Rapid Communication

Investigation of the Possible Role of a Novel Gene, *DPCD*, in Primary Ciliary Dyskinesia

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Primary ciliary dyskinesia (PCD) is an autosomal recessive disease caused by mutations that affect the proper function of cilia. Recently, deletion of DNA polymerase λ (*Poll*) in mice produced a phenotype characteristic of PCD (Kobayashi *et al.*, 2002, *Mol. Cell. Biol.* 22:2769–2776). Because it is unclear how a mutation in a DNA polymerase would result in a specific defect in axonemes, the targeting construct was examined further. Analysis of the genomic region surrounding the *Poll* gene revealed an uncharacterized gene, named *Dpcd*, that is predicted to be transcribed from the opposite strand relative to *Poll*. The deletion of *Poll* would also remove the first exon of *Dpcd*. Because it is possible that the PCD phenotype observed is due to the absence of either gene, the expression of these genes during ciliogenesis of human airway epithelial cells was examined. Northern analysis demonstrated that DPCD expression increases during ciliated cell differentiation; the expression of POLL decreases. To examine directly whether *DPCD* is mutated in cases of human PCD, the complete coding sequence of *DPCD* was sequenced from 51 unrelated PCD patients. No disease-causing mutations were confirmed; however, one variant could not be excluded. Therefore, *DPCD* remains a novel candidate gene for PCD.

Primary ciliary dyskinesia (PCD) is a genotypically heterogeneous disease, usually inherited as an autosomal recessive trait, caused by mutations that impair the function of cilia and flagella (1–3). The defect in ciliary function in the respiratory tract results in impaired or absent mucociliary clearance, which is believed to be responsible for the frequent and recurrent episodes of sinusitis and bronchitis that are typical of this disease. The phenotype of the disease is also variable; in severe cases patients develop end-stage bronchiectasis and require lung transplantation. In addition, the disease affects other organs and processes that require ciliary or flagellar motion, and patients with PCD may exhibit otitis media, situs inverses totalis, and/or infertility.

The respiratory cilium is a complex structure consisting of ~250 proteins (4, 5). In addition to the highly conserved 9+2 arrangement of microtubule doublets that form the basic axonemal structure, cilia contain inner and outer dynein arms (IDA, ODA) that provide the force for ciliary beating. Cilia also contain an unknown number of other proteins that may be involved in the assembly, maintenance, and regulation of ciliary function. In theory, a mutation in many of these genes would lead to the same phenotype, that is, impaired mucociliary clearance. However, most patients with PCD exhibit a structural defect in the outer and/or inner dynein arms. In a small percentage of patients with PCD, mutations have been identified in an ODA intermediate chain (DNAI1) (6–9) or an ODA heavy chain (DNAH5) (10, 11). These results confirm that PCD is a genetically heterogeneous disease and indicate that the causative mutation in many cases has yet to be identified.

Recently, a deletion of DNA polymerase λ (*Poll*) in mice was reported to produce a PCD phenotype that included a lack of inner dynein arms (12). This model exhibited many of the features associated with other models of PCD, including chronic sinusitis, situs inversus totalis, infertility, and hydrocephalus. Thus, *POLL* became a candidate gene for cases of human PCD. Because it is difficult to reconcile the function of a nuclear DNA polymerase with a specific defect in axonemal structure (missing IDA), we examined the deletion construct in more detail and determined that the expression of another gene was also likely disrupted in the mouse model. This novel gene, which we have named *Dpcd* (for deleted in a mouse model of primary ciliary dyskinesia), is predicted to code for a protein of 23 kD of unknown function. In addition, deletion of only the catalytic domain of pol λ in a separate study resulted in mice with a normal phenotype (13). Thus the PCD phenotype observed in the *Poll* knockout mice reported by Kobayashi and coworkers (12) is most likely due to the loss of Dpcd.

To investigate the possible role of DPCD in human cases of PCD, the expression of DPCD was examined in a panel of human tissues to determine if the gene was expressed in a specific pattern consistent with the PCD phenotype. The expression of DPCD and POLL were also examined during ciliogenesis of human bronchial epithelial (HBE) cells

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Abbreviations: deleted in a mouse model of PCD, Dpcd; human bronchial epithelial cells, HBE cells; inner dynein arm, IDA; outer dynein arm, ODA; primary ciliary dyskinesia, PCD; DNA polymerase λ, Poll.

