Germline Mutations in an Intermediate Chain Dynein Cause Primary Ciliary Dyskinesia

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Primary ciliary dyskinesia (PCD) is a genetically heterogeneous, autosomal recessive disorder caused by abnormal ciliary ultrastructure and function, characterized clinically by otosino-pulmonary disease. Mutations in an intermediate chain dynein (*DNAI1; IC78*) have recently been described in PCD patients, with outer dynein arm (ODA) defects. The aims of the current study were to test for novel *DNAI1* mutations in 13 PCD patients with ODA defects (from 7 unrelated families) and to assess genotype/phenotype correlations in patients and family members. A previously reported mutation (219+3insT) was detected in three PCD patients from two families. The opposite allele had the novel missense mutation G1874C (W568S) in both affected individuals from one family, and a nonsense mutation G1875A (W568X) in an affected individual from another family. The tryptophan at position 568 is a highly conserved residue in the WD-repeat region, and a mutation is predicted to lead to abnormal folding of the protein and loss of function. None of these mutations were found in 32 other PCD patients with miscellaneous ciliary defects. Mutations in *DNAI1* are causative for PCD with ODA defects, and are likely the genetic origin of clinical disease in some PCD patients with ultrastructural defects in the ODA.

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder, characterized by chronic middle ear, sinus, and lung disease (1, 2). The disease is usually inherited as an autosomal recessive trait (MIM242650), with an incidence of approximately 1 in 15,000–30,000 (3). Approximately 50% of the patients with PCD have situs inversus (SI), indicating that there is a tight (but random) association between SI and PCD, termed Kartagener's syndrome (4, 5).

The pathogenesis of the disease reflects defective epithelial ciliary structure and function in the eustachian tube, sinuses, and lower airways, leading to abnormal mucociliary clearance, an important component of host defenses in these organ systems (6, 7). A variety of ultrastructural abnormalities of cilia have been described. Since the ciliary axoneme is comprised of nearly 250 polypeptides, there are many candidate genes that could account for the genetic heterogeneity of PCD. The most common form of PCD is associated with defects (or absence) of the outer dynein arm (ODA), inner dynein arm (IDA), or both dynein arms (6, 8). Components of dynein arms include axonemal dyneins, which are large globular microtubule-activated ATPase motor protein complexes composed of several heavy, intermediate, and light chains.

Recently, a human intermediate chain dynein 1 (DNAI1) gene (GenBank accession no. AF091619), related to the *C. reinhardtii* IC78 gene, was cloned, and loss-of-function mutations were described in the DNAI1 gene in a PCD patient with ciliary ODA defects (9). In the same study, five other consanguineous families with defective ODA did not show linkage to the DNAI1 gene when intragenic single nucleotide polymorphisms (SNPs) were used, further supporting the hypothesis that PCD is a genetically heterogeneous disease.

Without careful categorization and phenotyping of PCD patients, genetic heterogeneity is a significant limitation for large-scale genetic testing. As part of an overall effort to study the phenotype and genetics of patients with PCD, we have assembled a large database of well-characterized PCD patients and family members. This effort includes the collection of clinical and physiologic data, including measures of nasal nitric oxide (NO), development of pedigrees, and analyses of ciliary structure and function. Patients can be segregated into cohorts, depending on the nature of the ciliary structural abnormality (inner versus outer dynein arm versus other ciliary abnormality), which facilitates genetic testing in a more selective and efficient fashion (10). For the current study, we initially focused on patients with defects in the ODA.

Materials and Methods

Patients: Clinical Evaluations and Ciliary Dynein Arm Evaluations

All patients with PCD, and their available family members, were seen and evaluated at the General Clinical Research Center of the University of North Carolina (UNC). Studies were performed under the auspices of the Committee for the Protection of the Rights of Human Subjects at UNC Chapel Hill. Clinical evaluations included a detailed medical history (including an extended family history), physical examination, sinus and/or chest radiographs (CXR), lung function testing, and sputum microbiology. Specific information was sought for neonatal respiratory distress, recurrent otitis media with a requirement for placement of drainage tubes, recurrent bronchitis and copious purulent sputum production, and situs inversus (10). To assess ciliary structure, epithelial cells were obtained from the inferior turbinates using a noninvasive nasal curettage technique (11). The specimens were fixed in buffered 2% glutaraldehyde + 2% paraformaldehyde (2+2) + 0.5% tannic acid and processed by standard techniques to epoxy resin blocks (11). Sections of 90 nM thick-

