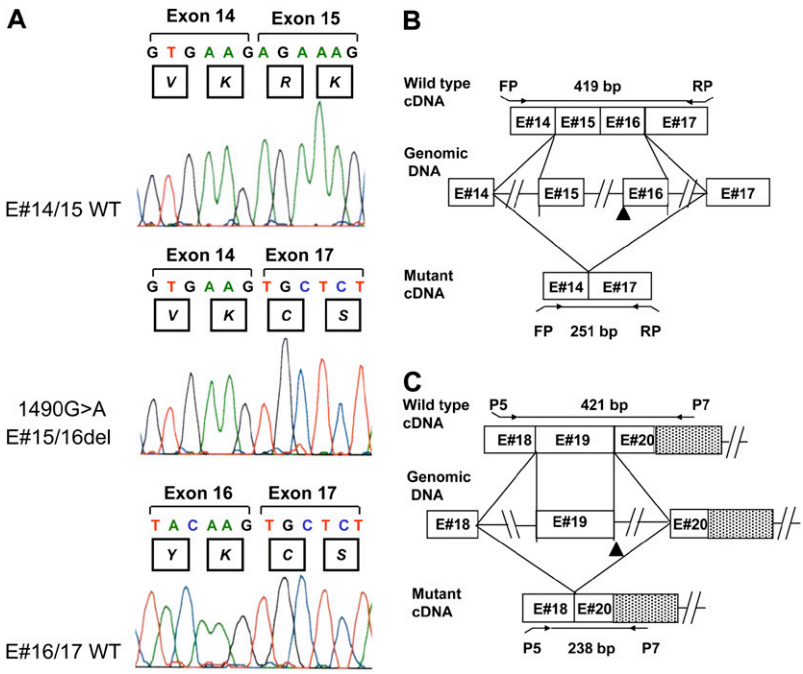

Figure 3. Effect of the 1490G>A and IVS19+1G>A mutations on mRNA. (*A*) Electropherogram showing the effect of the 1490G>A mutation on the *DNAI1* transcript and the cDNA sequence of the wild-type (*top* and *bottom*) and mutant (*middle*) *DNAI1* alleles. RNA from the parents was available for the cDNA analysis. cDNA amplification from the father, who carried a 1490G>A transition, generated a 419-bp (normal size) and a 251-bp fragment, whereas only a 419-bp fragment was obtained from the control subject. In the presence of the 1490A allele, exons 15 and 16 are deleted from the message, leading to the in-frame deletion of 56 amino acids (R468_K523del). Exons are indicated as E#. (*B*) Schematic representation of the 1490G>A splicing mechanisms leading to the normal (*top*) and aberrant (*bottom*) DNAI1 transcript. The size of the normal (*top*) and mutant (*bottom*) reverse transcriptase–polymerase chain reaction (RT-PCR) products generated with forward primer (FP) and reverse primer (RP) (*arrows*) are indicated (*see* online supplement). Exons are indicated as E#. *Closed arrowhead* indicates the mutation in the genomic DNA. (*C*) Schematic representation of the splicing mechanisms leading to normal (*top*) and abnormal (*bottom*) *DNAI1* transcripts, from the corresponding genomic DNA fragments (*middle*); the 3′ untranslated region is shown as a *shaded area*. The size of the normal (*top*) and mutant (*bottom*) RT-PCR products generated with primers P5 and P7 (*arrows*) are indicated. The IVS19+1G>A mutation (*closed arrowhead*) results in abnormal splicing with the generation of a small product (238 bp) lacking exon 19; this would lead to an in-frame deletion of the 61 amino acid residues (A607_K667del) encoded by exon 19. Normal splicing results in the generation of a 421-bp product (*see* online supplement). Exons are indicated as E#.

small number of patients with PCD (24–27). However, genotype–phenotype correlations and the prevalence of mutations in these genes have not been investigated. We undertook a mutation analysis of *DNAI1* in a large number of well characterized patients with PCD to test for the correlation of mutations with ciliary ultrastructural defects (ODA versus other defects) and clinical phenotype and to define the prevalence of *DNAI1* as a disease-causing gene in PCD. Determining the prevalence and distribution of mutations in *DNAI1* is essential if a clinical molecular genetic screening test for PCD is to be developed. Because PCD is a rare genetic disorder, we obtained DNA samples from six geographically different cities to increase the number of patients tested. All specimens came from the specialized PCD centers that are experienced with PCD diagnosis using established methods. The majority of the patients (141 of 179) had specific ultrastructural defects in cilia (Table E1). In addition, the patients without the defined ultrastructural abnormalities were included if they had a strong clinical phenotype, including oto-sinu-pulmonary disease, situs abnormalities, bronchiectasis, neonatal respiratory distress in term neonates, history of infertility, low nasal NO, or abnormal ciliary beat frequency. A case report on a patient with PCD and normal dynein arms has been reported previously (40).

