LETTER TO JMG

Identification of predicted human outer dynein arm genes: candidates for primary ciliary dyskinesia genes

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Background: Primary ciliary dyskinesia (PCD) is a severe inherited disorder characterised by chronic respiratory disease, male infertility, and, in ~50% of affected individuals, a left-right asymmetry defect called situs inversus. PCD is caused by defects in substructures of the ciliary and flagellar axoneme, most commonly loss of the outer dynein arms. Although PCD is believed to involve mutations in many genes, only three have been identified.

Methods: To facilitate discovery of new PCD genes, we have used database searching and analysis to systematically identify the human homologues of proteins associated with the *Chlamydomonas reinhardtii* outer dynein arm, the best characterised outer arm of any species.

Results: We find that 12 out of 14 known *Chlamydomonas* outer arm subunits have one or more likely orthologues in humans. The results predict a total of 24 human genes likely to encode outer dynein arm subunits and associated proteins possibly necessary for outer arm assembly, plus 12 additional closely related human genes likely to encode inner dynein arm subunits.

Conclusion: These genes, which have been located on the human chromosomes for easy comparison with known or suspected PCD loci, are excellent candidates for screening for disease-causing mutations in PCD patients with outer and/or inner dynein arm defects.

Primary ciliary dyskinesia (PCD; MIM 242650), also known as immotile cilia syndrome, is the name given to a group of genetic disorders of motile cilia that includes Kartagener syndrome (MIM 244400).[1] The disease, which affects about one in 20 000 humans,[2] usually involves a defect in a substructure of the "9+2" axonemes of the cilia and flagella (fig 1): patients have been reported with loss of the outer dynein arms, the inner dynein arms, the outer arms and the inner arms, the radial spokes, and the central pair of microtubules. As a result, ciliary and flagellar motility is abnormal, leading to a similar clinical profile that includes chronic or recurrent sinopulmonary infections, infertility in males, and a reversal in the placement of internal organs in about half of affected individuals. PCD is genetically heterogeneous and is likely caused by defects in many different genes; however, to date only three PCD genes have been identified.[3–5]

The discovery of additional genes involved in PCD would be greatly facilitated by more complete information on the human genes that encode axonemal structures. Proteomic analysis of isolated ciliary axonemes from primary cultures of human bronchial epithelial cells has resulted in a list of over 200 potential axonemal proteins,[6] but identification of the defective gene in a patient with PCD would be much simplified by knowledge of the subset of genes necessary for assembly of the specific axonemal substructure that is defective in the patient. Currently, data on the proteins that make up each of the substructures of the axoneme are most complete for the unicellular green alga *Chlamydomonas reinhardtii*, which has been used extensively to study the assembly, composition, and mechanism of action of cilia and flagella. The *Chlamydomonas* cell has two flagella extending from its anterior end that it uses for swimming. Structurally, these flagella are virtually indistinguishable from human airway cilia (fig 1), and biochemical and proteomic studies indicate that most of the axonemal proteins have been highly conserved throughout evolution,[7] so that their human orthologues can be readily identified. Moreover, mutations in *Chlamydomonas* are known that cause loss of each of the major axonemal substructures, thus mimicking nearly all of the ultrastructural defects seen in PCD. As a result, *Chlamydomonas* is an excellent model system for PCD, and information on the genes necessary for assembly of an axonemal structure in *Chlamydomonas* should be directly transferable to humans by identification of the human orthologues of these genes. Indeed, such information provided the basis for discovery, using a candidate gene approach, of the first two PCD genes.[3 4]

The most frequent cause of PCD is loss of the outer dynein arms,[8–10] and in this study we use known *Chlamydomonas* genes involved in assembly of the outer dynein arms to identify their likely human counterparts. Outer dynein arms are tightly bound to one outer doublet microtubule and use the energy of ATP hydrolysis to make transient interactions with an adjacent doublet microtubule to produce force for ciliary beating. The *Chlamydomonas* outer dynein arm is a 1.2 MDa complex composed of three heavy chains (HC), two intermediate chains (IC), and at least nine light chains (LC) (see fig 3). The HCs are the sites of the ATP hydrolysis required for ciliary motility. The ICs bind to the HCs and appear to be involved in regulation of HC activity and attachment of the dynein to the microtubule. The nine LCs are a diverse group of proteins including a Ca^{2+} binding protein, thioredoxin-like molecules, and an extremely highly conserved peptide that is also found in several other motor and non-motor complexes. The outer arm is bound to the doublet microtubule by a distinct structure termed the docking complex (DC), which is essential for outer arm assembly and contains two coiled-coil proteins and an EF-hand protein similar to calmodulin. An additional, less well characterised complex that includes a protein called ODA5 and an associated adenylate kinase (AK) also is essential for

Abbreviations: AK, adenylate kinase; DC, docking complex; HC, heavy chains, IC, intermediate chains, LC, light chains; PCD, primary ciliary dyskinesia

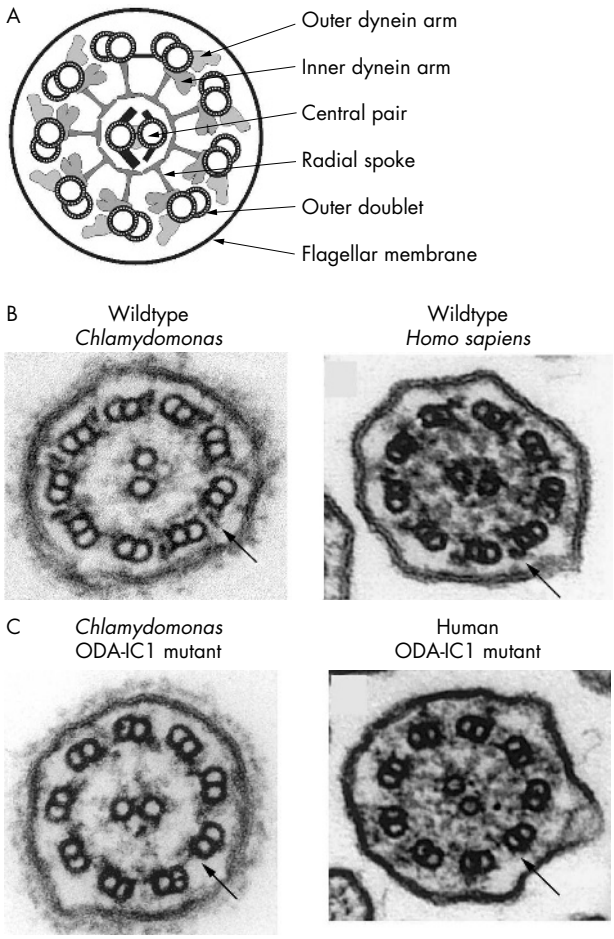

Figure 1 The ''9+2'' axoneme is structurally similar in *Chlamydomonas* and humans. (A) Schematic cross section of a 9+2 cilium showing the major axonemal substructures. (B, C) Cross sections of wildtype (B) and mutant (C) cilia from *Chlamydomonas* and humans. An outer dynein arm in each wildtype cilium is marked with an arrow. In the mutants of both species, a defect in the gene encoding the IC1 subunit of the outer arm dynein results in loss of the outer arms (arrows). The *Chlamydomonas* images are from Wilkerson *et al*,[24] and the human images are from Pennarun *et al*[3] and are used with permission.