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Abbreviations: base pair, bp; chest radiograph, CXR; aspartic acid, D; denaturing high performance liquid chromatography, DHPLC; dynein intermediate chain 1, DNAI1; diagnosis, DX; forced expiratory volume in 1 s, FEV_1; female, F; glycine, G; histidine, H; inner dynein arm, IDA; male, M; nitric oxide, NO; outer dynein arm, ODA; primary ciliary dyskinesia, PCD; polymerase chain reaction, PCR; serine, S; standard error of the mean, SEM; single nucleotide polymorphism, SNP; situs inversus, SI; tryptophan, W; University of North Carolina, UNC; wild type, WT.

* These authors performed equal amounts of work for the study.

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ness were obtained and post-stained with uranyl acetate and lead citrate, and examined and photographed in a Zeiss EM900 operating an accelerating voltage of 50 kV at a magnification of ×50,000. Ciliary images were considered acceptable for analysis based on criteria described elsewhere (12). Each subject was categorized as having a predominantly ODA defect, IDA defect, both dynein arms defective, or other microtubular defects (central pair abnormal, or radial spoke abnormalities). A defect was defined as absence or near-absence (short or stubby on all sections) of dynein arms. Those family members unable to travel to UNC for evaluation agreed to provide DNA by means of the buccal brush method; evidence for PCD was sought in these subjects by a careful history as described above.

A selected cohort of PCD patients with clear-cut defects in ODA were chosen for the initial genetic study of the DNAI1 gene, because mutations in this gene appear to be associated with PCD and ODA defects. This cohort included 13 PCD patients with clear-cut defects in ODA (± IDA defects), and, where available, first-degree relatives from the seven families. Two families (Families 7 and 11) had known consanguinity, whereas there was no known inbreeding in the other five families. This strategy allowed us to use intragenic SNPs in the DNAI1 gene in these families to exclude the ones where linkage to DNAI1 gene could be clearly ruled out.

Nasal NO Measures

As an additional phenotypic marker, we measured nasal NO in all subjects with PCD. Recent reports suggest that nasal NO is low in PCD patients, and effectively distinguishes between PCD and normal subjects, although the precise nature of the link between the observation and the molecular abnormality causing PCD remains unknown (13, 14). No subjects had acute nasal disease (infection/inflammation) at the time of measurement, and none were smokers. NO levels were measured with the velum (soft palate) voluntarily closed, at a constant sampling rate of 460–500 ml/min, channeled directly into a Sievers Model 270 chemiluminescent analyzer (Sievers, Boulder, CO). For each individual, the level of NO reported is derived from four readings (two per nostril), and reported as the product of steady state (plateau) NO concentration and nasal flow (expressed as nL/min). Nasal NO levels were also measured in a control group of nonallergic, nonsmoking, normal control subjects ($n = 16$, mean age 33 yr, range 24–50, four females) and compared with PCD using a two-tailed, two-sample t test, with the level of significance set at $P = 0.05$.

DNA Extraction

DNA was acquired from all subjects from either fresh whole blood or from buccal cell brushings. Genomic DNA extraction from blood was performed using standard protocol (15). QIAamp DNA mini kit (Qiagen, Valencia, CA) was used to extract genomic DNA from buccal samples, as per manufacturer's instruction.

Linkage Analyses

Two previously reported intragenic SNPs at nucleotide 1003 of exon 11 and nucleotide 42 of intron 11 (9) and one in intron 14 (B. Duriez, personal communication) were used to test for linkage of PCD to DNAI1 gene. Intronic primer sequences were those used by Pennarun and coworkers (9), unless otherwise stated. Polymerase chain reaction (PCR) was performed to amplify the fragments with reagents and AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA) using manufacturer's instructions. The initial denaturation was at 94°C for 5 min followed by 35 amplification cycles (94°C for 30 s, 55–65°C for 30–45 s, and 72°C for 30–45 s) and ended with a 5- to 10-min extension at 72°C. Direct DNA sequencing of PCR product was performed on ABI 310 automated DNA sequencer (PE Biosystems, Foster City, CA) using Prism BigDye Primer Cycle Sequencing Ready Reaction kit (PE Biosystems).