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in vitro. Finally, to examine whether mutations in *DPCD* or *POLL* could account for some cases of human PCD, we have sequenced the coding regions of both *DPCD* and *POLL* in a cohort of well-characterized PCD patients. A preliminary report of this work has been presented (14).

Materials and Methods

Database Analysis

The *Dpcd* gene was found in the Celera Discovery System mouse genomic database by searching the region surrounding *Poll*. Subsequent analysis identified the same gene in the National Center for Biotechnology (NCBI) databases (http://www.ncbi.nlm.nih.gov). Ensembl (http://www.ensembl.org) was used to visualize the gene relative to *Poll* on the chromosomes of mouse and human.

Analysis of DPCD and POLL Expression

RT-PCR primers were designed to amplify probes for DPCD (458 bp) and POLL (251 bp) based on sequences present in NCBI (XM_058358* and NM_013274). Primers for DPCD were 5′ GTTCACTATTTATTCCCAGACGGC 3′ and 5′ CACCACAA CCTCCTTTGGCTT 3′; primers for POLL were 5′ CCTAGG AGGGAAGAGGGAGA 3′ and 5′ TGACTTCACCAGCTGA GCAC 3′. The coding region of DPCD (687 nt) was amplified using primers 5′ TGCTTAGCAGGGGAAAGATG 3′ and 5′ GCCAAGCCTTGAAGTCTCAC 3′, cloned into TopoTA (InVitrogen, Carlsbad, CA) and sequenced (deposited in GenBank as accession number AY532267). Human multiple tissue northern blots were purchased from Clontech (Palo Alto, CA) and probed for DPCD expression according to the manufacturer's instructions. For analysis of expression during ciliogenesis, human bronchial epithelial cells (obtained under protocols approved by the University of North Carolina Institutional Review Board) were cultured using conditions described in detail elsewhere (15, 16). Total RNA was harvested from cells grown on plastic for 5 d, cells grown on collagen-coated Millicell-CM culture inserts (Millipore Corp., Bedford, MA) submerged in media for 3 d, and cells cultured on inserts at an air–liquid interface and harvested on Days 7, 29, and 40 of culture. RNA was isolated using RNeasy reagents (Qiagen Inc., Valencia, CA), fractionated on an agarose/formaldehyde gel as previously described (17), transferred to Hybond-N+ membrane (Amersham Biosciences, Piscataway, NJ), and hybridized with radioactively labeled probes in Rapid-hyb buffer (Amersham Biosciences) according to the manufacturer.

Clinical Evaluation and Ciliary Dynein Arm Evaluation of Patients with PCD

This study was approved by the committee for the protection of the rights of human subjects at the University of North Carolina (UNC)-Chapel Hill. The majority of the PCD families ($n = 44$) were seen and evaluated at General Clinical Research Center at UNC, whereas 7 families were evaluated at other institutions. Clinical evaluations, ciliary ultrastructural analysis, and genomic DNA isolations were performed as described earlier (6). Patients were categorized as outer dynein arm defects (ODA$^-$), inner dynein arm defects (IDA$^-$), both dynein arm defects (Both$^-$), and normal dynein arm (other). A cohort of 68 patients belonging to 51 independent families was selected. There was known consanguinity in three families. Ages ranged from 10 mo to 73 yr, and there were 30 males and 38 females. Of the 68 PCD patients, 28 (41%) presented with situs inversus and 1 patient presented with situs ambiguous. We selected one patient with PCD from each of the 51 families for the DPCD gene analysis, and 15 patients with PCD from 15 PCD families for the *POLL* gene analysis, focusing on patients with a defect in the IDA.

Mutation Profiling

DNA was extracted from either fresh blood or buccal brushings using standard procedures. All the coding exons of *DPCD* (exons 1–6) and *POLL* (exons 2–9) and the intron/exon boundries were amplified. All the primer sets were located in the flanking intronic sequences and are available upon request. Amplifications were performed using reagents and AmpliTaq polymerase from Perkin Elmer (Foster City, CA). The initial denaturation was 94°C for 5 min, followed by 35 cycles (94°C for 30 s, 60°C for 30 s, 72°C for 45 s) and final extension for 10 min at 72°C. Direct DNA sequencing of PCR products was performed on an ABI310 or ABI3100 automated DNA sequencer (PE Biosystem, Foster City, CA), using Prism BigDye primer Cycle Sequencing Ready Reaction kit (PE Biosystem). Intragenic polymorphisms of *DPCD* were used for exclusion mapping. To estimate the frequencies of variants in the general population, 100–110 chromosomes from non-PCD individuals were analyzed by sequencing.