outer arm assembly. All of these subunits have been cloned and sequenced (table 1). Importantly, mutational analysis indicates that defects in most of the above proteins, even including the LCs, can cause loss of the outer dynein arms in *Chlamydomonas* (table 1). Therefore, the human genes encoding orthologues of all of these proteins are candidates for causing PCD in those human patients with outer dynein arm defects. In addition to identifying these potential orthologues, we also place the outer dynein arm loci on the human genetic map for easy comparison with known or suspected PCD loci.

The nomenclature of dynein genes is complicated and convoluted with multiple names given to the same sequence and the same names given to different sequences. This has produced a situation where it is difficult for even an expert in the field to follow the literature. To overcome this problem, we have used the official names given to the genes by the HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/) and provided RefSeq numbers for the proteins (in the text) and corresponding mRNAs (in the tables) whenever possible. We have also extensively documented GenBank accession numbers (acc. no.) and NCBI GeneID tags throughout the document. Since even this may still leave uncertainty, we have also included the predicted peptide sequence encoded by each of the loci in the supplemental material (available at http://www.jmedgenet.com/supplemental).

Table 1 Known *Chlamydomonas* outer dynein arm subunits and associated proteins

Protein	Mutant gene	Mutant phenotype	Reference
Heavy chains			
DHCα	*oda11*	Loss of part of outer arm	15, 17, 18, 64
DHCβ	*oda4*	Loss of outer arm	14, 15, 17, 65
DHCγ	*oda2*	Loss of outer arm	15, 16
Intermediate chains			
IC1	*oda9*	Loss of outer arm	15, 24
IC2	*oda6*	Loss of outer arm	15, 23
Light chains			
LC1		Unknown	27
LC2	*oda12*	Loss of outer arm	28, 29
LC3		Unknown	34
LC4		Unknown	40
LC5		Unknown	34
LC6	*oda13*	No structural defect; slightly slow swimming	45
LC7a	*oda15*	Loss of outer arm	44, 45
LC7b		Unknown	46
LC8	*fla14*	Loss of outer arm+others	41, 43
Docking complex			
DC1	*oda3*	Loss of outer arm	15, 48
DC2	*oda1*	Loss of outer arm	15, 49
DC3	*oda14*	Loss of most outer arms	50
Associated proteins			
ODA5	*oda5*	Loss of outer arm	15, 51
ODA5-AK		Unknown	51

METHODS

Bioinformatics

BLAST searches were performed using the NCBI BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple sequences were aligned with ClustalW[11] and phylogenetic trees were drawn with NJPlot.[12] Human genome positions were identified using the NCBI human genome BLAST server (http://www.ncbi.nlm.nih.gov/genome/seq/HsBlast.html) and the Golden Path web server at the University of California at Santa Cruz (http://genome.ucsc.edu).

RESULTS

HC genes

Dynein HCs are large ~450 kDa proteins that convert the energy of ATP to force. These proteins have an N-terminal tail connected to a C-terminal globular head. The N-terminal tail binds ICs and most LCs and is thought to be involved in binding the dynein motor to microtubules or cargo in an ATP insensitive manner. The globular head is made up of six AAA domains organised in a hexameric ring with a short extension protruding from the ring that is postulated to bind microtubules in an ATP sensitive manner.[13] The *Chlamydomonas* outer dynein arm is composed of three HCs termed DHCα (Q39610), DHCβ (Q39565), and DHCγ (Q39575). Mutations in the *ODA4* and *ODA2* genes, which encode DHCβ and DHCγ, respectively, block assembly of the entire outer arm complex and cause an approximately two thirds reduction in swimming speed.[14–17] Cells carrying a mutation in *ODA11*, which encodes DHCα, assemble an outer dynein arm that lacks DHCα and the DHCα associated LC5. These cells swim only slightly slower than normal.[18] The number of HCs in flagellar outer arm dyneins varies with species. Like *Chlamydomonas*, *Tetrahymena* has three distinct HCs, while sea urchins have two HCs.[19] The organisation of human outer arm dynein has not been examined, but in two