Mutation Detection

All 20 exons of the DNAI1 gene were amplified and sequenced using intronic primers of Pennarun and colleagues (9), except forward primers for exons 1 and 20 which were as follows: IC78-Ex1F (forward) 5′-ATTCTATCCTGCAAGGGCA-3′ and IC78-Ex20F (forward) 5′-CTGGACAGGCTGGGCAGAGGA-3′. DNA from one patient with PCD from each of the families, not excluded by the linkage studies, was completely sequenced to detect the mutation(s) in the DNAI1 gene. After identification of the mutations, the remaining family members were also tested for the same mutations, either by restriction-enzyme (*Hpa1*) digestion for intron 1 or denaturing high performance liquid chromatography (DHPLC) for codon 568 of exon 17. The flanking primers utilized to amplify exon/intron 1 were IC78-Ex1F (forward) and IC78-Ex1R (reverse) 5′-TGATTAGTGGTCACTGGCGA CGA-3′. PCR-amplification of exon 17 for DHPLC analyses was performed using following intronic primers: IC78-Ex17F (forward) 5′-TTAAGGACAGGAGTCTAAGG-3′ and IC78-Ex17R (reverse) 5′-CAGAGGTGGAGGGTGGAAAG-3′ (9). Heteroduplex formation was performed as described earlier (16–18) using the Helix System (Varian Inc., Palo Alto, CA); temperature for detection of variants was 62°C. Heterozygous profiles were detected as distinct elution peaks from homozygous wild-type peaks.

After testing of the initial seven families was complete and mutations were identified in DNAI1 gene, an additional 32 other patients with PCD from 15 unrelated families with miscellaneous ciliary ultrastructural defects were analyzed for all known *DNAI1* mutations, using enzyme digestion assay or DHPLC as rapid screen-

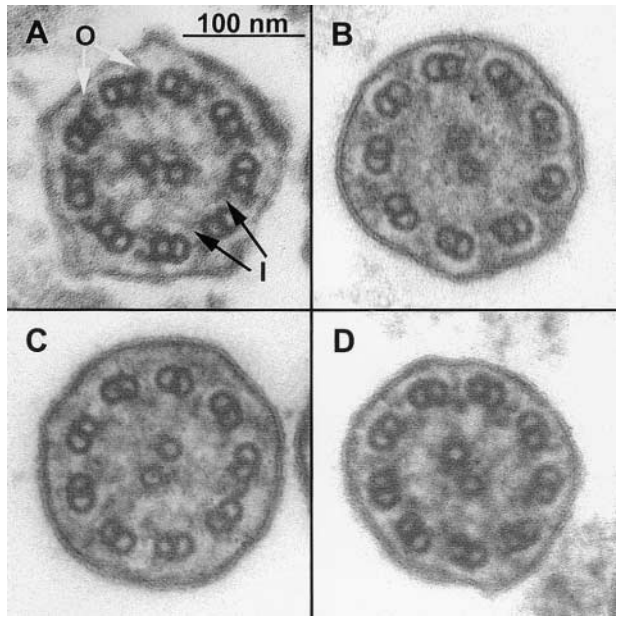

Figure 1. Representative electron microscopic images of a cross-section of a cilium. (*A*) Normal cilium from a clinically unaffected sibling II-1 (Family 16) with both wild-type alleles for the DNAI1 gene. "O" represents the outer dynein arm (*open arrow*) and "I" represents the inner dynein arm (*solid arrow*). The central doublets and radial spokes are also visible. (*B*, *C*, and *D*) Cilium from PCD subjects III-1 (Family 5), III-3 (Family 5), and II-2 (Family 16), respectively, demonstrating absence of outer plus abnormal inner dynein arms.

ing tools. Linkage studies with DNAI1 gene were not performed in these 32 patients. To estimate the frequency of mutations at codon 568 in the general population, 400 chromosomes from 200 non-PCD individuals were analyzed by DHPLC.

Results

Study Subjects

Overall, we have characterized 45 patients from 22 unrelated families with clinical disease consistent with PCD and defects of ciliary ultrastructure and function (10, 19). From this population, we selected 13 patients with PCD (seven male) with clear-cut defects in ODA (with or without IDA defects; Figure 1) in seven unrelated families ($n = 59$ subjects in all), initially to test for linkage to the DNAI1 gene. The 25 family members were evaluated in detail, and another 21 agreed to provide DNA using the buccal brush technique.

