Axonemal Beta Heavy Chain Dynein DNAH9: cDNA Sequence, Genomic Structure, and Investigation of Its Role in Primary Ciliary Dyskinesia

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Dyneins are multisubunit protein complexes that couple ATPase activity with conformational changes. They are involved in the cytoplasmatic movement of organelles (cytoplasmic dyneins) and the bending of cilia and flagella (axonemal dyneins). Here we present the first complete cDNA and genomic sequences of a human axonemal dynein beta heavy chain gene, DNAH9, which maps to 17p12. The 14-kb-long cDNA is divided into 69 exons spread over 390 kb. The cDNA sequence of DNAH9 was determined using a combination of methods including 5' rapid amplification of cDNA ends, RT-PCR, and cDNA library screening. RT- PCR using nasal epithelium and testis RNA revealed several alternatively spliced transcripts. The genomic structure was determined using three overlapping BACs sequenced by the Whitehead Institute/MIT Center for Genome Research. The predicted protein, of 4486 amino acids, is highly homologous to sea urchin axonemal beta heavy chain dyneins (67% identity). It consists of an N-terminal stem and a globular C-terminus containing the four P-loops that constitute the motor domain. Lack of proper ciliary and flagellar movement characterizes primary ciliary dyskinesia (PCD), a genetically heterogeneous autosomal recessive disorder with respiratory tract infections, bronchiectasis, male subfertility, and, in 50% of cases, situs inversus (Kartagener syndrome, KS). Dyneins are excellent candidate genes for PCD and KS because in over 50% of cases the ultrastructural defects of cilia are related to the dynein complex. Genotype analysis was performed in 31 PCD families with two or more affected siblings using a highly informative dinucleotide polymorphism located in intron 26 of DNAH9. Two families with concordant inheritance of DNAH9 alleles in affected individuals were observed. A mutation search was performed in these two "candidate families," but only polymorphic variants were found. In the absence of pathogenic mutations, the DNAH9 gene has been excluded as being responsible for autosomal recessive PCD in these families.

INTRODUCTION

The identification of candidate genes for monogenic disorders has been greatly facilitated by the recent sequencing of the human genome and the possibility of comparing human sequences to those of other species.

The various classes of genes for dyneins, which are components of the cilia, are an illustrative example of candidate genes for primary ciliary dyskinesia (PCD), an

etiologically heterogeneous group of autosomal recessive disorders (Afzelius and Mossberg, 1995; Blouin *et al.,* 2000).

Electron microscopy of cilia and flagella shows a characteristic pattern, of nine circumferential and two central paired microtubules, the axoneme that contains more than 250 different polypeptides (Dutcher, 1995). From the outer microtubule of the peripheral doublets protrude two linear lateral extensions, the outer and inner dynein arms. These arms are multisubunit protein complexes with ATPase activity composed of axonemal dyneins. They promote sliding between adjacent microtubules and the beating of cilia and flagella (Holzbaur and Vallee, 1994). Dynein complexes are also present in the cytosol (cytoplasmic dyneins) and are involved in various different functions (Karki and Holzbaur, 1999). Both axonemal and cytoplasmic dyneins are composed of heavy (two or three chains of ~500 kDa each), intermediate (two to five chains of ~70 kDa each), and light chains (about four chains of ~8 –22 kDa each) (Witman, 1992) (King *et al.,* 1998). The light/intermediate chains (~56 kDa) are an additional component of the cytoplasmic complex. The functional specificity of dyneins is partly achieved through the assembly of different isoforms of dynein subunits. The dynein arm isoform composition is both tissue-specific and outer arm- or inner arm-specific. There are least 17 different dynein heavy chain genes, 13 of which determine axonemal proteins and 4 of which determine cytoplasmic proteins (Asai *et al.,* 1994; Rasmusson *et al.,* 1994; Wilkerson *et al.,* 1994; Gibbons *et al.,* 1994; Tanaka *et al.,* 1995; Porter, 1996; Andrews *et al.,* 1996; Vaisberg *et al.,* 1996; Vaughan *et al.,* 1996; Chapelin *et al.,* 1997; Neesen *et al.,* 1997). The multiple dynein isoforms are produced by different genes, rather than resulting from alternative splicing of one or a limited number of genes.

Each heavy chain dynein consists of a tail (the N- terminal third of the protein) and a glomerular head (the C-terminal two-thirds) (Witman, 1992; Koonce and Samso, 1996). The tail binds the outer microtubule and the other proteins in the complex, while the head contains the nucleotide-binding sites, known as the motor domain (Ogawa, 1991), and the microtubule- binding sites (Koonce, 1997; Gee and Vallee, 1998). Activation of the motor domain changes the conformation of the dynein, provides force for movement, and bends the cilium (Koonce, 1997; Gee and Vallee, 1998). The heavy chain dynein isoforms are grouped into three classes: cytoplasmic, axonemal outer arm, and axonemal inner arm. In the axonemal subfamily, the heavy chains form heterodimers or heterotrimers (Gibbons, 1995).

A mutation in an axonemal heavy chain dynein gene (*Lrd*) was found in the *iv/iv* (inversum viscerum) mice; these animals have situs inversus, but no ultrastructural defects in tracheal cilia or flagella (Supp *et al.,* 1997). Moreover, targeted mutations in the *Lrd* motor domain produce a phenotype that overlaps with that of the *iv/iv* mouse, and the monocilia in the Hensen node are immotile (Supp *et al.,* 1999). Mutations in axonemal heavy chain dyneins have also been found in several immobile strains of *Chlamydomonas* (Kamiya, 1988; King and Dutcher, 1997; Pazour *et al.,* 1999), and male infertility in *Drosophila* is associated with loss of the outer dynein arms in sperm flagella (Gepner and Hays, 1993; Hardy *et al.,* 1981; Fossella *et al.,* 2000).

Since the axonemal heavy chain dyneins are involved in situs inversus and ciliary movements, they are excellent candidate genes for PCD (OMIM 242650; also known as immotile cilia syndrome), an autosomal recessive disorder (with rare exceptions; Narayan *et al.,* 1994). Ciliary motility is affected, with a severity ranging from dysmotility to complete immobility. The disorder is multisystemic, of varying penetrance and severity, with bronchiectasis, recurrent pulmonary and upper

respiratory tract infections, and male infertility. In about 50% of cases, dextrocardia is present, with or without situs inversus totalis (in which case the disorder is also referred to as Kartagener syndrome, OMIM 244400). It appears that in Kartagener syndrome, organ orientation is a random rather than a predetermined event (Afzelius, 1999). Monozygotic twins have been reported with situs inversus in one twin and normal visceral orientation in the second (Noone *et al.,* 1999; Blouin *et al.,* 2000). The most frequent ultrastructural ciliary defect, present in more than 50% of PCD patients, is the absence of outer and/or inner dynein arms (Afzelius, 1981). This supports the hypothesis that one or more mutant dynein genes may be involved in this phenotype. Genetic heterogeneity of PCD, previously proposed because of the complex architecture of cilia, has been demonstrated through linkage studies (Blouin *et al.,* 2000; Witt *et al.,* 1999; Meeks *et al.,* 2000). Recently, mutations were found in an intermediate chain dynein gene, DNAI1, in one patient with PCD (Pennarun *et al.,* 1999).

In the current study, we cloned and sequenced the cDNA of DNAH9, the first human heavy chain gene to be completely described, determined its genomic structure, and excluded this dynein gene as being responsible for PCD in a subset of families in which allelic segregation was concordant with a PCD locus in the DNAH9 genomic region.