Results

Analysis of mouse chromosome 19 in the region of the *Poll* gene revealed an uncharacterized gene (mCG127478 in the Celera mouse genomic database) predicted to be transcribed from the opposite strand relative to *Poll*. The predicted first exon of this novel gene, named *Dpcd*, lies only 75 bases from exon 1 of *Poll*, in the same region encompassed by the deletion of *Poll* reported to produce a PCD phenotype (12). The 6.4-kb deletion is predicted to remove the first exon of *Dpcd*, including the predicted first ATG, and therefore would likely disrupt the function of both *Dpcd* and *Poll* (Figure 1). Thus the phenotype observed could be due to the loss of either gene. For comparison, the deletion reported by Bertocci and colleagues (13) is also shown. This deletion, which replaced three exons of pol λ with a neo gene, did not produce a PCD phenotype, suggesting the disruption of *Dpcd* is likely responsible for the PCD phenotype.

The human homolog of *Dpcd* (accession number XM_058358*; designated *DPCD*) is located in a highly syntenic region of chromosome 10 (Figure 1). The predicted human DPCD protein (NP_056263) is 88% identical and 94% similar to the mouse protein. *DPCD* is predicted to code for a novel protein of 23 kD with no homology to any conserved protein domains. Because PCD typically affects a specific set of axoneme containing tissues, including the airways and reproductive organs, we examined a panel of human tissues to determine the pattern of DPCD expression. Probing northern blots containing samples from 16 different human tissues showed that the highest level of DPCD expression was in the testis (Figure 2). A single band close to the expected size of 821 bp was observed, indicating the absence of any major splice variants. Weak signals were also observed in RNA from pancreas, skeletal muscle, and

*This sequence record has been removed from the NCBI database (note added in proof).

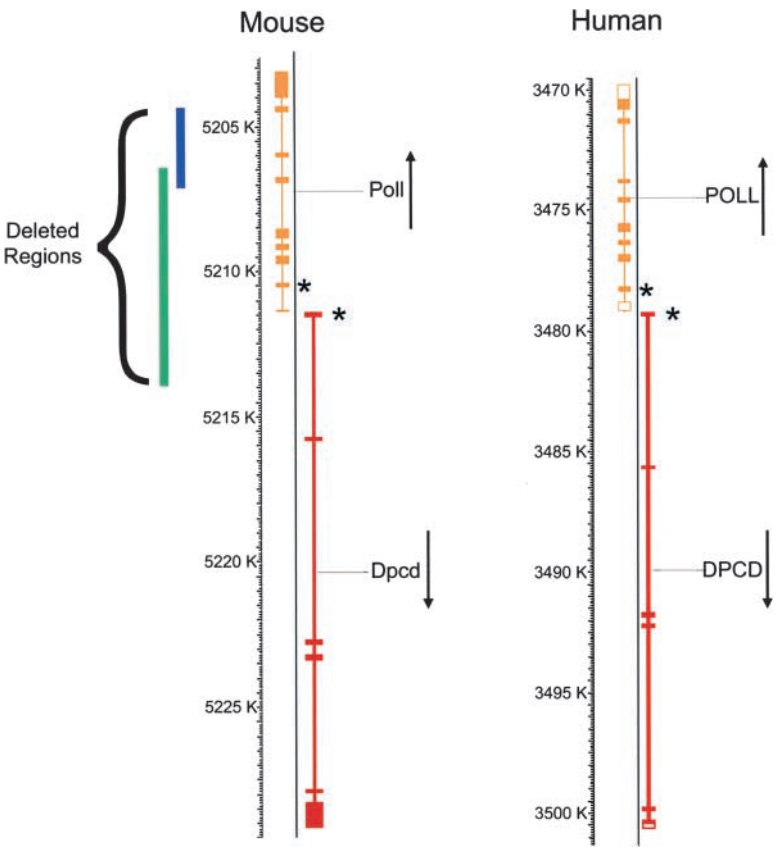

Figure 1. Diagram showing the chromosomal location of the DNA polymerase lambda gene (*Poll*) and the novel gene *Dpcd* on mouse chromosome 19. The syntenic region of human chromosome 10 is also shown. *Asterisks* indicate the start codons. Targeted deletion of the region indicated by the green line is predicted to disrupt both the *Poll* and *Dpcd* genes in a mouse model of PCD (12). Targeted deletion of the region indicated by the blue line produced animals with a normal phenotype (13), suggesting that the PCD phenotype is caused by disruption of the novel gene, *Dpcd*.