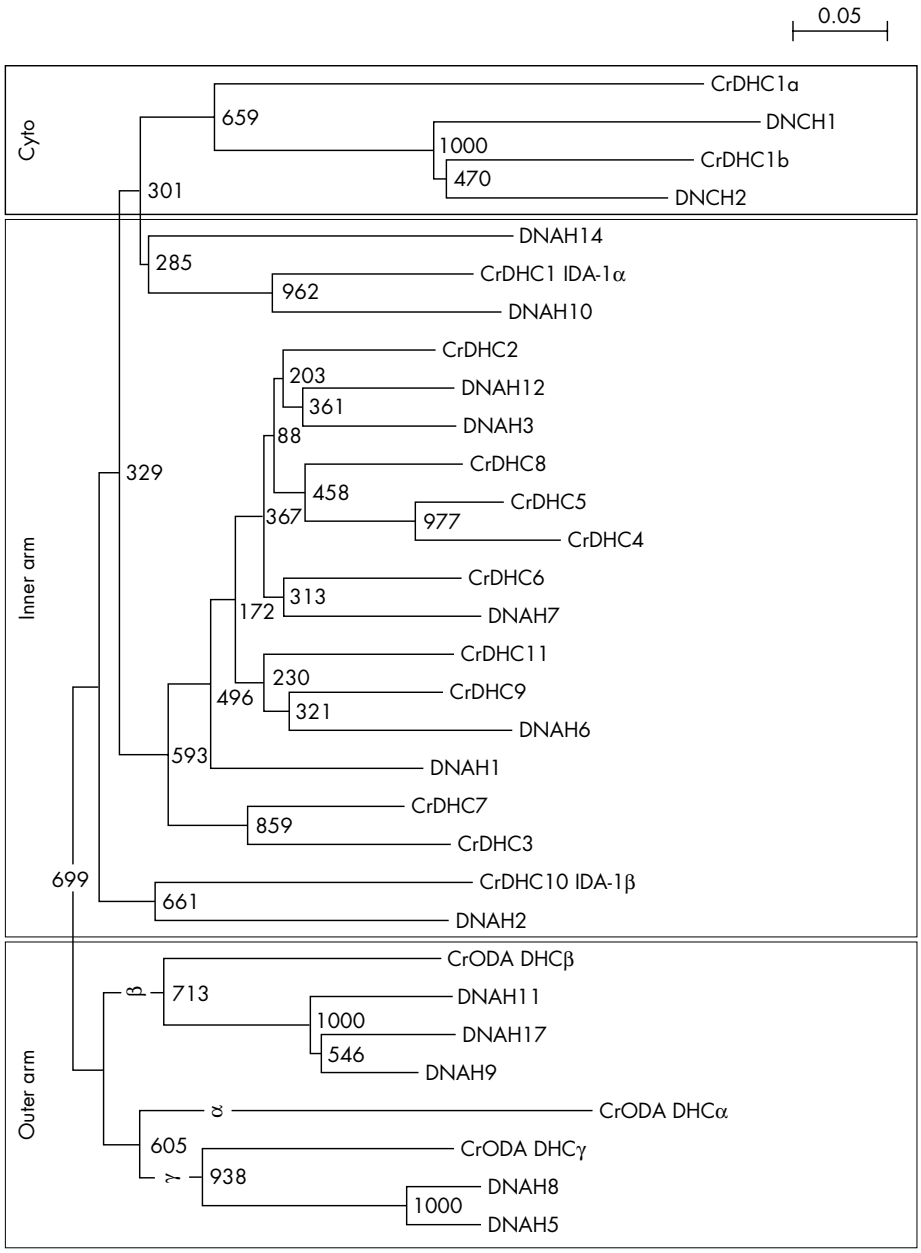

Figure 2 Phylogenetic tree showing the relationship between the dynein HCs of *Chlamydomonas* and humans. An ~100 amino acid peptide spanning the region from GPAG to FITMNP near the first P-loop of each of the dyneins was aligned by ClustalW and developed into a phylogenetic tree. The numbers show the bootstrap values from 1000 iterations. The outer dynein arm γ and β branches of the tree are marked near the bottom of the tree.

other vertebrates, trout and pigs, the outer arm appears to be composed of two HCs.[20 21]

The human dynein HC genes were identified by BLAST searching the non-redundant database with protein and nucleotide sequence from the region surrounding the first nucleotide binding P-loop of dynein. This region has been cloned from many species and is adequate to identify different dynein isotypes. Searches were initiated using *Chlamydomonas* ODA-DHCβ and repeated using sequences identified by this search until no new sequences were identified. Sixty three supposedly human dynein HC sequences were found. This list is highly redundant, as multiple laboratories have identified many of the dynein HC sequences independently.

Each of the 63 sequences was used to search the human genomic sequence using the NCBI human genome BLAST server. All the sequences except four (Z83804, Z83799, U61737, and Z83803) could be located in the human genomic sequence. These four are also missing from the Celera public human genome sequence, suggesting that they may not be of human origin. Supporting this idea, Z83804 and Z83799 are similar to mouse sequences, U61737 is similar to a rat sequence, and Z83803 is similar to a *Drosophila hydei* sequence.

The remaining 59 sequences aligned to 15 distinct genomic loci (tables 2–4). No additional dynein HC-like sequences were found in the genome. Some of the dynein cDNA sequences showed small polymorphisms with the genomic sequence. These probably reflect variation within the human population, or errors generated by PCR amplification of the cDNA when the HCs were originally cloned.

Sequences corresponding to the highly conserved dynein HC domain GPAGTGKT…FITMNP encoded by each of the

Table 2 Human outer dynein arm heavy chain loci

Chlamydomonas protein	NCBI acc. no.	Symbol*	NCBI gene ID	Location
ODA DHCγ	NM_001369	DNAH5	1767	5p15.2
	U61735	Dnahc5		
	AY049075	DNAH5		
	AY045575	DNAH5		
ODA DHCγ	NM_001371	DNAH8	1769	6p21
	AF356519	DNAH8		
	Z83806	hdhc9		
	AL034345	DNAH		
	AJ132090	DNAH5		
	AJ132091	DNAH8		
ODA DHCβ	NM_003777	DNAH11	8701	7p21
	AJ320497	DNAH11		
	AJ132087	DNAH11		
	U83569	Dnahc11		
ODA DHCβ	NM_003727	DNAH17	8632	17q25
	AJ000522	DNEL2		
	AL122077	DNEL2		
	AL832652			
ODA DHCβ	NM_001372	DNAH9	1770	17p12
	AJ404468	DNAH9		
	U61740	Dnahc9		
	AJ132088	DNAH9		
	AF015265			
	X99947			
	AF257737	DNAH9		
	NM_004662	DNAH17L		

**Homo sapiens* official gene symbols (http://www.gene.ucl.ac.uk/nomenclature/) are underlined.

human genomic loci and by each of the known *Chlamydomonas* dynein HC genes were aligned by ClustalW. If the published peptides did not span the sequence, the sequence was extended using genomic sequence from the region to predict the missing amino acids. The alignment was used to draw a phylogenetic tree (fig 2). There are five human genes that encode proteins similar to *Chlamydomonas* DHCβ and DHCγ (fig 2, table 2). Three of these proteins, DNAH11 (NP_003768), DNAH17 (NP_003718), and DNAH9 (NP_001363), encoded at 7p21, 17q25, and 17p12, are most similar to *Chlamydomonas* DHCβ.[17] DNAH5 (NP_001360) and DNAH8 (NP_001362), from 5p15 and 6p21, are most similar to *Chlamydomonas* DHCγ.[16] Humans do not appear to have an orthologue of *Chlamydomonas* DHCα.