MATERIALS AND METHODS

Cloning of cDNAs from the human DNAH9 gene. Partial cDNA sequences of the DNAH9 axonemal dynein have been previously reported: DNEL1 (later renamed DNAH17L), a 3.2-kb 3' end (X99947 (Milisav *et al.,* 1996)); AJ132087 a shorter 305-bp sequence covering the first P-loop; KIAA0357 (AB002355); and a 6094-bp 3' end partially overlapping with the first sequence (Nagase *et al.,* 1997). Radiation hybrid analysis and FISH demonstrated that DNAH9 maps to 17p12 (Bartoloni *et al.,* 1999). Additional 5' cDNA sequences were determined by several methods including 5' rapid amplification of cDNA ends (RACE), RT-PCR, and cDNA library screening. A *Sma*I fragment of KIAA0357, corresponding to the 5'-most sequence, was used to screen approximately 600,000 clones from a human testis λgt10 cDNA library (Clontech Catalog No. HL3024a). Clones with sequences identical to at least 100 bp of KIAA0357 were further analyzed, and the novel 5'-most sequence was subsequently used to obtain additional clones further upstream. PCR amplification of testis and fetal lung cDNA libraries with vector primers λgt10F or λgt10R and a DNAH9-specific primer was also used to obtain additional sequences of DNAH9. 5' RACE reactions were performed on mRNA from human nasal epithelium (after *in vitro* ciliogenesis (Jorissen *et al.,* 1990)) and fetal lung poly(A)$^+$ RNA using the Marathon cDNA amplification kit or the SMART-RACE cDNA kit (Clontech) according to the manufacturer's specifications. The genomic sequence of certain BACs from the Whitehead Institute/MIT Sequencing Center provided the opportunity to determine a number of DNAH9 exons by Blast analysis using dynein cDNAs and proteins from different species (Altschul *et al.,* 1997) and NIX analyses of confirmed by RT-PCR from cDNA obtained from nasal epithelium, testis, and fetal lung cDNA or cDNA libraries. The mRNA for the nasal epithelial library was derived from nasal epithelia biopsies, following *in vitro* ciliogenesis (Jorissen *et al.,* 1990).

Nucleotide sequencing was performed using the ABI 377 sequencer, and sequence assembly was performed using the gap4 program (Staden, 1996). Analysis of the resulting sequence was performed with numerous computer programs available through the "Tools" option of EXPASY (http://expasy.hcuge.ch/www/tools.html). Multiple sequence alignments with published full-length axonemal dyneins were

performed using the CLUSTALW program (Thompson *et al.,* 1994) and shaded with Genedoc (http://www.cris.com/ ~ketchup/genedoc.html).

Genomic structure of DNAH9. The genomic sequencing group of the Whitehead Institute/MIT Center for Genome Research is currently sequencing human chromosome 17 (http://www-seq.wi. mit.edu/public_release/). The sequenced clones containing the DNAH9 gene are hRPK.628_E_12 (AC005701), hRPK.628_O_18 (AC005209), and hRPK.1096_G_20 (AC005410). The sequence data were used to determine the genomic structure and the exon–intron junctions of the DNAH9 gene by comparison with dynein genes of other species, and comparison of cDNA sequences with the genomic sequence. Detailed analysis of the genomic sequence was performed using numerous programs, several of which interfaced with NIX at HGMP (http://www.hgmp.mrc.ac.uk/registered/webapp/NIX/).

Mutational analysis. We have collected more than 60 well-characterized families with PCD, 31 of which have at least two affected individuals (Blouin *et al.,* 2000). The genotypes of the DNAH9 intron 26 polymorphic locus were analyzed using touchdown PCR and primers SEA5058 (AGATCAACAGAGGCTCTGTC) and SEA5059 (TCC-ACTGGTACCGTCTCATG); the amplification product was approximately 200 bp. This polymorphic marker was used to identify families in which the allele segregation is concordant with a PCD locus in the DNAH9 genomic region. Such patients have been selected for mutational search in the DNAH9 gene.

All 69 DNAH9 exons were PCR-amplified from the DNAs of affected individuals from family GVA29 from Spain and family UCL15 from Canton, China, and the products were directly sequenced. Electropherograms were examined using gap4 from the Staden software package; when differences from the reference sequence were found, the appropriate exons were sequenced in 10 unrelated controls from CEPH families.

Exon 5 (where a C to T transition eliminates an *Hae*III restriction site) was also amplified in 623 controls (258 Caucasians, 365 Chinese). The PCR product was digested for 2 h at 37°C with 1.2 units of *Hae*III (NEB), in a 30-μl final volume. The 316-bp-long amplicon is normally cut into three fragments of 30, 145, and 171 bp. An additional band of 201 bp is detected when the C>T transition is present.

Partial cDNAs, derived from the KIAA0357 clone, RT-PCR products, and 5'RACE products were radioactively labeled and used as probes in Southern blot analyses.

RESULTS AND DISCUSSION

Cloning of the Human DNAH9 cDNAs

Using several different methods (see Materials and Methods), we were able to determine an almost full- length cDNA sequence of the DNAH9 gene. Published partial sequences, the genomic sequence, and homology searches were used to design oligonucleotide primers (Table 1) for RT-PCR experiments using testis, fetal lung, and nasal epithelium RNA. The almost full- length cDNA sequence was defined using overlapping fragments obtained from nasal epithelium or fetal lung, as shown in Fig. 1.

Despite using multiple methods (5'RACE, colony screening, RT-PCR), we were unable to clone and/or to sequence the 5'-most end of the DNAH9 cDNA (corresponding to the first 68 amino acids). These putative 68 amino acids were defined using the

available genomic sequence through protein sequence homology (shown in italics in Fig. 2).

TABLE 1

Primers Used in cDNA Extension

Product	Forward primer	Reverse primer	Exons
Clone 1	SEA4095-CTCTCCAAGCAGGATCATTACG	SEA4093-CGTTTTTCACCAGGTATGCCTC	31–38
Clone 2	SEA4177-GGCGTTACAACTATACCAC		27–31
RT-PCRL1	SEA4921-CCTCGCTTGGTGATCACACC	SEA4920-GCACAACTGGTAAAGAGTGATG	26–30
RT-PCRT1	SEA4924-GGAGGAAGGCTATGAGAGTG	SEA4923-CATAGACCAGGATGCCCAGTGC	25–27
RT-PCRT2	SEA5128-GGTTTGAAGGCATCGACATTG	SEA5063-GGTCTCCCTTGGAGAGCTGG	21–25
RT-PCRL2	SEA4928-GATGTTTGAGCCCTTAAAGCA	SEA5130-TCTGGGCATCATAAGCTAGCTG	18–21
RT-PCRT3	SEA4928-GATGTTTGAGCCCTTAAAGCA	SEA4925-CTGGAATCGCATCTTGGCCATGTTG	18–21, 22–23
RT-PCRT4	SEA5061-CTCCCCGGTGCACAAGAAC	SEA4929-CCAGCCGTCAAACACCTTGATG	10, 15, 17
RT-PCRL3	SEA5061-CTCCCCGGTGCACAAGAAC	SEA5256-CAGCAATTCAATAGTCTGC	10–18
RT-PCRL4	SEA4935-CAGGCCTCTAATTATCTCAGC	SEA5062-GCTGAAAGGACCCTGGATGC	6–10
RT-PCRT5	SEA4559-CTAAGGTGGAGTTGGAGTTC	SEA4934-CAGGAAGCCATCCAATCGCAC	3–6
RACE1		SEA6367-CTGGACTTGGTAGCTCCATTTGATCAC	1–3
RACE2		SEA6139-GCTGGAAGAGGCAGCAAAG	1–2

RT-PCR between exons using cDNA from nasal epithelium and testis resulted in products of different sizes, indicating potential alternative splicing. Sequencing of the PCR products confirmed the presence of an alternatively spliced product in testis that lacks at least six exons (exons 11–14, 16, and 20; 1762 bp). Since the resulting reading frame is no longer open, we suggest that there are additional alternatively spliced exons, undetected in our study. Moreover the published sequences of DNAH17L (from testis) and KIAA0357 (from fetal lung) differ for the presence or absence of exon 63, which is 228 nt long but whose absence does not alter the reading frame. Mutations in alternatively spliced exons could explain the occurrence of Kartagener syndrome without male sterility and/or isolated defects in spermatozoa in the absence of systemic PCD.