heart. Other tissues examined showed no detectable expression, even after prolonged exposure (14 d). The expression of POLL is also highest in testis, with lower levels of expression reported in ovary, skeletal muscle, pancreas, and heart (18–20). Thus the expression pattern of DPCD and POLL follow a similar pattern when analyzing RNA extracted from total tissues. However, the absence of a DPCD signal in lung and other tissues may be due to the low percentage of ciliated cells in these total tissue samples. To determine if DPCD was expressed in airway epithelial cells, RT-PCR was used to amplify a full-length cDNA from cultured HBE cells. The sequence of the cDNA was identical to that in the database, verifying the expression of the predicted transcript (not shown). To evaluate more specifically whether DPCD and/or POLL are expressed in ciliated airway epithelial cells and whether their expression correlated with ciliogenesis, HBE cells were grown at an air–liquid interface (15, 16). RNA was isolated from parallel cultures at different stages of differentiation and analyzed by Northern blotting. At early time points in these cultures, the HBE cells are undifferentiated and resemble basal cells. With time, the cells differentiate to form a heavily ciliated epithelium similar to that observed *in vivo*. The expression of ciliated cell–specific genes has previously been shown to correlate with the development of ciliated cells in this model system (e.g., Figure 5 in Ref. 21). The results demonstrate that DPCD is expressed in airway cultures and that it becomes more abundant during ciliated cell differentiation, consistent with a potential role in cilia structure or function (Figure 3, *top panel*). Reprobing of the blot with a POLL-specific probe showed a low level of expression early in culture, which appeared to decrease as differentiation occurred (Figure 3, *middle panel*).

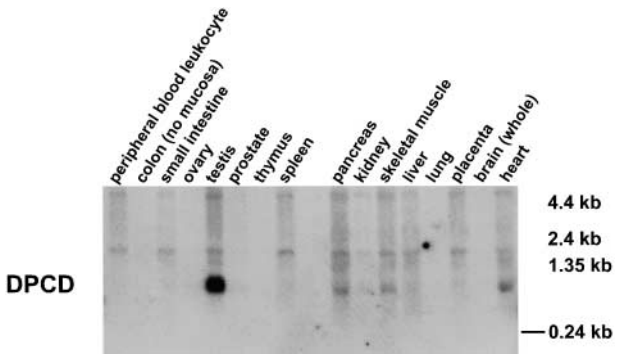

Figure 2. Expression of DPCD in human tissues. Northern blots containing polyadenylated RNA from the indicated human tissues were probed for DPCD expression. DPCD message was most abundant in testis, with low levels of expression observed in pancreas, skeletal muscle, and heart. Exposure time for the figure shown was 14 d. Molecular size markers are indicated.

To examine whether mutations in *DPCD* could account for some cases of human PCD, the coding region was sequenced from a group of 68 patients with well-characterized PCD. Initially, we sequenced the coding region of *DPCD* from 51 unrelated patients with PCD. During the course

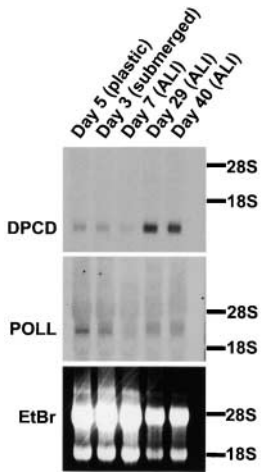

Figure 3. Expression of DPCD and POLL RNA during ciliogenesis of HBE cells. RNA was isolated from cultures of HBE cells cultured on plastic for 5 d or at an air–liquid interface for the times indicated. Northern analysis was performed using probes specific for the novel gene DPCD (*top panel*) or DNA polymerase lambda (POLL) (*middle panel*). The ethidium bromide stained gel showing the amount of RNA loaded is shown (*bottom panel*). The expression of DPCD increases at later time points when ciliated cell differentiation is occurring, whereas the expression of POLL appears to decrease during differentiation.

of sequence analysis, six sequence variants were detected (Table 1).