Clinical Evaluations of Patients with PCD and ODA Defects

Table 1 depicts the 13 patients with PCD (mean age 20 yr; range 3–44, six females) from the seven families. All had clinical evidence of sino-pulmonary disease, chronic middle ear disease in childhood, and/or neonatal respiratory pulmonary problems, consistent with clinical PCD. Five (three males) had SI totalis. The mean forced expiratory volume in 1 s (FEV_1) (% predicted) was 80 (range 51–123), and bronchiectasis was present on CXR and/or chest computed tomography (CT) in 10 of 13 patients. The 13 patients with PCD had nasal NO levels (nl/min; mean ± SEM) that were very low compared with disease-free control subjects ($n = 16$); 12 ± 5 versus 380 ± 38 ($P < 0.0001$), consistent with previously reported levels in PCD (10, 13). These phenotypic data support the diagnosis of PCD in these individuals, and the levels are similar to other PCD patients with miscellaneous defects in DA (data not shown). None of the family members evaluated, including the parents and unaffected siblings of patients with PCD, had clinical disease suggestive of the PCD phenotype, including unremarkable history and physical examinations ($n = 25$), normal CXR ($n = 14/15$; one had changes consistent with hyperinflation and asthma), normal sinus films ($n = 7/8$; one had evidence of mild unilateral maxillary mucosal thickening) and normal lung function ($n = 15$, mean ± SEM, $FEV_1 = 102.1 \pm 4\%$ predicted).

Ciliary Analyses

All evaluated patients with PCD had qualitative defects in the ODA. The ODA was absent in 8/13, and near-absent in 5/13. The IDA was also absent in 3/13, near-absent in 6/13, and normal in 4/13. Figure 1 illustrates cross-sectional images of cilia from the probands of two families (III-1 and III-3 from Family 5, and II-2 from Family 16) with missing ODA, variably absent/abnormal IDA, compared with a normal control image from a sibling with no clinical disease (II-1, Family 16).

Linkage Analyses

In the seven families, there were a total of 59 subjects from whom the DNA was available, of whom 14 were parents, and 32 were first- or second-degree relatives (siblings, aunts, uncles, or grandparents). To test the involvement of the DNAI1 gene for the PCD phenotype, three intragenic SNPs

TABLE 1

Details of 13 patients with primary ciliary dyskinesia and defects in outer dynein arm (ODA)

Patient No.	Family No.	Age (*yr*)	Age at DX	Sex	Situs Status*	FEV (%)	RDS†	Bronchiectasis‡	Sputum Microbiology	DA Defect
1	5	8	5	M	SS	96	+	−	NA§	ODA shortened
2	5	5	Birth	M	SI	123	+	−	NA	Absent ODA¶
3	16	44	25	M	SS	90	+	+	*P. aeruginosa* *H. influenza*, NTM‖	Absent ODA¶
4	3	30	26	F	SS	75	+	+	*H. influenza*	Absent ODA
5	3	26	25	F	SI	61	+	+	*H. influenza*	Absent ODA¶
6	7	7	Birth	M	SI	99	+	+	*P. aeruginosa* *H. influenza, S. aureus*	ODA shortened¶
7	7	11	5	M	SS	103	+	−	*S. pneumonia* *S. aureus*	ODA shortened¶
8	8	23	13	F	SS	65	+	+	*H. influenza* *S. aureus*	Absent ODA¶
9	8	14	5	F	SS	51	+	+	*S. aureus*	Absent ODA¶
10	11	15	6	F	SS	68	0	+	*H. influenza* *S. aureus*	Absent ODA¶
11	11	11	Birth	M	SI	90	+	+	*H. influenza* *S. aureus*	Absent ODA
12	18	33	25	M	SS	65	+	+	*H. influenza*	Absent ODA
13	18	30	27	F	SI	121	0	+	*P. aeruginosa*	Absent ODA

Patients 1, 2 and 3 have mutations in the DNAI1 (IC78) gene. Patients 1 and 2, 4 and 5, 6 and 7, 8 and 9, 10 and 11, 12 and 13 are siblings from the same families.
*SS = situs solitus (normal); SI = situs inversus.
†RDS = respiratory symptoms in neonatal period.
‡ = bronchiectasis present on CXR or CT.
§NA = sample not available.
¶Inner dynein arm missing or shortened also.
‖NTM = non-tuberculous mycobacteria cultured.