Eight human genes encode inner arm dynein HCs (table 3) and are tabulated here because they are candidates for causing PCD in patients with defects in the inner dynein arm. Among the inner arm dynein HCs, DNAH10 and DNAH2 correspond to DHC1α and DHC1β of the *Chlamydomonas* inner arm dynein 1. This inner arm also contains the ICs IC140 and IC138 (see below) as well as at least two LCs.

A single human gene encodes each of the cytoplasmic dynein HCs DHC1 and DHC2 (table 4). DHC1 is the conventional cytoplasmic dynein HC isoform and corresponds to DHC1a in *Chlamydomonas*. DHC2, the orthologue of *Chlamydomonas* DHC1b, is involved in the process of intraflagellar transport, which is necessary for assembly of cilia and flagella.

IC genes

The outer dynein arm ICs are WD-repeat proteins that are part of a family of proteins that includes cytoplasmic and inner dynein arm ICs (table 5). *Chlamydomonas* IC1 (formerly IC78 or IC80) (Q39578) binds to α-tubulin and is thought to be involved in binding the base of the outer dynein arm to the flagellar microtubule.[22] *Chlamydomonas* IC2 (formerly IC69 or IC70) (P27766) is thought to be involved in regulation of the motor activity of the outer dynein arm.[23] Defects in the *ODA*9 and *ODA*6 genes, which encode *Chlamydomonas* IC1 and IC2,

Table 3 Human inner dynein arm heavy chain loci

Chlamydomonas protein*	NCBI acc. no.	Symbol**	NCBI gene ID	Location
NI		DNAH14	1772	1q42.12
	U61741	Dnahc14		
NI	NM_018897	DNAH7	56171	2q33.1
	AF327442	DNAH7		
	Z83801	hdhc2		
	AB023161	KIAA0944		
	AJ132084	DNAH7		
	AJ132093	DNAH7		
NI	NM_015512	DNAH1	1764	3p21
	AJ132083	DNAH1		
	U83571	Dnahc1		
	AB037831	KIAA1410		
	U61738	Dnahc1		
	AJ132094	DNAH1		
NI		DNAH12	8679	3p21.1
	Z83802	hdhc3		
	U83573	Dnahc3		
	U53532	DHC3		
	U61739	Dnahc3		
IDA HC1α	NM_207437	DNAH10	196385	12q24.31
NI	NM_017539	DNAH3	55567	16p12
	AF494040	DNAH3		
	Z83805	hdhc8		
	U83574	Dnahc3b		
	AJ132085	DNAH3		
	AJ132092	DNAH3		
IDA HC1β		DNAH2	1765	17p13
NI	U83570	Dnahc2		
		DNAH6	1768	2p11.2
	XM_211702	LOC284945		
	U61736	Dnahc6		
	AJ132086	DNAH6		

**NI, not identified.*
***H sapiens* official gene symbols (http://www.gene.ucl.ac.uk/nomenclature/) are underlined.

respectively, block assembly of the outer dynein arm; as a result, the cells swim at about one third normal speed.[15 23 24]

Pennarun *et al*[3] identified DNAI1 (NP_036276) as the human orthologue of *Chlamydomonas* IC1 (Q39578). The *Chlamydomonas* and human proteins are 43% identical and have a BLAST E value of 1e-150. Pennarun *et al*[25] and Bartoloni *et al*[26] identified DNAI2 (NP_075462) as the human orthologue of *Chlamydomonas* IC2 (P27766). The *Chlamydomonas* and human proteins are 48% identical and the comparison has a BLAST E value of 1e-154.

Searching the human genomic sequence with *Chlamydomonas* IC1 and IC2, or human DNAI1 and DNAI2

Table 4 Human cytoplasmic dynein heavy chain loci

Chlamydomonas protein	NCBI acc. no.	Symbol*	NCBI gene ID	Location
DHC1a	NM_001376	DNCH1	1778	14q32
	U53530	DHC1		
	L23958	DHC1 (p22)		
	AB002323	KIAA0325		
	AL833600			
DHC1b	XM_370652	DNCH2	1779	11q21-q22.1
	Z83800	hdhc11		
	U20552	DYH1B		
	U53531	DHC2		

**H sapiens* official gene symbols (http://www.gene.ucl.ac.uk/nomenclature/) are underlined.

Table 5 Human dynein intermediate chain genes

NCBI acc. no.	Symbol*	GeneID	NCBI genomic location	Statistics**	Notes
Outer arm					
NM_012144	DNAI1	27019	9p21-p13	43%/658aa/1e-147/IC1	Expressed
NM_023036 AJ295276 AF250288	DNAI2	64446	17q25	48%/556aa/1e-153/IC2	Expressed
Inner arm					
NM_024763 AK026782 AL133617	WDR78	79819	1p31.3	35%/457aa/1e-72/IC138	Expressed
NM_145172 AY049724	WDR63 NYD-SP29	126820	1p22.3	30%/837aa/5e-93/IC140	Expressed
Cytoplasmic					
NM_004411	DNCI1	1780	7q21.3-q22.1		Expressed
NM_001378	DNCI2	1781	2q31.1		Expressed
XM_208483			10q11.23		Has stops, pseudogene?

H sapiens official gene symbols (http://www.gene.ucl.ac.uk/nomenclature/) are underlined.
**Percent identity/number of residues aligned/BLAST E value/*Chlamydomonas* protein used for comparison.

identified four regions of significant homology on chromosomes 1, 9, 10, and 17. Reiteratively searching identified additional loci on chromosomes 1, 2, and 7. The locus at chromosome 9p21-p13 encodes outer arm IC DNAI1, while the locus at 17q25 encodes outer arm IC DNAI2. The loci at 7q21 and 2q31 encode cytoplasmic dynein ICs DNCI1 and DNCI2, respectively. The locus at chromosome 10q11 encodes a relatively uncharacterised region of sequence (XM_208483) that at the peptide level is almost identical to cytoplasmic dynein intermediate chain DNCI2. This locus is likely a pseudogene derived from integration of a DNCI2 cDNA as it is almost continuous with the DNCI2 cDNA sequence and has a stop codon within the predicted coding region. The locus at chromosome 1p31 encodes a novel protein that is similar to dynein ICs. There are two cDNA clones from this region: AK026782 appears to be full length while AL133617 is from the 3′ end of the gene. The peptide encoded by AL133617 should be included within the protein encoded by the longer AK026782 cDNA but is not due to a frame shift in AK026782. This protein has now been curated as WDR78. The locus at 1p22 encodes a testis expressed protein called WDR63.