Genomic Structure of DNAH9

Comparison of the DNAH9 cDNA sequence to that of three overlapping BACs (AC005701, AC005209, and AC005410) resulted in the determination of genomic structure, intron– exon boundaries, and the precise mapping of DNAH9. The gene is composed of 69 coding exons, extending over 373 kb of genomic sequence. Most exons are from 100 to 288 bp long. The exceptions are 3 exons around 400 bp (exons 17, 50, and 68), exon 11, which is 69 bp, and exon 20, which consists of 871 bp (and is alternatively spliced in testis) (Table 2). A CpG island precedes exon 1 (Fig. 1). Introns range in size from 109 bp (intron 16) to 19,381 bp (intron 67).

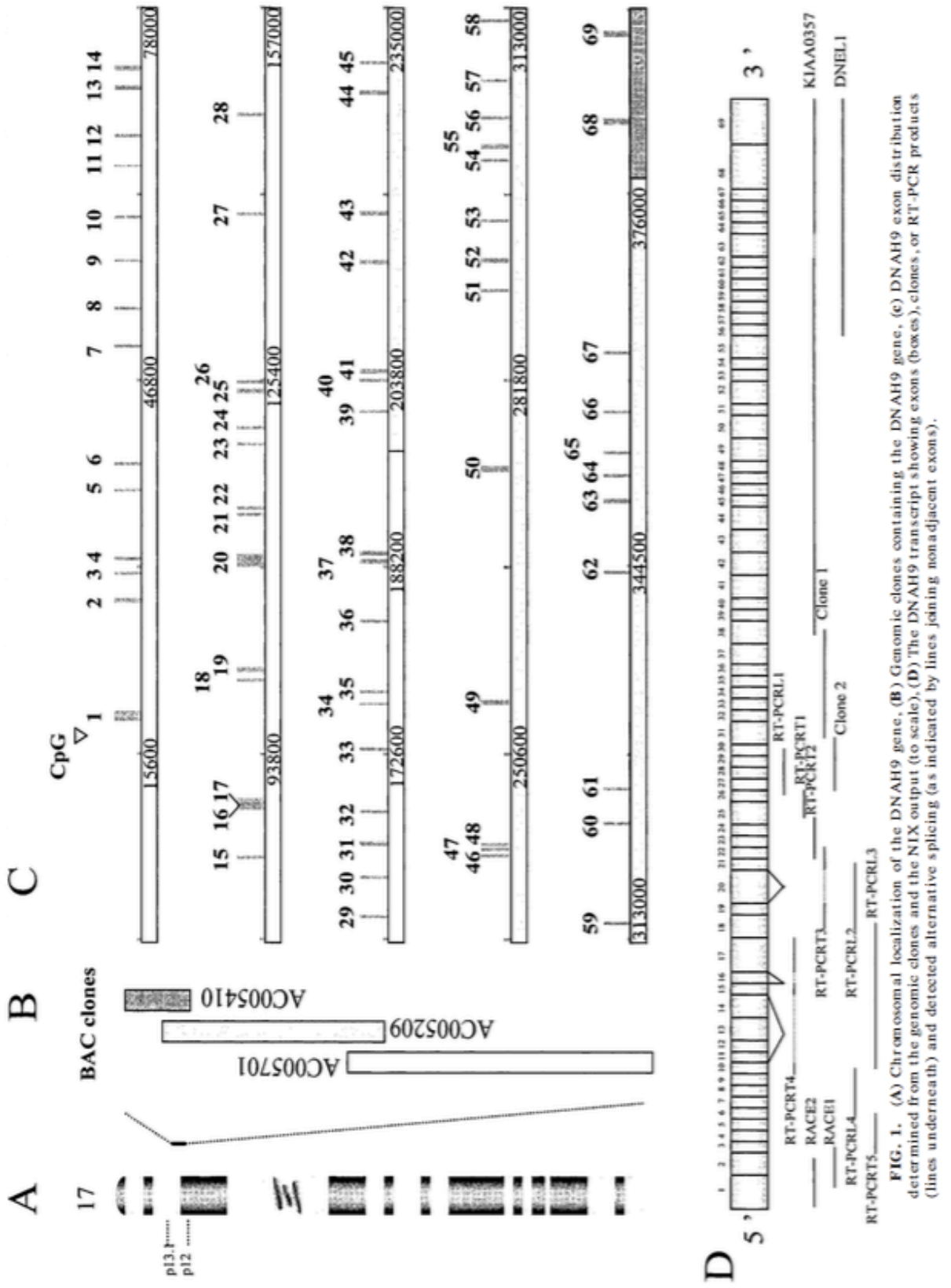

FIG. 1. (A) Chromosomal localization of the DNAH9 gene, (B) Genomic clones containing the DNAH9 gene, (C) DNAH9 exon distribution determined from the genomic clones and the NIX output (to scale), (D) The DNAH9 transcript showing exons (boxes), clones, or RT-PCR products (lines underneath) and detected alternative splicing (as indicated by lines joining nonadjacent exons).