were determined by direct genomic DNA sequencing. As shown in Figure 2A, Family 7 was excluded from linkage between the DNAI1 gene and the PCD phenotype, because two affected individuals (II-1 and II-3) and an unaffected sister (II-2) shared the same genotype (TT) at intron 11 locus. Family 18 was excluded from linkage because the two children with PCD carried different *DNAI1* genotype at polymorphic loci in intron 14 (data not shown). Families 11 and 16 were partially informative (mothers in both families were homozygous for SNPs) for intragenic polymorphisms at nucleotide 1003 of exon 11 and nucleotide 42 of intron 11, respectively, since affected children displayed different genotypes when compared with their healthy siblings (*see* Figure 2B for Family 11). In Family 8, both affected siblings shared the same homozygous genotype for intron 11 SNP (father was heterozygous at this locus), but due to the unavailability of DNA from an unaffected brother, linkage status could not be established (data not shown); both the parents in this family were homozygous for the other polymorphisms. The remaining two families (Families 3 and 5) were uninformative since both the parents were homozygous at all three intragenic loci (*see* Figure 2C for Family 3); thus, linkage could not be excluded.

Mutation Detection

We completely sequenced the DNAI1 gene from one patient with PCD from each of the five families (3, 5, 8, 11, 16) in whom we could not exclude linkage between the DNAI1 gene and the PCD phenotype (i.e., who were either partially informative, or uninformative, for linkage). Mutations in the DNAI1 gene were found in two families (Families 5 and 16). One mutation was the insertion of a T nucleotide at +3 position of intron 1 (219+3insT) in both the families, as reported earlier (9), which can be detected by the mutation-induced *HpaI* restriction site (Figure 3A). Novel mutations on codon 568 were detected on the second allele of both the families. Family 5 had the G1874C transversion in exon 17 (missense mutation), designated as W568S, and Family 16 had a G1875A transition in exon 17 (nonsense mutation), designated as W568X (Figure 3B).

Figures 3C and 4 depict the segregation of mutations in both the families. There was no known consanguinity in either family. The probands in Family 5 (III-1 and III-3) were two male patients aged 3 and 6 yr, both with absent ODA. Both patients were found to have 219+3insT on one allele and W568S on the opposite allele. The 219+3insT was in-

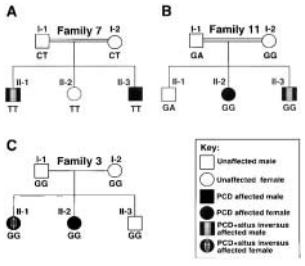

Figure 2. Linkage analyses at exon/intron 11 polymorphic loci in DNAI1 gene. (*A*) Both affected patients (II-1 and II-3) and their unaffected siblings (II-2) from Family 7 shared the same genotype (TT) at intron 11 locus, hence the family was excluded from linkage. (*B*) Both affected individuals (II-2 and II-3) from Family 11 shared the same genotype (GG), whereas their unaffected sibling (II-1) had a different genotype (GA) at exon 11 locus. Since the mother (I-2) was homozygous at the polymorphic locus, this family was partially informative. (*C*) Family 3 was uninformative, since both the parents were homozygous (GG) at the exon 11 polymorphic locus.