Phylogenetic analysis (not shown) supports the published assignment of DNAI1 and DNAI2 as outer arm dynein ICs and DNCI1 and DNCI2 as cytoplasmic dynein ICs. The presumed pseudogene XM_208483 also groups with the cytoplasmic dynein ICs. The remaining two human sequences, WDR63 and WDR78, group with *Chlamydomonas* inner dynein arm IC140 (AAD45352) and IC138 (AAU93505), suggesting that they are subunits of the inner dynein arm.

LC genes

LC1

Chlamydomonas outer dynein arm LC1 (AAD41040) is a leucine rich repeat protein. No mutations in the gene encoding LC1 are known to exist in *Chlamydomonas*, so the importance of this LC to arm structure is unknown.

Benashski and colleagues[27] identified EST AA923426 as a potential human orthologue. This EST has now been curated as a hypothetical protein C14orf168 (table 6). Searching the human genomic sequence with *Chlamydomonas* LC1 or human C14orf168 identified three regions of homology on chromosomes 14, 2, and 12. The locus at 14q24 encodes C14orf168. The locus on chromosome 2 (Entrez Gene reports the locus at 15q26) encodes a small nuclear ribonucleoprotein polypeptide A′ (NP_003081), whereas the locus at 12q21 encodes a glycoprotein called lumican (NP_002336). *Chlamydomonas* LC1 and human C14orf168 are 55% identical and have BLAST E values of 3e-38, supporting the idea that they are orthologues. The BLAST E values for comparisons between *Chlamydomonas* LC1 and the ribonucleoprotein or lumican are relatively poor (3e-06 and 7e-05), indicating that they are not likely to be orthologues.

LC2

Chlamydomonas LC2 (AAB58383) is homologous to mouse TCTEX2 and is very distantly related to TCTEX1.[28] Murine TCTEX1 and TCTEX2 are encoded within the *t* complex. This region of chromosome 17 is involved in an extreme form of meiotic drive whereby in +/*t* males the *t* version of chromosome 17 is transmitted to >90% of the offspring. The meiotic drive is thought to be the result of differential phosphorylation of flagellar dynein affecting sperm motility.[28] Mutations in *oda12*, which encodes LC2 in *Chlamydomonas*, block outer dynein arm assembly.[29] A second *Chlamydomonas* LC2-like protein called Tctex2b (DAA05278) was recently found associated with the inner dynein arm. Mutant analysis indicated that the protein is not required for inner arm assembly but is required for arm function as cells lacking this protein swam more slowly than normal.[30]

Searching the human genome database with *Chlamydomonas* LC2 identified two regions of homology on chromosome 1 and additional regions of homology on chromosomes 3 and 6 (table 7). The loci at 1p31 and 3q29 encode poorly characterised proteins called FLJ40873 (NP_689878) and MGC33212 (NP_689986), while the locus at 6q27 encodes a protein called TCTE3 (NP_777570). The locus at 1p34.1 has been predicted to encode an uncharacterised

Table 6 Human dynein light chain 1 loci

NCBI acc. no.	Symbol*	GeneID	NCBI genomic location	Statistics**	Notes
NM_031427	C14orf168	83544	14q24.3	55%/154aa/3e-38/LC1	Expressed

H sapiens official gene symbol (http://www.gene.ucl.ac.uk/nomenclature/).
**Percent identity/number of residues aligned/BLAST E value/*Chlamydomonas* protein used for comparison.

Table 7 Human dynein light chain 2 loci

NCBI acc. no.	Symbol*	GeneID	NCBI genomic location	Statistics**	Notes
Tctex2					
NM_152665	FLJ40873	200132	1p31.3	34%/123aa/2e-14/LC2 29%/113aa/4e-09/Tctex2b	Expressed
NM_174910	TCTE3	6991	6q27	39%/108aa/1e-15/LC2 26%/115aa/2e-07/Tctex2b	Likely outer arm, expressed
NM_152773	MGC33212	255758	3q29	34%/119aa/7e-20/LC2 49%/114aa/9e-26/Tctex2b	Likely inner arm, expressed
NM_001013632	LOC343521	343521	1p34.1	32%/108aa/8e-13/LC2 31%/115aa/2e-12/Tctex2b	Expressed
TcTex1					
NM_006520	TCTE1L	6990	Xp21	45%/114aa/4e-27/Tctex1	Expressed
NM_006519	TCTEL1	6993	6q25.2-q25.3	62%/108aa/2e-37/Tctex1	Expressed
XM_293797			4p16.1	56%/108aa/8e-29/Tctex1	No ESTs
Hs20_11544			20p11	40%/114aa/5e-21/Tctex1	No ESTs
Hs2_5236			2q21.1	47%/68aa/2e-13/Tctex1	No ESTs
Hs17_10875			17p11-p12	56%/106aa/3e-28/Tctex1	Has stops, no ESTs

*_H sapiens_ official gene symbols (http://www.gene.ucl.ac.uk/nomenclature/) are underlined.
**Percent identity/number of residues aligned/BLAST E value/_Chlamydomonas_ protein used for comparison.

protein called LOC343521 (NP_001013654). This locus and the other three appear to be expressed, as there are EST sequences corresponding to all four loci in the databank. These four proteins group phylogenetically with *Chlamydomonas* LC2 and Tctex2b, as well as with a sea urchin homologue of LC2 (BAA24185) (data not shown). TCTE3 groups closely with *Chlamydomonas* LC2 and thus is assigned to the outer dynein arm; MGC33212 groups closely with the *Chlamydomonas* Tctex2b and thus is assigned to the inner dynein arm. The trees do not definitively group FLJ40873 and LOC343521 with either the outer or the inner dynein arm LC2s; nevertheless, they should be considered candidates for PCD genes that map to chromosome 1p31-34.

Tctex1 is not found in the *Chlamydomonas* flagellar outer dynein arm but is found in the inner dynein arm of the *Chlamydomonas* flagellum[31] and the outer dynein arm of the sea urchin flagellum.[32] It is not known if TCTEX1 is a subunit of human outer arm dynein, but since it is found in the sea urchin outer dynein arm, database searches were carried out to identify the human homologues. Six Tctex1-like sequences are encoded by the human genome on chromosomes X, 6, 17, 4, 20, and 2 (table 7). The loci at 6q25 and Xp21 encode proteins TCTEL1 (NP_006510) and TCTE1L (NP_006511), respectively. The gene encoding the latter protein was considered a candidate for the retinitis pigmentosa 3 gene and is sometimes referred to as RP3. However, it is now known that the real RP3 gene encodes the retinitis pigmentosa GTPase regulator.[33] The other four genomic loci do not appear to be expressed at a significant level, as these sequences could not be found in the protein or EST databanks. The locus on chromosome 17 is likely to be a pseudogene as there are stop codons within the predicted coding region.