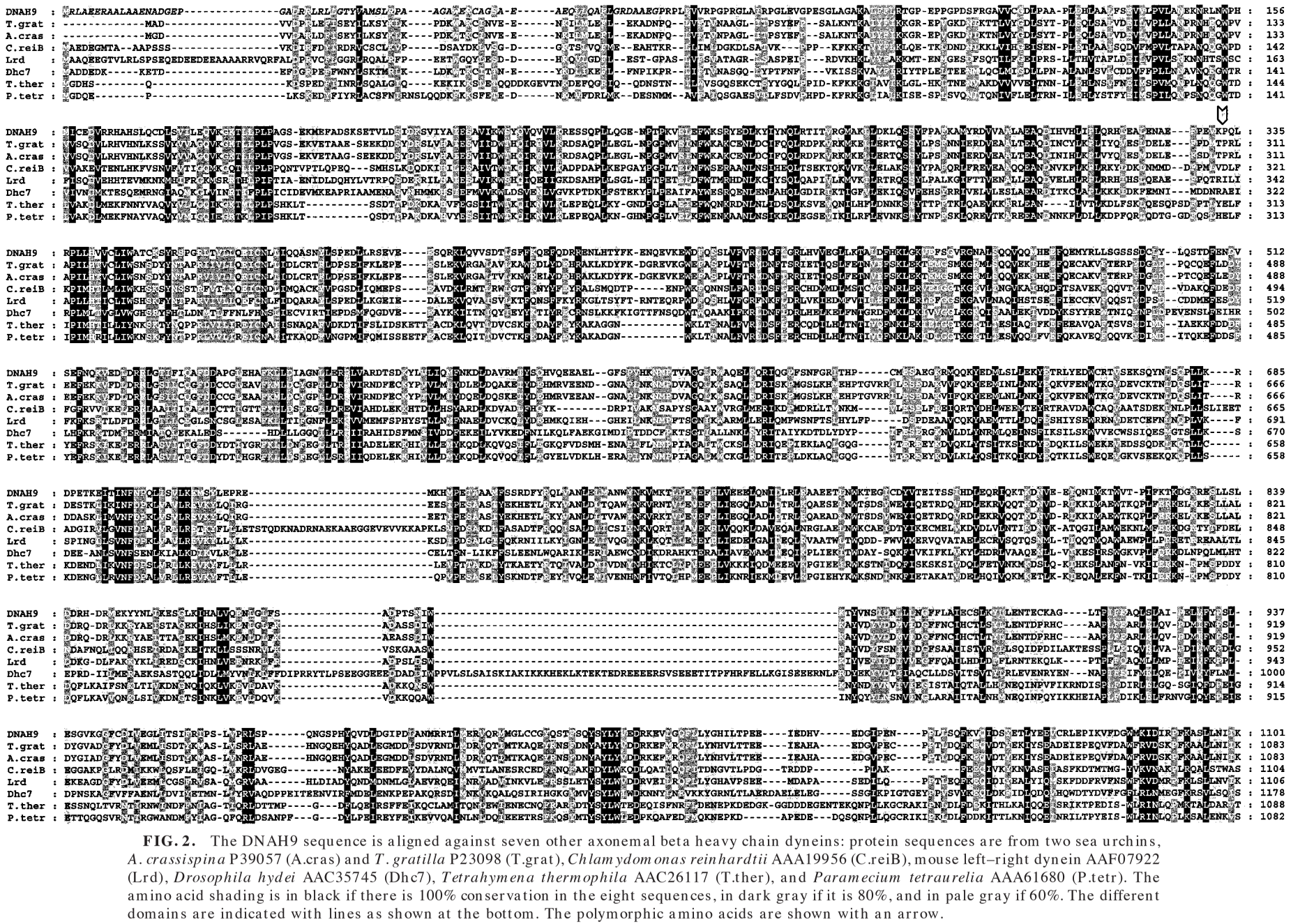

FIG. 2. The DNAH9 sequence is aligned against seven other axonemal beta heavy chain dyneins: protein sequences are from two sea urchins, *A. crassispina* P39057 (A.cras) and *T. gratilla* P23098 (T.grat), *Chlamydomonas reinhardtii* AAA19956 (C.reiB), mouse left–right dynein AAF07922 (Lrd), *Drosophila hydei* AAC35745 (Dhc7), *Tetrahymena thermophila* AAC26117 (T.ther), and *Paramecium tetraurelia* AAA61680 (P.tetr). The amino acid shading is in black if there is 100% conservation in the eight sequences, in dark gray if it is 80%, and in pale gray if 60%. The different domains are indicated with lines as shown at the bottom. The polymorphic amino acids are shown with an arrow.

FIG. 2—*Continued*

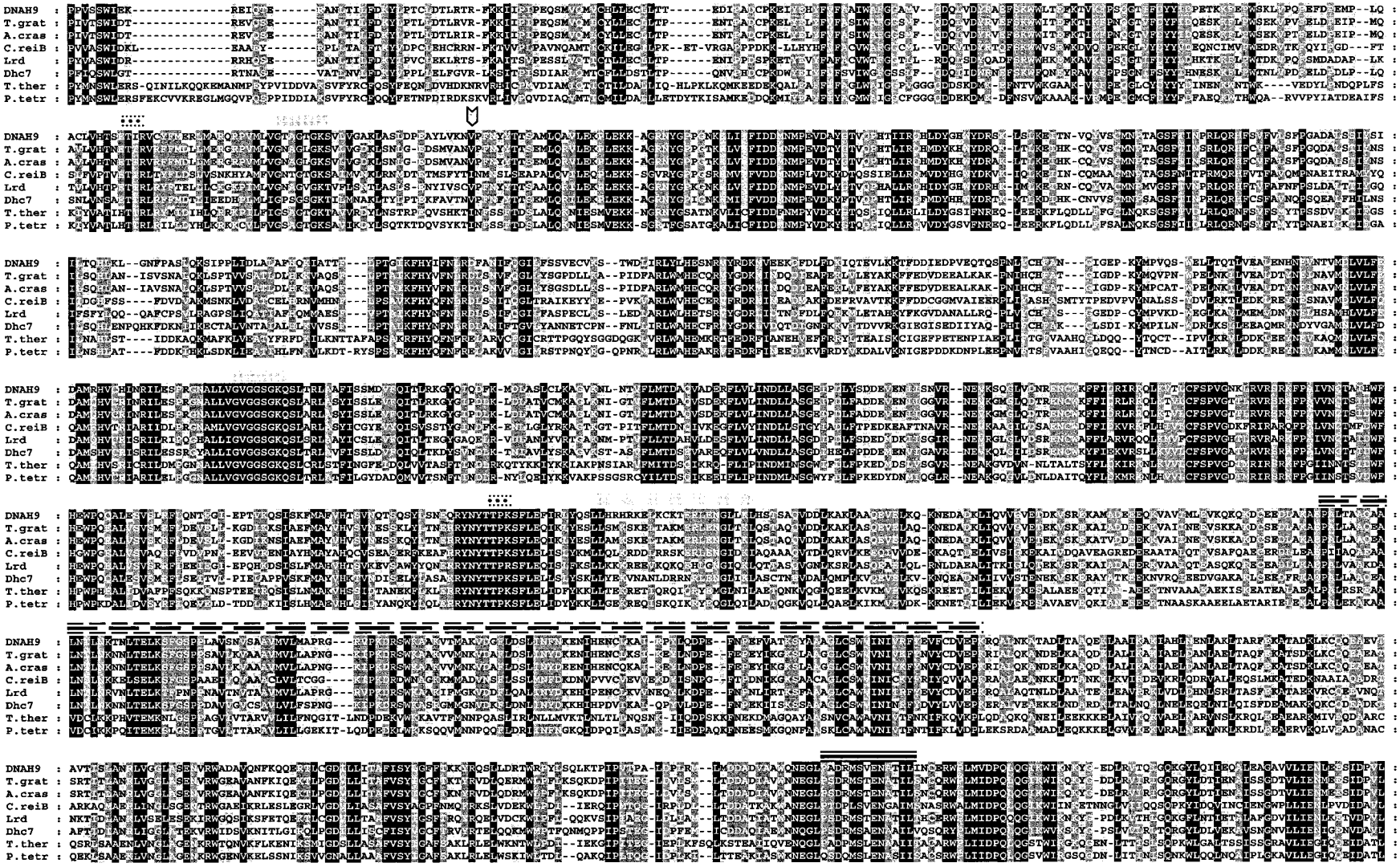

FIG. 2—*Continued*

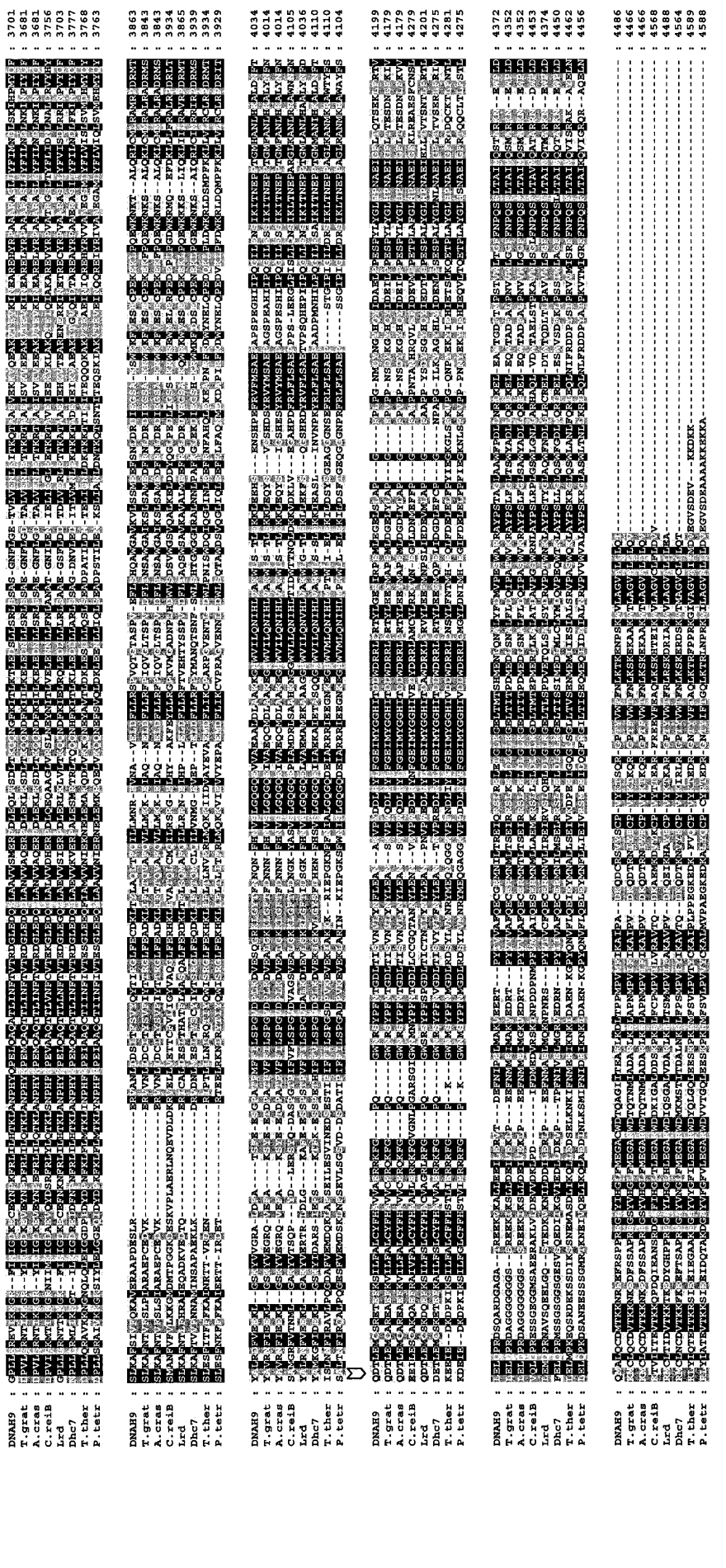

FIG. 2—*Continued*

The predicted domains of DNAH9 are shown in Fig. 2 and are as follows:

ATP/ GTP-binding site (P-loop). The pattern [AG]- x(4)-G-K-[ST] forms a flexible loop that interacts with one of the phosphate groups of the ATP. The motor domain of the heavy chain dynein harbors several P- loop domains, the first of which is highly conserved among cytoplasmic and axonemal dyneins and represents the major ATP catalytic site for force production (Gibbons *et al.,* 1991). In DNAH9, the first P-loop is between residues 1870 and 1877 and has the sequence **G**PAGT**GKT.** The other P-loops likely bind nucleotides, but it seems they act more as reservoirs of nucleotides than true ATPases. In DNAH9 they are at positions 2151–2158 (**G**GAGT**GKS**) and 2478 –2485 **G**TAGT**GKS**). In cytoplasmic dyneins, there is an additional, fourth 3' canonical P-loop. At the corresponding site in DNAH9 (aa 2825–2832) and in other axonemal dyneins, a highly conserved sequence is found (**G**VGGS**GK**Q), which may be an unconventional P-loop or the vestige of an ancestral functional site.

Microtubule-binding motif. In heavy chain dyneins, some microtubule-binding activity is located within the predicted alpha-helical region, immediately following the last P-loop and/or in the proximity of P-loops (Gee and Vallee, 1998; Koonce, 1997). Two models of microtubule-binding sites have been proposed. The first model (Vallee and Gee, 1998) involves a stalk, the product of interactions between coiled-coil domains, at the apex of which the domain with linking activity might be located. The authors observed only one stalk protruding from the globular head, which indicates that each heavy chain dynein has only one site with strong affinity for tubulin. The microtubule-binding domain should reside about 340 residues downstream from the last P-loop element. The putative stalk region, flanked by two prolines, is about 200 residues long and forms two alpha-helices that can interact in an antiparallel way, giving a coiled-coil structure. A similar domain is present in DNAH9. From approximately aa 3050 to 3155 and from 3284 to 3390, there are two coiled-coil domains, in the middle of which there is a region delimited by two prolines (P3155 and P3284). Some amino acids scattered in this region are conserved in all heavy chains, and some groups of amino acids are conserved in the axonemal beta chains. An interesting group of 3 charged amino acids is present in all the axonemal heavy chains and located in the middle of the region. In DNAH9 these amino acids are K3204, D3205, and R3206. These residues could be part of the microtubule-binding site.

The second model (Koonce, 1997) suggests a motif shared by dyneins and kinesins, the core consensus of which is P-X6-E-X4-L, surrounded by several conserved hydrophobic, polar, and charged regions of approximately 125 amino acids; these regions are well conserved among dynein heavy chains. In DNAH9 we identified three such sequences: **P** AGTGKT**E**TTKD**L** at residues 1871–1883 (conserved in all the heavy chains), **P** GYAGRT**E**LPEN**L** at position 1974 –1986 (conserved in all the axonemal heavy chains), and **P** ADRMSV**E**NATI**L** at position 3444 –3456 (conserved in beta axonemal heavy chains).

Leucine zipper pattern. There is one leucine zipper domain at position 3055–3076 (**L**HRHRKE**L**KCKTER- **L**ENGLLK**L**). This domain is conserved in only some axonemal beta heavy chains.

Phosphorylation sites. There are several [ST]-x- [RK] consensus sequences for protein kinase C in DNAH9, five of which are conserved in all heavy chains dyneins: **T**D**R** (1849–1851, N-terminal to the first P- loop), **T**G**K** (1872–1874, internal to the first P-loop), **T**T**K** (1878 –1880, just following the first P-loop), **T**I**R** 2457–2459), and **T**P**K**