Our current estimates of 218 subjects from 179 unrelated families indicate that approximately 9% of all patients with PCD have disease-causing mutations in *DNAI1* and that approximately 13% of patients with PCD with ODA defects have *DNAI1* mutations (Table 2). None of the 32 unrelated patients in the current study without defined ODA defects had any *DNAI1* mutation. Thus, *DNAI1* mutations are detected exclusively in association with the ODA defects. Similar observations have been made in *Chlamydomonas*, where IC78 (ortholog of *DNAI1*) mutations lead to ODA defects in flagella (17). We also observed a similar prevalence of mutations in *DNAI1* when we combined our current study with three previous studies (24–26) involving 47 families (10% of all the 226 families had *DNAI1* mutations, and of those 14% with a known ODA defect harbored *DNAI1* mutations; Table 2). We believe that this observation is a realistic frequency and that there is no ascertainment bias because all 89 unrelated patients with PCD evaluated at UNC were sequenced without preselection, and mutations were seen in 9% (n = 8 patients). In addition, the current study shows that 14% of patients with PCD with defined ODA defects have mutations in the *DNAI1*, and because approximately 66% of patients with PCD present with ODA defects (1, 3, 21), approximately 9% of patients with PCD are likely to have mutations in *DNAI1* gene.

The use of polymorphisms(s) to "exclude" families is useful in a genetically heterogeneous disorder like PCD, especially when dealing with the large genes, because it reduces the need for full gene sequencing in a large number of subjects. During this study, we found nine polymorphisms that were useful for exclusion mapping because they permitted us to exclude 13 families from linkage to *DNAI1*.

The pattern of inheritance of *DNAI1* mutations in PCD is autosomal recessive (Figure 2). Of 22 patients with *DNAI1* mutations from the current and previous studies (24–26), 20 had biallelic mutations (five patients were homozygous, and nine patients were compound heterozygous). Furthermore, the segregation pattern, if available (Table 1 and References 24–26), showed that these mutations were transallelic. In two patients from the current study, only monoallelic mutations were observed. In these two patients, it is likely that the mutation on the trans allele was missed because only genomic DNA sequence analysis was performed. There is no clinical evidence of dominant inheritance in these two patients because a parent from each of these patients carried the same mutation but was clinically unaffected. In addition, one patient carried the common mutation in the heterozygous state, and we know that this mutation segregates in a recessive manner. Although we have not observed

evidence for a digenic pattern in PCD, it cannot be ruled out. There are other genetically heterogeneous disorders where this mode of inheritance has been described (41–43).

The type of mutations in *DNAI1* varied. Of the 42 mutant *DNAI1* alleles from 22 unrelated patients from current and previous studies (24–26), 18 different mutations have been described, including frameshift/stop mutations, base substitutions, and splice mutation. Twelve of the mutations described in the current study are novel mutations (Figure 1). There were two missense variants (V408M and A538T), which we believe are likely to be disease-causing mutations because (*1*) one of the variants (A538T) was found to be present in two unrelated families, (*2*) segregation analysis showed that they were transallelic with respect to the second mutation, (*3*) both variants involved residues that are highly conserved across the species and belong to the conserved tryptophan and aspartic acid (WD) repeat domain of the gene, and (*4*) neither variant was detected in more than 100 control chromosomes from the general population (Table 3). Similarly, none of the splice mutations was found in any of the control chromosomes (Table 3). Although mutations were present throughout the *DNAI1*, most mutations occurred in intron 1 (IVS1+2_3insT was present in 55%) and in exons 13, 16, and 17 (29% of the mutant alleles). Moreover, nearly half of the families (46%) carried at least one mutant allele in these three exons. Having a high prevalence of mutations in discrete regions (intron 1 and exons 13, 16, and 17) of *DNAI1* will aid in the development of the clinical screening panel for PCD.

The status (mutation vs. polymorphism) of two heterozygous variants (526_528delGAA [176delE] and 1177G>A [V393M]) discovered during this study is uncertain. Both variants are evolutionarily conserved among some species, and they were not present in control chromosomes from the general population (108 chromosomes for 176delE and 394 chromosomes for V393M), suggesting that they may be true mutations. However, it seems more likely that these are polymorphisms for several reasons: (*1*) a patient who carried these variants did not have an ODA defect (based on EM data), and mutations in *DNAI1* have been associated with ODA defects; (*2*) a patient who carried 176delE in a heterozygous state was born to consanguineous parents, and such a patient would likely depict "identity by descent" and hence homozygosity for the mutations or polymorphisms at the disease locus; and (*3*) in rats, methionine is used at the equivalent codon of V393M, suggesting that methionine is the normal variant and hence V393M represents a rare polymorphism (*see* online supplement).