LC3 and LC5

Chlamydomonas LC3 (Q39592) and LC5 (AAB03681) are members of the thioredoxin family.[34] Searching the databanks with these proteins identified the sea urchin outer dynein arm IC1 protein (BAA09934), *Ciona* dynein intermediate chain 3 (BAB68388), human TXNDC3 (also known as NM23-H8) (NP_057700), human TXNDC6 (also known as thioredoxin-like 2 TXL-2) (NP_835231), and a large number of thioredoxin-like proteins. The sea urchin and *Ciona* dynein ICs and the two human proteins are composed of both thioredoxin-like domains and a nucleoside diphosphate kinase-like domain.[35 36] No mutations in the *Chlamydomonas* genes encoding LC3 or LC5 have been found so it not known how mutations in these genes will affect assembly of the outer dynein arm.

Searching the human genomic sequence with *Chlamydomonas* LC3 and LC5 and the LC3/5-like domain of TXNDC3 identified four regions with homology on chromosomes 3, 4, 7, and 18. These four regions were each used to search the protein database to determine which thioredoxin-like protein is encoded at that region. The locus on chromosome 4 encodes a widely expressed isoform of thioredoxin termed TXN (NP_003320), while the locus on chromosome 18 encodes a sperm specific isoform of thioredoxin termed TXNDC2, also known as Sptrx-2[37] (NP_115619), that has been localised to the fibrous sheath of the sperm flagellum.[38] The locus on chromosome 7 encodes TXNDC3 whereas the locus on chromosome 3 encodes TXNDC6, which is expressed in ciliated cells and binds microtubules[39] (table 8). Phylogenetic analysis (using the thioredoxin domains) suggests that human TXNDC3 and TXNDC6 are outer dynein arm subunits as only these proteins group with the known *Chlamydomonas* and sea urchin outer arm polypeptides in a tree containing thioredoxin-like molecules (data not shown).

LC4

Chlamydomonas LC4 (Q39584) is a calcium binding EF-hand protein.[40] No mutations in the gene encoding LC4 are known in *Chlamydomonas*.

Searching the human genomic sequence with *Chlamydomonas* LC4 identified 14 regions of homology ($E<0.001$). The top two matches are located on chromosomes 18 and 10. The locus on chromosome 18 encodes centrin-1 (NP_004057), while the locus on chromosome 10 encodes

Table 8 Dynein light chain 3 and 5 loci

NCBI acc. no.	Symbol*	GeneID	NCBI genomic location	Statistics**	Notes
NM_016616	TXNDC3	51314	7p14.1	42%/104aa/2e-16/LC5	Expressed
NM_178130	TXNDC6	347736	3q22	40%/83aa/8e-11/LC5	Expressed

*_H sapiens_ official gene symbol (http://www.gene.ucl.ac.uk/nomenclature/).
**Percent identity/number of residues aligned/BLAST E value/_Chlamydomonas_ protein used for comparison.

Table 9 Dynein light chain 6 and 8 loci

NCBI acc. no.	Symbol*	GeneID	NCBI genomic location	Statistics**	Notes
NM_003746	DNCL1	8655	12q24.23	92%/85aa/2e-41/LC8	Expressed
NM_005740	DNAL4	10126	22q13.1	24%/111aa/0.01/LC6	Expressed
NM_080677	DLC2	140735	17q23.2	94%/85aa/3e-42/LC8	Expressed
Hs1_77990			1p36.13	82%/85aa/2e-36/LC8	No ESTs
Hs7_8090			7q21.12	79%/44aa/2e-11/LC8	No ESTs
Hs4_16510			4q23	70%/85aa/1e-25/LC8	Has stop codons, no ESTs
Hs14_26604	LOC390498	390498	14q31.1	79%/84aa/1e-32/LC8	Has stop codons, no ESTs
Hs14_26604	LOC246720	246720	14q31.1	83%/85aa/4e-36/LC8	No ESTs
Hs3_5769			3q25	63%/82aa/2e-23/LC8	Has stop codons, no ESTs

**H sapiens* official gene symbols (http://www.gene.ucl.ac.uk/nomenclature/) are underlined.
**Percent identity/number of residues aligned/BLAST E value/*Chlamydomonas* protein used for comparison.

calmodulin-3 (NP_005176). It is possible that the human orthologue of LC4 has diverged to the point that it is not readily recognisable above a background of small EF-hand proteins, that the human outer arm lacks an LC4 orthologue, or that centrin-1 or calmodulin-3 perform the functional role of LC4 in the human outer arm.

LC6 and LC8

Chlamydomonas LC6 (Q39579) and LC8 (Q39580) are members of a highly conserved class of dynein LCs. LC8 is one of the most highly conserved proteins known. *Chlamydomonas* LC8 is 92% identical to human LC8.[41] In addition to being a component of the outer dynein arm, LC8 has been found associated with inner arm dynein, myosin V, and cytoplasmic dynein, along with numerous other non-motor proteins including radial spokes, nitric oxide synthetase, and BIM death activator proteins.[42] Mutations in the *Chlamydomonas FLA14* gene, which encodes LC8, result in multiple flagellar defects, including lack of outer and inner arm dyneins and radial spokes, and loss of retrograde intraflagellar transport.[43] Deletion of the gene encoding *Chlamydomonas* LC6 (*ODA13*) does not prevent assembly of the outer dynein arm and has very little or no effect on swimming (Pazour and Witman, unpublished results).

Searching the human genomic databank with *Chlamydomonas* LC8 and LC6 identified two regions of similarity on chromosome 14 and single regions on chromosomes 1, 3, 4, 7, 12, 17, and 22 (table 9). Phylogenetic analysis indicates that DNAL4 (NP_005731) groups with LC6, while all the others group with LC8 (data not shown). Within the LC8 group, DLC2 (NP_542408) is most closely related to *Chlamydomonas* LC8.