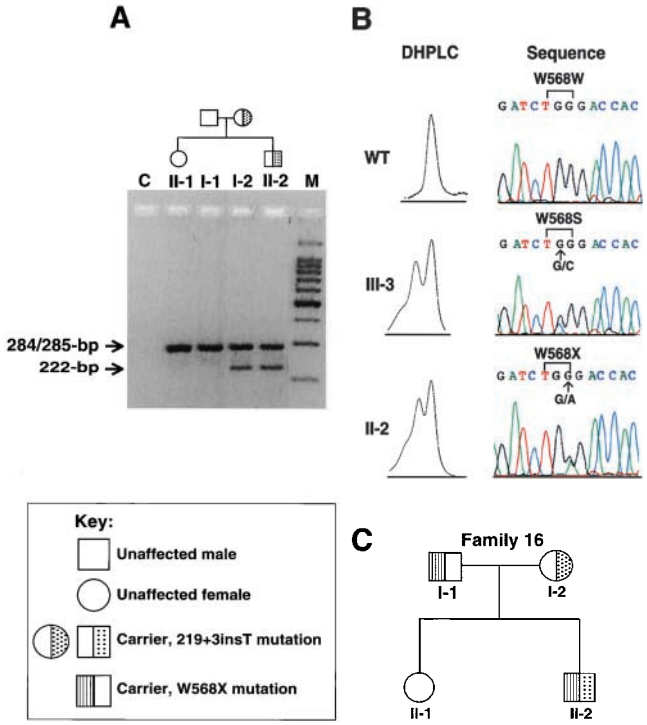

Figure 3. Familial mutations in DNAI1 gene. (*A*) Segregation analysis of the 1-bp insertion of T at +3 position of intron 1 (splice site mutation, 219+3insT) in Family 16 identified by mutation-induced cleavage at a *HpaI* site. A 284/285-bp region of the DNAI gene was amplified using IC78-Ex1F and IC78-Ex1R primers followed by restriction digestion with *HpaI*. PCR-product from normal allele is not cut, whereas the mutant allele cleaves into 222- and 63-bp fragments. Heterozygous individuals presented with one uncut and another digested allele on 2.5% agarose gel. From Family 16, the father (I-1) and his unaffected daughter (II-1) are wild type; the mother (I-2) and an affected son (II-2) are heterozygous. The mutation was confirmed by sequencing both the strands in all the individuals. *Lane M* corresponds to 1-kb ladder from Promega and *lane C* corresponds to control without DNA. (*B*) DHPLC (*left panel*) and sequence analyses (*right panel*) to detect novel mutations at codon 568. Mutations were detected by direct bidirectional sequencing of PCR-amplified product of exon 17 with intronic primers (IC78-Ex17F and IC78-Ex17R). DHPLC analyses at a run temperature of 62°C were compatible with the sequencing analyses. Distinct elution peaks were obtained for heterozygous and homozygous wild type (WT), which was analyzed visually (*left panel*). WT sequence at codon 568 from a control subject (*top panel*). A novel paternal missense mutation in PCD patient III-3 from Family 5 was found, which was heterozygous 1874 G→C transversion and was designated as "W568S" (*middle panel*). A novel paternal nonsense mutation in PCD patient II-2 from Family 16 was also found, which was heterozygous 1875 G→A transition and was designated as "W568X" (*bottom panel*). (*C*) A novel nonsense mutation (W568X) was found in an affected subject (II-2) from Family 16, which was heterozygous for 1875 G→A transition in exon 17. The maternal mutation in the affected individual is the same previously reported splice mutation (219+3insT). Neither mutation was present in the unaffected sibling (II-1).

herited from the mother (II-4) and was not present in the unaffected sibling (III-2), who had normal ciliary ultrastructure. This mutation was also present in the maternal aunt and the maternal grandmother (II-6 and I-4, respectively). The W568S mutation was present in the unaffected sibling (III-2), the father (II-3), a paternal aunt (II-2), and the paternal grandmother (I-2), all with normal ODA (Figure 4). Both these mutations segregated only in affected individuals in Family 5. In Family 16, a 45-yr-old male (II-2) with PCD had the 219+3insT mutation on one allele, also present in the mother (I-2) (Figure 3C). A W568X nonsense mutation was identified on the second allele, which was inherited from the father (I-1). Both these mutations segregated only in the affected individual; a clinically normal female sibling (II-1), aged 50, did not carry either mutation, and had normal ciliary ultrastructure.

To assess the allele frequency of codon 568 mutations in the general population, we screened DNA from 200 anonymous individuals with hemophilia. The allele frequency of the W568X nonsense mutation in these individuals was 0.0025 (1 in 400 chromosomes analyzed), whereas none of the 200 individuals (400 chromosomes) analyzed harbored the W568S missense mutation. The splice site (219+3insT) mutation frequency was tested previously in 50 individuals (100 chromosomes) and none of them had the mutation (9).

All additional 32 patients with PCD (from 15 families) with miscellaneous ciliary defects were found to be wild type when analyzed for codon 568 and 219+3insT mutations, presumably because of mutations in genes encoding for other ciliary defects, or indeed mutations elsewhere in the DNAI1 gene. This scenario is consistent with the genetic heterogeneity associated with PCD (9).