TABLE 2

Intron–Exon Junctions of the DNAH9 Gene

Exon No.	Exon length	5′ Splice donor	Intron length	3′ Splice acceptor
1	485[a]	TTCTCGGAG**GT**GAGGGTGGGTTAG	9,213	GCACCTCTGTTCC**AG**GTTGTTCTACC
2	197	TGAGACAGT**GT**AAGTACCGCCAGC	2,070	TTTTGTCTTTCAC**AG**CTTGGATTCTA
3	159	GAAGAGCAG**GT**AGGCAAGAAGGCA	1,095	TTCATGATATTGC**AG**GTATGAAGATC
4	131	TTGTTGCAG**GT**GAGGACCAGCAAG	5,630	TCCAATTCCTTCC**AG**CTCTAGCAGAG
5	212	ATCCAGCAG**GT**GGGCTGCCCTGGG	1,925	CTGTTTGTATACC**AG**GCCTCTAATTA
6	234	GTGGTGGAG**GT**GAGTGCGCACCTC	9,635	GAATTGTCCCCAT**AG**GGTCTTCTGAA
7	168	CAAAGCACG**GT**AGGGTTGGGAAGG	3,002	ATGGTGTCTCTTC**AG**GACTTTGAAAA
8	117	GCCTTTAAG**GT**TTGTGTAAATGGG	3,930	CCACCTTCCCTAC**AG**CTGCTAGACAT
9	151	CAGAACTTG**GT**AGGCTTGTTAAAG	3,485	GTGCCATGTCTCC**AG**GGTTCTCCCCG
10	115	CACACACCC**GT**GAGTATTGTGTTC	4,246	CTTTTGTACGAGT**AG**TTGCATGGAAT
11	69	GCTAGAAAA**GT**AAGCAACTTCTGG	2,372	CTCTGACCCTTGC**AG**GTATGAGACAA
12	127	AACCCACAG**GT**CAGTTGGCTGACA	3,870	GCTTCTTTTCTCC**AG**CTGATTTCAGT
13	256	AAACAGAAG**GT**AACAGGGCACCAT	1,436	TCTTTTGGGGAAC**AG**GCATTTGCGAT
14	242	CTTGTTCAG**GT**AATAACCCAGCCA	11,830	TTCTTCCTTTTAT**AG**GAAAACCTGGG
15	136	AAAATACTG**GT**ACTTACTGGCTTA	4,095	TTCTGTGTTTTTC**AG**AGTGTAAGGCA
16	197	CACTATCAG**GT**ACTGGAGCTGCAG	109	GTCTCGCTTCCCC**AG**GTCGACCTGGA
17	425	CACTCACAG**GT**ACAACAGTTGTTT	9,962	GTTTGTGTTGATT**AG**CTTGGCCAACC
18	223	CAGCTGGAG**GT**CAGTGCATTTATG	743	AGGTTTCCTTTGC**AG**GAGCTGCCTGA
19	167	CCCGTTCAG**GT**ATGGGCCGAACAC	8,676	CACTGTGTTTTGT**AG**GTTTGATAGCA
20	871	CTACCCCAG**GT**ACCTGCTAAGGAA	3,431	CATTTTTATTTGC**AG**GATTCTAAAAG
21	131	TCAGGGCAG**GT**GAGGGTCCGCCCA	322	GTTCTGTGCCCTC**AG**ATTGTGCCTGT
22	127	CCACAACAG**GT**AAGCTGGAGGAGC	5,283	GGTTTCCCTTCAT**AG**GTTCAACGTCA
23	153	AGCGGGCAG**GT**AACACAGTAGCCC	1,238	GAGTTGTTCTTTC**AG**GTAGAAATATG
24	126	CCAGCTCAG**GT**ATTCTCCTAATGG	2,955	CTCTTGCTGCCAC**AG**GTGGCCCTGAC
25	240	GCTCAGAAG**GT**GGGTCCCAAACAT	582	CCACTCTCTCTTT**AG**GTAGACAATGC
26	161	GACTGACAG**GT**GAGCACTGGTGTC	14,148	TCCCGTGTTTGGC**AG**GTGCTACATCA
27	157	GATTACAAG**GT**ACAGTTCCACCCG	8,327	TGCATTTTCTTGC**AG**TCTTGTGGCAA
28	105	GCAGTGCAG**GT**AAGGGCCAGAAGT	10,957	TGATTGGTTCACC**AG**GTAAAAAGCAT
29	158	TCTCTTCAG**GT**GAGTGTCAGTTCT	3,137	TCTTTCTATACTC**AG**GCCTTGTGCAA
30	139	TCCAAACAG**GT**AGGATCTCTAGGG	2,483	TAACACTGCCCAC**AG**GATCACTACGA
31	288	GTGCTCAAG**GT**ACATGTGGTTTTT	2,471	TTCCTGCCTCCTC**AG**GTGGTCCAGCT
32	211	GGAAGGATG**GT**AAGAGTGGGATTC	5,066	AGTGGTTCCTTTT**AG**GATTGTTCTCT
33	128	GATAACAAG**GT**ATGAAATTGGGGG	3,607	CCCTGTTGTCTCC**AG**GTGCTGACATT
34	109	CTAGAGCAG**GT**ACGGCCCAAGAAG	868	TCTCTGTGCTCAC**AG**GGATCTTGTAC
35	148	CAGAACCAG**GT**AGGCCAAGAAACA	5,747	GGTCTCTCTTTGC**AG**GTTTAAGAAGA
36	175	CAAGATCAG**GT**AAGGAGATATGTT	4,838	TCTTTGTTTTTGC**AG**CTTGTGGACTA
37	174	CCCTTGCAG**GT**GAGTGCAGCTGAG	495	GTGCTCCCATTCC**AG**GCGTGTTTGGT
38	208	TGCTGCAGG**GT**AAGCAGCCCAGGG	11,679	TGGCTCTCTCCCC**AG**CTGTCCTGGAG
39	166	TGGCCACTG**GT**AAGAGCGCCCATG	2,471	TTCTGACTGTTCC**AG**GTATGATCGGA
40	103	CGGCTTCAG**GT**ACAGGAGGAGCCA	551	TTGTTTCCTGTAC**AG**CGTCACTTCAG
41	240	ATTTTCCAG**GT**GAGTTCATCGGCT	8,963	CTGTTCCCCTTGA**AG**GGCATTCTCTT
42	162	ACTTTTGAT**GT**GAGTATTGCCCTA	3,932	GTGTCTCATCACC**AG**GATATTGAAGA
43	197	GCGCCATGT**GT**AAGTGTGCTTCTC	9,938	CTTCCTTCTCTTT**AG**CTGCCATATCA
44	163	GACTTCAAG**GT**AAAAGGTCAGGCA	2,351	TGACCATTCCTAC**AG**ATGGACCTGGC
45	124	TGGCATCTG**GT**AAGAGATTCCTTG	11,550	TCCATTTTTTCCC**AG**GGGAGATCCCA
46	143	CAGCTGAAG**GT**AAAGAGCATTTAC	375	CTTCTGACTTCTC**AG**GTGACTCTCTG
47	162	GGCATTGAG**GT**GAGAGAGAAAAGG	201	GCTCTTTGGTTTT**AG**CCCACAGTAAA
48	234	TCTGCCCAG**GT**GAGCAATGTCCCG	11,603	GCTTCTCCAATGC**AG**GTGGATGATCT
49	261	CTGAACAAG**GT**AGGAGGACTGTCT	19,104	CTTCTCTTTTCCC**AG**ACCAACCTGAC
50	426	AAGATCGCT**GT**GAGTGACCCCAGA	14,705	TTCCTTCCTAAAC**AG**CACCTTAATGA
51	120	AACCGCCTG**GT**GAGTGTAAGCCAC	2,344	CTTTTGAAATGGT**AG**GTTGGAGGACT
52	189	CAGCTGAAA**GT**ACGTATGGCCTGA	3,162	TTTCTTCTTCACC**AG**ACTCCCATTCC
53	236	TCAGAAAGG**GT**AAGTGGTTGAGCA	4,893	TACTTGTTTCCCT**AG**CTACCTTCAAA
54	123	AAAAGGACG**GT**AAGACTCAGCTGT	1,008	GCCTTTATGTTCC**AG**ATTCATTAAAA
55	205	CAGCTGAAG**GT**GAGGACAGAAGGG	2,172	GCTTGATATTTGC**AG**TCCGATCTCAC
56	165	GAGAAAAAG**GT**AAAACTCCTCTGG	3,074	TTATTTTTCCAAC**AG**GTCCAGGAGGC
57	141	TCTCTCAAG**GT**GACTTACACCTGG	4,811	GTCTTCTGTCCCC**AG**GCCTTCAGTAT
58	171	ACCTTTCAG**GT**AAAAGTGGATTGA	2,426	TTCTCCACATTGC**AG**ATTCTCCTCAT
59	123	GCTGTCAAG**GT**CAGTATTGACCCC	8,222	TCTGAAATTTCCC**AG**GTACTTTCATC
60	194	TGCTTTGCG**GT**AGGAAACAGGGTG	2,748	CTTTTTTTCTGGC**AG**AGATTTTGTTG
61	149	AAAGTCAAG**GT**GAGAAAGGCGTCC	18,008	CCTCCTTTTAGGA**AG**AAAACTTGGAT
62	124	ATTTTGCAG**GT**ATGTTCCTCTACC	5,924	CTTCCTTCCCACT**AG**AACATTCACCT
63	228	TTCACTCAG**GT**ACGGCCCCGGGAG	1,920	ACACTCTCATTTC**AG**GACACTCTGGA
64	192	AACGCAAAG**GT**AAAGGCCATGGAC	1,674	TCCCCCATCACTC**AG**GTCCCCTATGA
65	192	TATCATCAG**GT**GAGACTCTGCTCT	3,280	GCCCTTGATTTTC**AG**TACATCGATGC
66	171	GAAGAAAAG**GT**GTGTGTGGTGGGG	4,782	CCTTCTGTGGGGC**AG**GTCAAGGCACT
67	181	GGCTTAAAG**GT**GAGCGCGGTCTTG	19,381	ATTCTTTATCTTC**AG**GGGGAGCTGAC
68	390	GACACACAG**GT**AAAGCTTGGAATG	7,043	ATGTTTTATCCTC**AG**GCTGGGATCAT
69	462[b]			

Note. The invariant AG/GT nucleotides at the end of exons and at the beginning of introns are shown in boldface type.

[a] ATG at nt 69.

[b] Stop codon at nt 226.

TABLE 3

Polymorphisms Detected during the Mutation Search

	Exon										
	2	5	7	31	32	38	50	56	63	63	64
Sequence variant	C > T	C > T	A > G	C > T	A > G	G > T	G > C	C > T	A > G	G > A	G > A
Patient UCL15	C/T	C/T	A/A	T/T	A/G	G/T	G/G	T/T	A/G	G/A	A/G
Patient GVA29	C/T	C/C	A/G	C/C	A/A	G/G	C/G	C/C	A/G	G/A	A/G
Codon change	CCC > CCT	CCC > CTC	GAG > GAA	AAT > AAC	AAT > AGT	GTG > TTG	CCG > CCC	CTG > TTG	CCA > CCG	ACG > ACA	GAC > AAC
aa position	143	333	485	2110	2195	2505	3248	3619	3994	4020	4036
aa change	Pro > Pro	Pro > Leu	Glu > Glu	Asn > Asn	Asn > Ser	Val > Leu	Pro > Pro	Leu > Leu	Pro > Pro	Thr > Thr	Asp > Asn
Conserved aa		Yes			No	Yes					No
10 CEPH controls		0T/20C		8C/12T	5G/13A	20G/2T			15A/5G	15G/5A	14G/6A
Caucasian controls		0T/496C									
Ethnic controls		3T/727C									
Parental origin		Father				Mother					

(3040 –3042). Interestingly, alpha outer arm dynein heavy chains could be phosphorylated at a minimum of six sites, and two of these regions have been mapped *in vivo* in *Chlamydomonas* (King and Witman, 1994).