Each of the chromosomal regions was searched against the EST databank to determine if the genes were expressed. Three of these loci, 14q31, 4q23, and 3q25, have stop codons within the predicted open reading frames, suggesting that they are pseudogenes. Consistent with this, they are not found in the EST database. Surprisingly, even though the other six sequences are highly conserved, only three of them are represented in the EST databank. These encode DNCL1 (NP_003737) on chromosome 12, DLC2 on chromosome 17, and DNAL4 on chromosome 22. It will be of interest to determine if DLC2 and DNCL1 have similar roles in distinct protein complexes.

LC7

Chlamydomonas LC7a (AAD45881) is a member of the LC7/Roadblock family of proteins.[44] Mutations in the *Chlamydomonas* LC7a gene (*ODA15*) partially block assembly of the outer dynein arm and cause the cells to swim at about one third normal speed[45] (Pazour and Witman, unpublished results). A second LC7/Roadblock family member, named LC7b, was recently found. It appears that both LC7a and LC7b can associate with the outer dynein arm and also with the inner dynein arm I1.[46]

Two groups of human ESTs (HUM424e02b and AA446298) were previously identified that encoded homologues of LC7/Roadblock; these proteins were named ROBL1 and ROBL2.[44] These proteins show differential expression with ROBL2 more highly expressed in testis. Antibodies to ROBL2 label sperm flagella, consistent with interaction with axonemal dynein. Both ROBL1 and ROBL2 appear to interact with cytoplasmic dynein, indicating that these proteins are subunits of multiple dynein isoforms.[47] Screening the human genome sequence with *Chlamydomonas* LC7a, ROBL1, and ROBL2 identified four regions of homology, on chromosomes 20, 16, 12, and 18 (table 10). The locus at chromosome 20q11 encodes ROBL1 and has been curated as protein DNCL2A (NP_054902). The locus at 16q23 encodes ROBL2 and has been curated as protein DNCL2B (NP_570967). The loci on chromosomes 12 and 18 are likely pseudogenes as the regions of homology contain stop codons, and no ESTs corresponding to these genes are in the EST database. Phylogenetic analysis (not shown) did not clearly distinguish the closest interspecies homologues between *Chlamydomonas* LC7A and LC7B and human DNCL2A and DNCL2B; in any case, both DNCL2A and DNCL2B are candidates for being subunits of both human outer arm and inner arm dynein.

Docking complex genes

DC1

Chlamydomonas DC1 is a coiled-coil protein.[48] Defects in the *Chlamydomonas* gene encoding DC1 (*ODA3*) block assembly of the outer dynein arm and the outer dynein arm docking complex, leading to reduced beat frequency and slow swimming.

Searching the databanks with *Chlamydomonas* DC1 (AAC49732) identified only low significance matches to coiled-coil proteins such as cingulin and myosin. Thus, it

Table 10 Dynein light chain 7 loci

NCBI acc. no.	Symbol*	GeneID	NCBI genomic location	Statistics**	Notes
NM_014183	DNCL2A	83658	20q11.21	58%/99aa/1e-25/LC7a	Expressed
NM_130897	DNCL2B	83657	16q23.3	63%/98aa/8e-29/LC7a	Expressed
Hs12_9916			12p13	43%/100aa/7e-15/LC7a	Has stop codons, no ESTs
Hs18_25184			18q21	41%/89aa/7e-12/LC7a	Has stop codons, no ESTs

**H sapiens* official gene symbol (http://www.gene.ucl.ac.uk/nomenclature/).
**Percent identity/number of residues aligned/BLAST E value/*Chlamydomonas* protein used for comparison.

Table 11 Dynein DC2 loci

NCBI acc. no.	Symbol	GeneID	NCBI genomic location	Statistics*	Notes
NM_144577	FLJ32926	93233	19q13.33	25%/488aa/5e-34/DC2	Expressed
NM_152591	FLJ35843	160762	12q24.13	26%/403aa/3e-31/DC2	Expressed

*Percent identity/number of residues aligned/BLAST E value for *Chlamydomonas* DC2 compared to BAB71508+BAB71448 or FLJ35843. *Chlamydomonas* protein used for comparison.

does not appear that there are any potential human orthologues of DC1 in databanks at this time.

DC2

Like DC1, DC2 is a coiled-coil protein[49] and mutation of the *Chlamydomonas* gene (*ODA1*) encoding this protein blocks assembly of the outer dynein arm and outer dynein arm docking complex, leading to slow swimming.

Takada *et al*[49] identified two human proteins (BAB71448 and BAB71508) as potential orthologues of *Chlamydomonas* DC2 and named these HsDC2-1 and HsDC2-2. These two polypeptides are likely to be the N- and C-terminal ends of the same protein; only BAB71508 has been assigned an interim gene symbol (FLJ32926) and RefSeq number (NP_653178). Searching the databanks with *Chlamydomonas* DC2 (AAK72125) or the peptide that results from combining HsDC2-1 and HsDC2-2 identified a locus on chromosome 19 that encodes HsDC2-1 and HsDC2-2 and a locus on chromosome 12 that encodes a hypothetical protein called FLJ35843 (NP_689804) (table 11). FLJ23926 (HsDC2-1+HsDC2-2) is 25% identical to *Chlamydomonas* DC2 over 488 residues (BLAST E value of 5e-34). FLJ35843 is 26% identical to *Chlamydomonas* DC2 over 403 residues (BLAST E value of 3e-31). FLJ32926 and FLJ35843 are only slightly similar to one another (24% identity over 220 amino acids, BLAST E value of 7e-15).

Searching the non-human databases with these sequences identified a number of matches in lower eukaryotes as previously reported[49] but also identified a significant match to an unpublished *Ciona* protein called axonemal p66 (BAB88833) as well as uncharacterised proteins from *Xenopus* and other vertebrates. Interestingly, *Chlamydomonas* DC2 is distantly related to the *Chlamydomonas ODA5* gene product (23% identical over 413 residues, BLAST E value of 9e-22). ODA5 will be discussed below.

DC3

Chlamydomonas DC3 (AAP49435) is an EF-hand protein related to calmodulin and centrin.[50] Searching the human genomic sequence with *Chlamydomonas* DC3 identified two regions of homology (E<0.001) on chromosome 13, and one region on chromosome 18. The two loci on chromosome 13 are most similar to calmodulins but are likely pseudogenes as they contain stop codons within the predicted regions of homology and are not represented by EST clones. The locus on chromosome 18 encodes centrin-1 (NP_004057). Thus, we cannot identify a definitive orthologue of *Chlamydomonas* DC3 in the human genome at this time.