Discussion

PCD is characterized by respiratory distress at birth, chronic middle ear disease during childhood, chronic sinusitis, and frequent respiratory infections leading to bronchiectasis in later life (2). The differential diagnosis is broad and may be confused (especially in children) with other causes of chronic airways disease. Defining the genetics of PCD will be useful for earlier and more definitive diagnosis, and in the long term for the development of novel therapies. One of the primary challenges for the study of PCD is the genetic heterogeneity of the disorder.

To counter this limitation, we have characterized a large number of patients with PCD in considerable detail, which allows more rigorous categorization of the phenotype. Patients with PCD commonly have a history of neonatal respiratory distress/pneumonia, recurrent otitis media, and chronic cough (20). In addition, analyzing the ultrastructural defects is also discriminatory for PCD, with defects in the ODA specifically seen in PCD. Although the precise nature of the relationship between nasal NO levels and PCD has not been elucidated, the initial reports of very low nasal NO levels in patients with PCD are borne out in the subjects with PCD in this report. Using this approach, it is possible to better categorize patients, and to systematically test those subjects who have similar disease and/or electron microscopic findings (ultrastructural defects in cilia) for genetic mutations associated with PCD. Multiple family members were also evaluated for clinical disease, and the presence of mutations in the gene. No stigmata of PCD were evident on evaluation or by history in those subjects carrying one mutation in the DNAI1 gene, including parents, sibling, grandparents, or aunts. One sibling was clinically normal, and was not a carrier of any mutation. Thus, carriers of loss-of-function mutations in the DNAI1 gene do not appear, at least in this small sample, to be predisposed to any discernable clinical disease.

The recent description of loss-of-function mutations in the DNAI1 gene located on chromosome 9 was the first genetic link to clinical PCD. Ciliary ultrastructure in that patient showed an ODA defect in airway epithelial ciliary structure. One of the mutations was a 4 base-pair (bp) insertion causing a frameshift in exon 5, and leading to a premature translation termination. The mutation on the other allele was a splice mutation (219+3insT) of intron 1. This mutation disrupts normal splicing, leading to the insertion of 132-bp of intron 1 sequences in mRNA followed by cryptic splice-donor site, which leads to the termination signal at position 73 (9). Very recently Guichard and colleagues reported the same 219+3insT mutation in five patients with PCD from three families (21). In the cohort of patients and family members in the present study, the previously described 219+3insT mutation was detected on one allele in three PCD patients, belonging to two unrelated families. The mutation on the second allele was W568S in two patients (III-1 and III-3) from Family 5 and W568X in the patient (II-2) from Family 16. All three patients have disease typical of PCD, with recurrent sino-pulmonary dis-

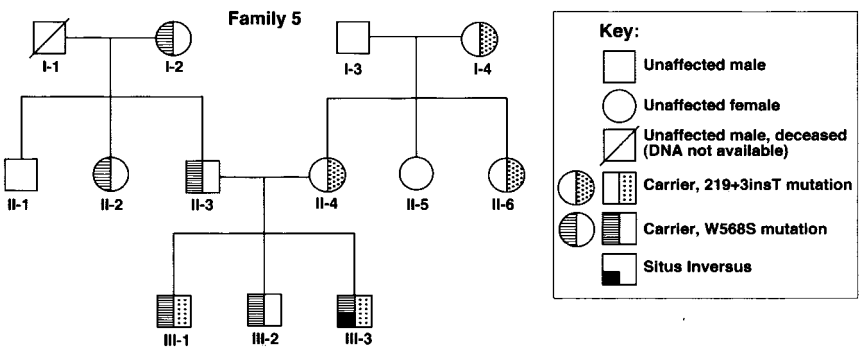

Figure 4. Pedigrees demonstrating recessive inheritance of the mutations. A novel missense mutation (W568S) was found in the affected individuals (III-1 and III-3) from Family 5, which was heterozygous 1874 G→C transversion in exon 17. This mutation was also found in the father, unaffected sibling, paternal aunt, and paternal grandmother. The previously reported splice mutation, which was an insertion of a T at +3 position of intron 1 (219+3insT), was identified in both the affected individuals. This mutation was present in the mother, maternal aunt, and maternal grandmother.

ease, recurrent ear infections, with SI totalis in one. All three are males; fertility status has not been established in the younger patients (III-1 and III-3), though the spouse of the adult male patient (II-2) achieved conception through artificial insemination of his sperm, which is in accordance with previous reports (22).