Mutational Analysis of Candidate PCD Families

Linkage analysis using DNAs from 31 multiplex families with PCD failed to demonstrate a single locus common to the majority of these families and indicated considerable genetic heterogeneity. In addition, there was no strong evidence for linkage on chromosome 17p12, the mapping position of DNAH9 (Blouin *et al.,* 2000). However, given the extensive genetic heterogeneity of PCD, we performed mutational analysis of the entire coding region and the intron–exon junctions in 2 families in which affected individuals have concordant inheritance of alleles for a $(CA)_n$-$(GA)_n$ microsatellite within intron 26 of DNAH9. This marker was first genotyped in the parents of the 40 unrelated CEPH families, revealing a heterozygosity rate of 86%, due to nine distinct alleles.

The segregation of the alleles of this polymorphic site in 31 PCD families revealed 2 families for which the microsatellite was fully informative and in which the affected offspring demonstrated the same genotypes. Affected individuals in family GVA29 shared alleles 1 and 3, with the genotypes in the parents being 1-2 (father) and 3-4 (mother). Similarly, both affecteds from family UCL15 shared the 2-3 genotype, while the parents were 1-3 and 2-4. In family GVA29, the affected siblings show dextrocardia and absence of inner and outer dynein arms.

The sequencing of the PCR products revealed 11 nucleotide changes that are likely to be polymorphic variants and not deleterious mutations, including the changes in exons 5, 32, 38, and 64 that result in amino acid substitutions (Table 3).

The base change in exon 64 involves the first nucleotide of the exon and could possibly alter normal splicing; in addition, it results in a D4036N substitution. However, sequence of 10 unrelated controls revealed that 75% of alleles contained a G, whereas the remaining 25% had an A, excluding a pathogenic role for this base change.

The exon 38 G to T transversion results in V2505L substitution. This is also likely to be a polymorphism since the codon for leucine was found in 2 of 22 control alleles. In exon 32 a serine codon is substituted for a nonconserved asparagine (N2195S), resulting from an A to G transition, in 25% of alleles in the control population. In exon 5 a C to T transition, leading to a P333L substitution, was present in a heterozygous state in both patients from family UCL15. The proline was present in all the axonemal dyneins analyzed, while it was not conserved in cytoplasmic heavy chains. The first group of 10 unrelated CEPH controls failed to show the leucine allele. Since this change abolishes a restriction *Hae*III site, allowing rapid population screening, we extended the analysis to 58 PCD patients and 182 Caucasian controls, not one of whom had the Leu allele. Since family UCL15 was from south China, we analyzed the DNAs from 365 Chinese controls. In a Taiwanese subgroup (189 individuals), we found 3 individuals heterozygous for the leucine allele. We concluded that this change represents a relatively rare variant in this population; in addition we failed to detect a potential “second” mutation in the patients from family UCL15. To investigate further the significance of this base change, and to exclude its pathogenicity, systematic screening of Taiwanese PCD patients would be necessary. Large DNAH9 rearrangements were excluded by Southern blot analyses of DNAs from the 2 selected families.

The absence of mutations in our set of patients does not necessarily exclude DNAH9 as a candidate gene for PCD/Kartagener phenotypes, since the disease is highly heterogeneous. The polymorphic microsatellite in intron 26 of DNAH9 can be used to test for concordance of DNAH9 alleles in additional families with Kartagener syndrome. Moreover, due to its expression in testis DNAH9, mutations could be associated with nonsyndromic male sterility. The study of additional axonemal dynein genes is still necessary to investigate further the molecular basis of PCD.

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Note added in proof. During the evaluation of this article, the sequence of DNAH9 was also deposited with the public databases (GenBank Accession No. AF257737) by investigators from the University of North Carolina (Chapel Hill). There is no difference be- tween the sequence reported here (AJ404468) and that from the other group (AF257737).

REFERENCES

Afzelius, B. A. (1981). Genetical and ultrastructural aspects of the immotile-cilia syndrome. *Am . J . Hum . Genet*. **33:** 852– 864.

Afzelius, B. A. (1999). Asymmetry of cilia and of mice and men. *Int. J . Dev. Biol*. **43:** 283–286.

Afzelius, B. A., and Mossberg, B. (1995). Immotile-cilia syndrome (primary ciliary dyskinesia), including Kartagener syndrome. *In* "The Metabolic and Molecular Bases of Inherited Disease" (C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, Eds.), pp. 3943–3954, McGraw-Hill, New York.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997). Gapped BLAST and PSI- BLAST: A new generation of protein database search programs. *Nucleic Acids Res*. **25:** 3389–3402.

Andrews, K. L., Nettesheim, P., Asai, D. J., and Ostrowski, L. E. (1996). Identification of seven rat axonemal dynein heavy chain genes: Expression during ciliated cell differentiation. *Mol. Biol. Cell*. **7:** 71–79.

Asai, D. J., Beckwith, S. M., Kandl, K. A., Keating, H. H., Tjandra, H., and Forney, J. D. (1994). The dynein genes of *Paramecium tetraurelia*. Sequences adjacent to the catalytic P-loop identify cytoplasmic and axonemal heavy chain isoforms. *J . Cell. S ci*. **107:** 839 – 847.

Bartoloni, L., Blouin, J., Sainsbury, A. J., Gos, A., Morris, M. A., Affara, N. A., DeLozier-

Blanchet, C. D., and Antonarakis, S. E. (1999). Assignment of the human dynein heavy chain gene DNAH17L to human chromosome 17p12 by in situ hybridization and radiation hybrid mapping. *Cytogenet. Cell Genet.* **84:** 188 –189.

Blouin, J -L., Meeks, M., Radhakrishna, U., Sainsbury, A. J ., Gehrig, C., Duriaux Sail, G., Bartoloni, L., Dombi, V., O'Rawe, A., Walne, A., Chung, E., Afzelius, B. A., Armengot, M., Jorissen, M., Schid- low, D. V., Van Maldergem, L., Walt, H., Gardiner, R. M., Probst, D., Guerne, P-A., Delozier-Blanchet, C. D., and Antonarakis, S. E. (2000). Primary ciliary dyskinesia: A genome-wide linkage analysis reveals extensive locus heterogeneity. *Eur. J. Hum. Genet.* **8:** 109 –118.

Chapelin, C., Duriez, B., Magnino, F., Goossens, M., Escudier, E., and Amselem, S. (1997). Isolation of several human axonemal dynein heavy chain genes: Genomic structure of the catalytic site, phylogenetic analysis and chromosomal assignment. *FEBS Lett.* **412:** 325–330.

Dutcher, S. K. (1995). Flagellar assembly in two hundred and fifty easy-to-follow steps. *Trends Genet.* **11:** 398 – 404.

Fossella, J., Samant, S. A., Silver, L. M., King, S. M., Vaughan, K. T., Olds-Clarke, P ., Johnson, K. A., Mikami, A., Vallee, R. B., and Pilder, S. H. (2000). An axonemal dynein at the hybrid sterility 6 locus: Implications for t haplotype-specific male sterility and the evolution of species barriers. *Mamm. Genome* **11:** 8–15.

Gee, M., and Vallee, R. (1998). The role of the dynein stalk in cytoplasmic and flagellar motility. *Eur. Biophys. J.* **27:** 466–473.

Gepner, J., and Hays, T. S. (1993). A fertility region on the Y chromosome of *Drosophila m elanogaster* encodes a dynein microtubule motor. *Proc. Natl. Acad. Sci. USA* **90:** 11132–11136.