We also observed the consequence of three splice mutations on mRNA levels. We confirmed that the IVS1+2_3insT mutation causes aberrant splicing, as previously reported (24). Another mutation, 1490G>A, predicts an amino acid substitution; however, we demonstrated that it also affects splicing, leading to in-frame deletion of exons 15 and 16 (56 amino acids). Finally, a splice mutation in intron 19 resulted in an in-frame deletion of exon 19 (61 amino acids). Although approximately 15% of the known disease-causing mutations are splice mutations (44), we found no (deep intronic) mutations by the analysis of RT-PCR products (Table 4).

The mutations in *DNAI1* seem to be most common in white individuals with PCD. Combining data from the current study with previous studies (24–26), ethnicity information is available on 182 unrelated patients with PCD (364 alleles). Most of the 364 alleles were in patients with Caucasian origin (315 alleles), and 34 alleles carried *DNAI1* mutations (11%). Of the alleles from the patients of non–Caucasian ethnicity (n = 47 alleles), only one allele carried a *DNAI1* mutation (2%). The non–Caucasian dataset is small but suggests that *DNAI1* mutations may be found more commonly in patients with a Caucasian ethnicity. One reason for *DNAI1* mutations in white patients could be the fact that the IVS1+2_3insT mutation was present in 55% (23 of the total 42) of the mutant alleles; moreover, 82% (18 of 22) of unrelated patients had IVS1+2_3insT on at least one allele. Based on the high frequency of the IVS1+2_3insT mutation, founder effect analysis was carried out, and several lines of evidence strongly suggest the presence of a founder effect. First, a careful examination of the nucleotide environment did not reveal structural features that would favor the insertion of one base pair at this position. Second, all the patients who carried this mutation and who were genotyped at the closely linked *D9S1805* marker were shown to carry the same polymorphic allele bearing 19-CA repeats. The association of the disease-causing mutation with the 19-CA *D9S1805* allele was clearly demonstrated in two homozygous and one compound heterozygous patient. Although the parental DNA samples were not available for the other compound heterozygous patients, all these patients were heterozygous for the 19-CA *D9S1805* allele. These data strongly indicate that the IVS1+2_3insT alleles originate from an ancestral mutational event.

Taken together, the prevalence of the IVS1+2_3insT mutation and the cluster of mutations in exons 13, 16, and 17 would allow identification of most patients with *DNAI1* mutations. Specifically, 21 of all 22 unrelated patients described in the present and past studies (24–26) that carry mutations of *DNAI1* would be detected by screening for IVS1+2_3insT and mutations in exons 13, 16, and 17. This suggests that the development of clinical molecular genetic testing is a realistic and useful goal. Similar data are available for another gene encoding an ODA protein, *DNAH5* (28), which has a frequency of mutation in all PCD of 28% (38 of 134 unrelated families with PCD). Moreover, in 47% of all mutant *DNAH5* alleles (31 of 66), the mutations were located in clusters of five exons. Combining the data for both genes (*DNAI1* or *DNAH5*), it seems that approximately 38% of all the patients with PCD are estimated to carry mutations in *DNAI1* (22 families) or *DNAH5* (38 families). For feasibility of genetic screening, it is important to note that over half (66 of 108) of the mutant alleles for both genes fall into the mutation cluster of four exons in *DNAI1* and five exons in *DNAH5*. Thus, screening for only these nine exons would permit detection of 41 of 60 (68%) families that harbor mutations in at least one allele. A reasonable approach for developing a clinical test panel would be to test by "tiered" approach, for example, screening for mutation cluster region of the *DNAI1* and *DNAH5* in phase I, followed by full gene sequencing of the appropriate gene for the patients with monoallelic mutations in a second phase. Such genetic analysis would help expedite and improve the diagnosis of PCD and subsequent counseling for patients and families.

In conclusion, we show that mutations in *DNAI1* are estimated to occur in up to 14% of patients with PCD and reflect an autosomal recessive mode of inheritance. Most mutations in *DNAI1* are truncating mutations and seem to occur more commonly in patients with Caucasian ethnicity. These data, coupled with the data for *DNAH5* mutations (28), indicate that development of a clinical molecular genetic panel is feasible and would improve diagnosis and counseling for PCD.

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