To determine if any of these variants could be pathogenic, a population study was performed in individuals without PCD (Table 2). Four of these variants were found to be present in individuals without PCD, with varying frequencies. Five non-PCD individuals were homozygous for 467T>C, demonstrating that this variant is clearly not pathogenic. IVS2+59A>G and 399C>T (N133N) variants were not detected in a homozygous state in the control population; however, given their high minor allele frequencies (30% and 22%, respectively), they are most likely polymorphisms. Also, the 399C>T and IVS2+59A>G variants were found to be in a homozygous state in the unaffected sibling of PCD1 (Figure E1 in the online supplement). The unaffected father of PCD151 was also homozygous for the 399C>T variant. IVS2–22_40 del had a minor allele frequency of only 1% in the normal population, but this variant was not detected in an affected sibling of PCD163 (Figure E1 in the online supplement). Therefore, this variant is most likely a rare polymorphism. IVS5–48_50 del was not found in the 106 alleles analyzed from the disease-free individuals. However, it also is probably a rare polymorphism because it was detected in one allele from an affected sibling of PCD354, who was wild-type at this locus (Figure E1 in the online supplement). The 168T>G (S56R) variant was not detected in 100 alleles analyzed from the control population. The role of this variant is less certain because it was detected in a heterozygous state in only one affected individual (PCD222), who was wild-type for all the other coding nucleotides and intron–exon junctions in this gene. It is possible that a mutation in the noncoding region of the other allele of *DPCD* may be responsible for PCD in this individual. Further studies will be required to test this hypothesis.

The discovery of intragenic polymorphisms was useful for carrying out exclusion mapping analysis to exclude the *DPCD* gene from linkage to PCD, assuming an autosomal recessive mode of inheritance. For PCD1, affected and unaffected siblings shared the same genotype at the polymorphic loci in intron 2 (IVS2+59A>G) and in exon 4 (399C>T), and both the parents were heterozygous. Thus *DPCD* was excluded from linkage to PCD in this family. PCD373 was borne of consanguineous union and was heterozygous for IVS2+59A>G; therefore, linkage was also excluded in this family (Figure E1 in the online supplement). For PCD Patients 10, 16, 34, 108, 163, and 354, the affected and unaffected siblings were discordant for either one or more polymorphic loci, hence these families were also excluded. Given the proximity of the *POLL* gene to *DPCD* gene, all the above-mentioned families are probably excluded for *POLL* gene as well.

Because the ultrastructural analysis of the *Poll* knockout mouse revealed an absence of IDA (12), we sequenced the coding region of the *POLL* gene in a patient from all 13 PCD families with solely an IDA defect, and in two patients with PCD with normal dynein arms. During the course of sequence analysis, eight sequence variants were detected (Table E1 in the online supplement); all of these have been identified as intragenic polymorphisms (Table E2 in the online supplement).

Discussion

Although a small number of mutations have recently been identified as causes of PCD, in the majority of cases the underlying mutation is still unknown. Recently, a deletion of DNA polymerase λ was reported to produce a PCD phenotype in a mouse model (12). The COOH terminal region of DNA polymerase λ (pol λ) shows 33% sequence identity with DNA polymerase β, and recombinant pol λ displays DNA polymerase activity (19, 20). In addition, a pol λ-GFP fusion protein has been shown to localize predominantly to the nucleus (20). Because it is difficult to reconcile the function of a nuclear DNA polymerase with a defect in axonemal structure (missing IDA), we examined the deletion construct in more detail and determined that another gene was also likely disrupted in the mouse model. Other reports have also documented the disruption of more than one gene in mouse models (22, 23). Therefore, the results from any genetically modified animal should be interpreted with caution, especially if the phenotype observed does not agree with the known function of the targeted gene. Interestingly, an independent deletion of *Poll* targeting the catalytic domain produced mice with a normal phenotype (13). This deletion would not be predicted to disrupt the coding sequence of the novel gene. Thus the PCD phenotype is most likely due to disruption of the novel gene.

The novel gene, which we have named *Dpcd*, is predicted to code for a protein of 23 kD of unknown function. Searches of databases as well as protein analysis programs have not revealed any significant homology to known proteins or any conserved functional domains to date. The predicted protein contains several potential phosphorylation sites and potential amidation and glycosylation sites, but further studies of the location and function of this novel protein are clearly required.