Gibbons, B. H., Asai, D. J., Tang, W. J., Hays, T. S., and Gibbons, I. R. (1994). Phylogeny and expression of axonemal and cytoplasmic dynein genes in sea urchins. *Mol. Biol. Cell* **5:** 57–70.

Gibbons, I. R. (1995). Dynein family of motor proteins: Present status and future questions. *Cell Motil. Cytoskeleton* **32:** 136 –144.

Gibbons, I. R., Gibbons, B. H., Mocz, G., and Asai, D. J. (1991). Multiple nucleotide-binding sites in the sequence of dynein beta heavy chain. *Nature* **352:** 640–643.

Hardy, R. W., Tokuyasu, K. T., and Lindsley, D. L. (1981). Analysis of spermatogenesis in *Drosophila melanogaster* bearing deletions for Y-chromosome fertility genes. *Chromosoma* **83:** 593– 617.

Holzbaur, E. L., and Vallee, R. B. (1994). DYNEINS: Molecular structure and cellular function. *Annu. Rev. Cell Biol.* **10:**339–372.

Jorissen, M., Van der Schueren, B., Van den Berghe, H., and Cassiman, J. J. (1990). Ciliogenesis in cultured human nasal epithelium. *ORL J. Otorhinolaryngol. Relat. Spec.* **52:** 368–374.

Kamiya, R. (1988). Mutations at twelve independent loci result in absence of outer dynein arms in *Chylamydomonas reinhardtii. J . Cell Biol.* **107:** 2253–2258.

Karki, S., and Holzbaur, E. L. (1999). Cytoplasmic dynein and dynactin in cell division and intracellular transport. *Curr. Opin. Cell Biol.* **11:** 45–53.

King, S. J., and Dutcher, S. K. (1997). Phosphoregulation of an inner dynein arm complex in *Chlamydomonas reinhardtii* is altered in phototactic mutant strains. *J. Cell Biol.* **136:** 177–191.

King, S. M., Barbarese, E., Dillman, J. F., Benashski, S. E., Do, K. T., Patel-King, R. S., and Pfister, K. K. (1998). Cytoplasmic dynein contains a family of differentially expressed light chains. *Biochemistry* **37:** 15033–15041.

King, S. M., and Witman, G. B. (1994). Multiple sites of phosphorylation within the alpha heavy chain of *Chlamydomonas* outer arm dynein. *J . Biol. Chem .* **269:** 5452–5457.

Koonce, M. P . (1997). Identification of a microtubule-binding domain in a cytoplasmic dynein heavy chain. *J . Biol. Chem .* **272:** 19714 – 19718.

Koonce, M. P., and Samso, M. (1996). Overexpression of cytoplasmic dynein's globular head causes a collapse of the interphase micro- tubule network in *Dictyostelium . Mol. Biol. Cell* **7:** 935–948.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* **44:** 283–292.

Meeks, M., Walne, A., Spiden, S., Simpson, H., Mussaffi-Georgy, H., Hamam, H. D., Fehaid, E. L., Cheehab, M., Al-Dabbagh, M., Blau, H., O'Rawe, A., Mitchison, H. M., Gardiner, R. M., and Chung, E. (2000). A locus for primary ciliary dyskinesia maps to chromosome 19q. *J . Med. Genet.* **37:** 241–244.

Milisav, I., Jones, M. H., and Affara, N. A. (1996). Characterization of a novel human dynein-related gene that is specifically expressed in testis. *Mam m . Genome* **7:** 667– 672.

Mitchell, D. R., and Brown, K. S. (1994). Sequence analysis of the *Chlamydomonas* alpha and beta dynein heavy chain genes. *J. Cell Sci.* **107:** 635– 644.

Nagase, T., Ishikawa, K., Nakajima, D., Ohira, M., Seki, N., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N., and Ohara, O. (1997). Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. *DNA Res.* **4:** 141–150.

Narayan, D., Krishnan, S. N., Upender, M., Ravikumar, T. S., Ma- honey, M. J., Dolan, T. F. J., Teebi, A. S., and Haddad, G. G. (1994). Unusual inheritance of primary ciliary dyskinesia (Kartagener's syndrome). *J . Med. Genet.* **31:** 493– 496.

Neesen, J., Koehler, M. R., Kirschner, R., Steinlein, C., Kreutzberger, J., Engel, W., and Schmid, M. (1997). Identification of dynein heavy chain genes expressed in human and mouse testis: Chromosomal localization of an axonemal dynein gene. *Gene* **200:** 193–202.

Noone, P. G., Bali, D., Carson, J. L., Sannuti, A., Gipson, C. L., Ostrowski, L. E., Bromberg, P. A., Boucher, R. C., and Knowles, M. R. (1999). Discordant organ laterality in monozygotic twins with primary ciliary dyskinesia. *Am. J. Med. Genet.* **82:** 155–160.

Ogawa, K. (1991). Four ATP-binding sites in the midregion of the beta heavy chain of dynein. *Nature* **352:** 643–645.

Pazour, G. J., Dickert, B. L., and Witman, G. B. (1999). The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. *J. Cell Biol*. **144:** 473– 481.

Pennarun, G., Escudier, E., Chapelin, C., Bridoux, A. M., Cacheux, V., Roger, G., Clement, A., Goossens, M., Amselem, S., and Duriez, B. (1999). Loss-of-function mutations in a human gene related to *Chlamydomonas reinhardtii* dynein IC78 result in primary ciliary dyskinesia. *Am. J. Hum . Genet*. **65:** 1508 –1519.

Porter, M. E. (1996). Axonemal dyneins: Assembly, organization, and regulation. *Curr. Opin. Cell Biol*. **8:** 10 –17.

Rasmusson, K., Serr, M., Gepner, J., Gibbons, I., and Hays, T. S. (1994). A family of dynein genes in *Drosophila melanogaster*. *Mol. Biol. Cell* **5:** 45–55.

Staden, R. (1996). The Staden sequence analysis package. *Mol. Bio- technol*. **5:** 233–241.

Supp, D. M., Brueckner, M., Kuehn, M. R., Witte, D. P., Lowe, L. A., McGrath, J., Corrales, J., and Potter, S. S. (1999). Targeted deletion of the ATP binding domain of left–right dynein confirms its role in specifying development of left–right asymmetries. *Development* **126:** 5495–5504.

Supp, D. M., Witte, D. P., Potter, S. S., and Brueckner, M. (1997). Mutation of an axonemal dynein affects left–right asymmetry in inversus viscerum mice. *Nature* **389:** 963–966.

Tanaka, Y., Zhang, Z., and Hirokawa, N. (1995). Identification and molecular evolution of new dynein-like protein sequences in rat brain. *J. Cell Sci*. **108:** 1883–1893.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. **22:**4673–4680.

Vaisberg, E. A., Grissom, P. M., and McIntosh, J. R. (1996). Mammalian cells express three distinct dynein heavy chains that are localized to different cytoplasmic organelles. *J. Cell Biol*. **133:** 831– 842.

Vallee, R. B., and Gee, M. A. (1998). Make room for dynein. *Trends Cell Biol*. **8:** 490 – 494.

Vaughan, K. T., Mikami, A., Paschal, B. M., Holzbaur, E. L. F., Hughes, S. M., Echeverri, C. J., Moore, K. J ., Gilbert, D. J ., Copeland, N. G., Jenkins, N. A., and Vallee, R. B. (1996). Multiple mouse chromosomal loci for dynein-based motility. *Genomics* **36:** 29 –38.

Wilkerson, C. G., King, S. M., and Witman, G. B. (1994). Molecular analysis of the gamma heavy chain of *Chlamydomonas* flagellar outer-arm dynein. *J. Cell Sci*. **107:** 497–506.

Witman, G. B. (1992). Axonemal dyneins. *Curr. Opin. Cell Biol*. **4:** 74 –79.

Witt, M., Wang, Y., Wang, S., Sun, C., Pawlik, J., Rutkiewicz, E., Zebrak, J., and Diehl, S. R. (1999). Exclusion of chromosome 7 for Kartagener syndrome but suggestion of linkage in families with other forms of primary ciliary dyskinesia. *Am. J. Hum. Genet*. **64:** 313–318