We identified two novel mutations at codon 568 in both these families. The nonsense mutation (Family 16, W568X) would presumably lead to a truncated protein, missing part of exon 17 and exons 18–20. The allele frequency for this mutation was 0.0025 (1 in 400 chromosomes) in non-PCD individuals. The missense mutation (W568S; Family 5), affected individuals (III-1, III-3) is likely to be a true genetic mutation and not a polymorphism, since this mutation appears to occur at a very low gene frequency in the general population. It is pertinent that both missense and nonsense mutations occurred at the codon 568, which is conserved residue in the protein. Hence, the DNA sequence in that region is likely to be a hotspot for the mutations. Further studies are needed to confirm this hypothesis. Our findings are consistent with the autosomal recessive mode of inheritance commonly reported in PCD families.

The PCD patients in this study had different mutations on opposite alleles, and this compound heterozygous status is consistent with the lack of consanguinity in these families. The presence of the 219+3insT mutation on one allele in nine patients with PCD from six different families (including the previous studies [9, 21]) indicates a relatively high prevalence of 219+3insT mutation. These observations suggest that this is either a common site for spontaneous mutations, or the mutation could be the result of a founder effect. The data in this study are insufficient to differentiate between these possibilities, and future studies will be necessary to answer this question.

In this study, the DNAI1 gene was not linked to the PCD phenotype in five of seven unrelated families, based on the results of intragenic SNPs and DNA sequencing. This supports the earlier reports of locus heterogeneity for the disease (9, 23). A locus on chromosome 5p has been described in a large inbred Lebanese kindred with PCD and defective ODA, using a homozygosity mapping strategy (24), and DNAH5 (an axonemal heavy chain dynein) is presumably a candidate gene. Another study provides evidence for a PCD locus on chromosome 19q (between the markers D19S572 and D19S218) in three Arab kindred. This 15-cM critical region is gene rich, but a single candidate gene is not yet defined (25).

The human DNAI1 gene encodes for a protein related to an intermediate chain dynein, and is a member of the axonemal protein family that exhibits evolutionary conservation. The intermediate chains are involved in the assembly of the outer arm complex (26, 27) and attachment to the outer doublet microtubules, the process being ATP-independent (28, 29). The DNAI1 gene is homologous with sea urchin IC2 (GenBank accession no. D38538) and *Chlamydomonas reinhardtii* IC78 (GenBank accession no. U19120), and the IC78 gene was found to be deleted, or disrupted by a large insertion, in an ODA-deficient mutant of *Chlamydomonas* (30). The previous data and the current findings support the hypothesis that the DNAI1 gene mutations will continue to be identified in patients with PCD (9). Intermediate chain dyneins, including IC78, are members of the WD family of proteins, which regulate ciliary function via protein–protein interactions (30–33). The crystal structure of WD containing G-β transducin (Gβ) protein (34) is a symmetrical structure made up of repeats, which adopts a β-propeller fold, each comprised of a four-stranded antiparallel β sheet. Glycine-Histidine and Tryptophan-Aspartate (GH-WD) flank the repeats. Neither the GH nor the WD is absolutely conserved; the aromatic amino acid W, which is also called a trademark tryptophan, was found to be conserved only 79% of the time (n = 776 WD repeats analyzed), and 17% of the time, it is replaced by other aromatic amino acids (phenylalanine and tyrosine). Both of the novel mutations (W568X and W568S) occur at the same tryptophan residue. The replacement of a bulky aromatic W residue by serine (S) may cause abnormal protein folding, since a bulky residue is usually associated with the D to end the last strand of the repeat and stabilize the folding of the turn between the third and fourth strand. In other WD proteins, where this W or aromatic amino acid is not conserved, S has not replaced it. All aligned WD repeats can be found at http://bmerc-www.bu.edu/wdrepeat/.

In conclusion, we detected a previously described mutation (219+3insT) in the DNAI1 gene, and two novel mutations in the same gene on the opposite allele in patients with clinical and cell biologic evidence of PCD. Related family members, including carriers of mutations in *DNAI1* had no phenotypic expression of PCD-like disease. Utilization of screening methods for rapid detection of the sequence variants at codon 568 and the 219+3insT mutation can expedite the process of mutation detection in PCD patients with ODA abnormalities. An increased understanding of the underlying molecular and cell biologic mechanisms will help in the development of more targeted therapies.

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