Because PCD is caused by an inherited defect in the structure or function of cilia, a candidate gene would most likely be expressed in tissues that contain cilia or flagella. DPCD and POLL are expressed at high relative levels in the testis, which is consistent with a possible role in the proper function of cilia and flagella. Both DPCD and POLL

TABLE 1
A summary of DPCD *sequence analysis in patients with PCD*

PCD No.	Ultrastructure	IVS2+59 A>G	IVS2–22_40del	(S56R) 168T>G	(N133N) 399C>T	(L156S) 467T>C	IVS5–48_50 delGAG
PCD 1	IDA$^-$	GG	WT/WT	TT	TT	CC	WT/WT
*PCD 10	IDA$^-$	AA	WT/WT	TT	CC	TC	WT/WT
*PCD 16	Both$^-$	AG	WT/WT	TT	CT	TC	WT/WT
PCD 26	Unknown	AG	WT/WT	TT	CC	TT	WT/WT
*PCD 34	Unknown	AA	WT/WT	TT	CC	TC	WT/WT
PCD 62	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 66	IDA$^-$	AA	WT/WT	TT	CC	TT	WT/d
†PCD 71	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 78	Other	AA	WT/WT	TT	CC	TT	WT/WT
PCD 79	IDA$^-$	AG	WT/WT	TT	CC	TC	WT/WT
*PCD 108	Other	AG	WT/WT	TT	CC	TT	WT/WT
PCD 116	IDA$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 140	Unknown	AA	WT/WT	TT	CC	TT	WT/WT
PCD 151	IDA$^-$	AG	WT/WT	TT	CT	TC	WT/WT
PCD 157	Other	GG	WT/WT	TT	TT	CC	WT/WT
PCD 158	Both$^-$	AG	WT/WT	TT	CT	TC	WT/WT
*PCD 163	Other	AA	WT/d	TT	CC	TT	WT/WT
PCD 182	Both$^-$	AG	WT/WT	TT	CC	TT	WT/WT
PCD 190	IDA$^-$	AG	WT/WT	TT	CT	TC	WT/WT
PCD 204	Both$^-$	AG	WT/WT	TT	CT	TC	WT/WT
PCD 221	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 222	Both$^-$	AA	WT/WT	TG	CC	TT	WT/WT
PCD 224	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 227	Both$^-$	AA	WT/WT	TT	CC	TC	WT/WT
PCD 254	IDA$^-$	AG	WT/WT	TT	CT	TC	WT/d
PCD 255	IDA$^-$	AA	WT/WT	TT	CC	TT	WT/d
PCD 256	Unknown	AG	WT/WT	TT	CT	TC	WT/WT
PCD 261	IDA$^-$	GG	WT/WT	TT	CT	TC	WT/WT
PCD 262	Both$^-$	AA	WT/WT	TT	CC	TT	WT/d
PCD 264	Other	AA	WT/WT	TT	CC	TT	WT/d
PCD 274	Unknown	GG	WT/WT	TT	CT	TC	WT/WT
PCD 290	Both$^-$	AG	WT/WT	TT	CC	TT	WT/WT
†PCD 299	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 306	Other	GG	WT/WT	TT	TT	CC	WT/WT
PCD 333	ODA$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 340	Both$^-$	GG	WT/WT	TT	CC	TT	WT/WT
PCD 345	Other	AG	WT/WT	TT	CT	TC	WT/WT
*PCD 354	Other	AG	WT/WT	TT	CT	TC	WT/WT
PCD 365	Other	AG	WT/WT	TT	CT	CC	WT/WT
PCD 367	Other	AG	WT/WT	TT	CC	TC	WT/WT
PCD 372	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
*†PCD 373	Both$^-$	AG	WT/WT	TT	CC	TT	WT/WT
PCD 383	Other	AA	WT/WT	TT	CC	TT	WT/d
PCD 388	IDA$^-$	AA	WT/WT	TT	CC	TC	WT/WT
PCD 429	IDA$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 460	Unknown	AG	WT/WT	TT	CT	TC	WT/WT
PCD 467	Both$^-$	GG	WT/WT	TT	TT	CC	WT/WT
PCD 481	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 483	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT
PCD 515	IDA$^-$	AG	WT/WT	TT	CT	TC	WT/WT
PCD 526	Both$^-$	AA	WT/WT	TT	CC	TT	WT/WT

Definition of abbreviations: d, deletion polymorphism; IDA$^-$, inner dynein arm defect; ODA$^-$, outer dynein arm defect; Both$^-$, inner + outer dynein arm defect; Other, dynein arms normal; WT, wild-type.

Sequence analysis of the *DPCD* gene. DNA was isolated from a group of PCD patients using standard procedures and the coding region of the *DPCD* gene, including intron-exon boundaries, was sequenced.

*Excluded based on SNP.

†Consanguineous family.

TABLE 2
Population frequency of DPCD *variants in PCD and non-PCD individuals*

Polymorphisms	Population	Subject Analyzed	Wild-type	Heterozygous	Homozygous	Frequency of Allele 1	Frequency of Allele 2
IVS2+59A>G	Control	51	20	31	0	0.696	0.304
[Intron 2]	PCD	51	25	19	7	0.676	0.323
IVS2–22_40del	Control	50	49	1	0	0.990	0.010
[Intron 2]	PCD	51	50	1	0	0.990	0.010
168T>G (S56R)	Control	50	50	0	0	1	0
[Exon3]	PCD	51	50	1	0	0.990	0.010
399C>T (N133N)	Control	52	29	23	0	0.780	0.220
[Exon4]	PCD	51	33	14	4	0.784	0.216
467T>C (L156S)	Control	55	22	28	5	0.655	0.345
[Exon5]	PCD	51	27	19	5	0.716	0.284
IVS5–48_50del	Control	53	53	0	0	1	0
GAG (Intron 5)	PCD	51	45	6	0	0.941	0.059

Single nucleotide polymorphism consortium: http://snp.cshl.org/.399C>T (N133N): rs7874, 1,488 chromosomes analyzed. C allele: 0.944. T allele: 0.056. 467T>C (L156S): rs7006, 308 chromosomes analyzed. T allele: 0.828; C allele: 0.172.

are expressed at low levels in a wide variety of other tissues, based on this and previous studies and EST data (18–20). However, in cultures of normal HBE cells undergoing ciliogenesis, DPCD increases in a pattern consistent with other cilia-specific genes (21, 24), whereas the expression of POLL remains constant or decreases. These data suggest that DPCD plays a role in the formation or function of ciliated cells.

Because the above data suggest that DPCD may be responsible for some cases of PCD, we examined a group of PCD patients for mutations in *DPCD*. Thirteen of these patients had a defect in the IDA, which is the same ciliary abnormality observed in the mouse model, and 20 patients had defects in both the IDA and ODA. Six sequence variants were identified, but none were confirmed as disease causing. Although several PCD patients were homozygous for the IVS2+59A>G and 399C>T variants, based on exclusion analysis, these are most likely polymorphisms and not disease causing. In addition, RT-PCR was performed on three patients (PCD 1, 157, and 158) and yielded a product of normal size with no mutations detected (not shown).

Although no causative mutations were positively identified in *DPCD*, one variant, 168T>G, which results in a serine to arginine change, cannot be eliminated without further studies. It is possible that in this patient a mutation in the other *DPCD* allele may have occurred outside the region sequenced. For example, a mutation in the promoter region may prevent expression of the wild-type allele, creating a *DPCD* null phenotype.

The fact that no disease causing mutations were identified in *DPCD* is not unexpected. PCD is known to be a heterogeneous disease, and only small numbers of samples are available for analysis. Further, mutations that cause PCD in mouse models may be different than those responsible for human disease. It is interesting to note that hydrocephalus is a common feature of many animal models of PCD, but is uncommon in the human disease. It is possible that mutations in genes like *DPCD* may not be compatible with life, and so may not be found in the PCD population.

A subset of the patients with PCD, including thirteen with only an IDA defect, were also examined for possible mutations in the *POLL* gene. No mutations were detected in the coding region or splice junctions. A recently reported study also failed to identify mutations in the *POLL* gene in patients with PCD (25). This is perhaps not surprising, as it appears unlikely that the PCD phenotype observed by Kobayashi and coworkers (12) was caused by the disruption of the *Poll* gene.

In conclusion, our results strongly suggest that the deletion of *Dpcd* may be responsible for the PCD like phenotype in the mouse model previously reported. This conclusion is supported by the expression pattern of Dpcd, the absence of a known function of a DNA polymerase in the structure/function of cilia, and by the lack of a PCD phenotype in mice in which the catalytic region of *Poll* was specifically deleted. Our results also show that mutations in the coding region and splice junctions of *DPCD* do not account for a large percentage of PCD cases, although it is possible that isolated cases may still be found. Further studies are needed to identify the role of DPCD in cilia structure and function.

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