Other genes

All of the proteins known to purify with the *Chlamydomonas* outer dynein arm have been cloned and sequenced and are discussed above. However, genetic analysis indicates that additional genes are required for assembly of the outer dynein arm. One of these, *ODA5*, has been cloned and characterised; its product is associated with an adenylate kinase (AK) and the product of another gene, *ODA10*, which has not been cloned.

ODA5

ODA5 (AAS10183) is a coiled-coil protein that associates with the flagellar axoneme independently of the outer dynein arm and the outer dynein arm docking complex.[51] Mutations in *ODA5* block assembly of the outer arm but not the docking complex; as a result, the mutants swim at approximately one third normal speed.[15 51] The only significant match in the human genome is to the FLJ35843 protein that was identified above as a potential orthologue of *Chlamydomonas* DC2. The match between ODA5 and FLJ35843 (21% identical over 321 residues, BLAST E value of 4e-12) is less significant than the match between DC2 and FLJ35843 (26% identical over 403 residues, BLAST E value of 3e-31), indicating that FLJ35843 is more likely to be orthologous to DC2 than to ODA5.

ODA5 associated AK

Analysis of *oda5* mutant cells identified an AK (AAS10182) that was reduced in the flagella of *oda5* and *oda10* mutant cells.[51] AKs increase the efficiency of ATP utilisation by converting two molecules of ADP to one AMP and one ATP; the resulting ATP can then be used by an ATPase like the outer dynein arm. Because no mutations in the gene encoding this AK have been reported, it is not known if this protein is required for outer arm assembly; however, because it is likely to be part of an axonemal complex (the ODA5/ODA10/AK complex) necessary for outer arm assembly, it is included here.

The human genome encodes multiple isoforms of AK. The *Chlamydomonas* ODA5 associated AK is composed of three AK motifs. No human proteins have this structure, but human AK5-1 and AK5-2 have two AK motifs.[51] Searching the human NR protein and nucleotide databases with the *Chlamydomonas* ODA5 associated AK identified AK1 (NP_000467) encoded on chromosome 9q34.1 and the two isoforms of AK5 (NP_777283, NP_036225) encoded on chromosome 1p31 as the best matches[51] (table 12).

Table 12 ODA5 associated AK loci

NCBI acc. no.	Symbol*	GeneID	NCBI genomic location	Statistics**	Notes
NM_000476	AK1	203	9q34.1	47%/185aa/2e-39/ODA5-AK	Expressed
NM_174858	AK5	26289	1p31	34%/420aa/4e-57/ODA5-AK	Expressed
NM_012093				34%/420aa/4e-57/ODA5-AK	Expressed

**H sapiens* official gene symbol (http://www.gene.ucl.ac.uk/nomenclature/).
**Percent identity/number of residues aligned/BLAST E value/*Chlamydomonas* protein used for comparison.

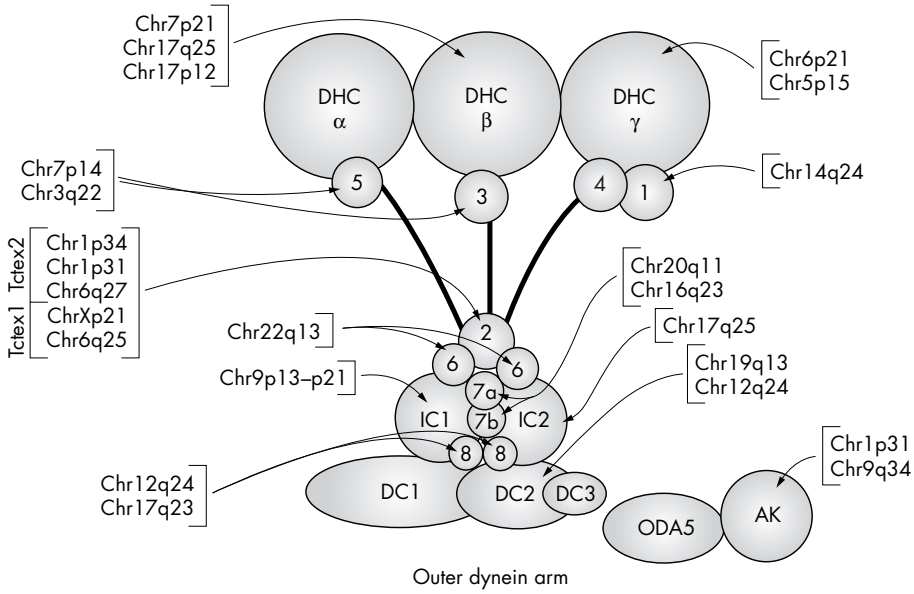

Figure 3 Human loci corresponding to the subunits of the *Chlamydomonas* outer dynein arm. Each of the loci identified in the human genome is linked to the corresponding subunit. Only genes that are likely to be expressed are included.

DISCUSSION

Human orthologues of *Chlamydomonas* outer dynein arm genes

A remarkable finding of the present study is that 12 out of 14 subunits of the *Chlamydomonas* outer dynein arm have readily identifiable homologues in humans (fig 3). This provides further evidence that the proteins as well as the structure of the ''9+2'' axoneme of cilia and flagella have been highly conserved throughout evolution, and confirms the value of *Chlamydomonas* as an excellent model system for elucidating the molecular basis for human diseases that affect cilia and flagella.

Another important finding is that several *Chlamydomonas* outer dynein arm proteins have multiple expressed homologues in humans (figs 3 and 4). This was particularly striking for the HCs: *Chlamydomonas* DHCβ has three close homologues in humans, and *Chlamydomonas* DHCγ has two close homologues. Inasmuch as the HCs are not known to be shared by different dyneins, it is likely that there exist multiple isoforms of outer arm dynein in humans. If some isoforms are expressed specifically in the testis and others in somatic tissues such as the lung, this could explain cases of human patients in which defects in respiratory tract cilia but not sperm flagella, or vice versa, have been reported.[1] In addition, *Chlamydomonas* LC2, LC7a/b, and LC8 each have two or more close homologues in humans. In *Chlamydomonas*, these LCs or their close relatives are subunits of more than one type of dynein: for example, the LC2 relative Tctex1 is a component of inner arm dynein and cytoplasmic dynein,[31 52] LC7a and LC7b are subunits of both inner arm and outer arm dynein,[46 47] and LC8 is a subunit of outer arm dynein, the inner arm I1 dynein, and cytoplasmic dynein.[31 42 53] Thus, it

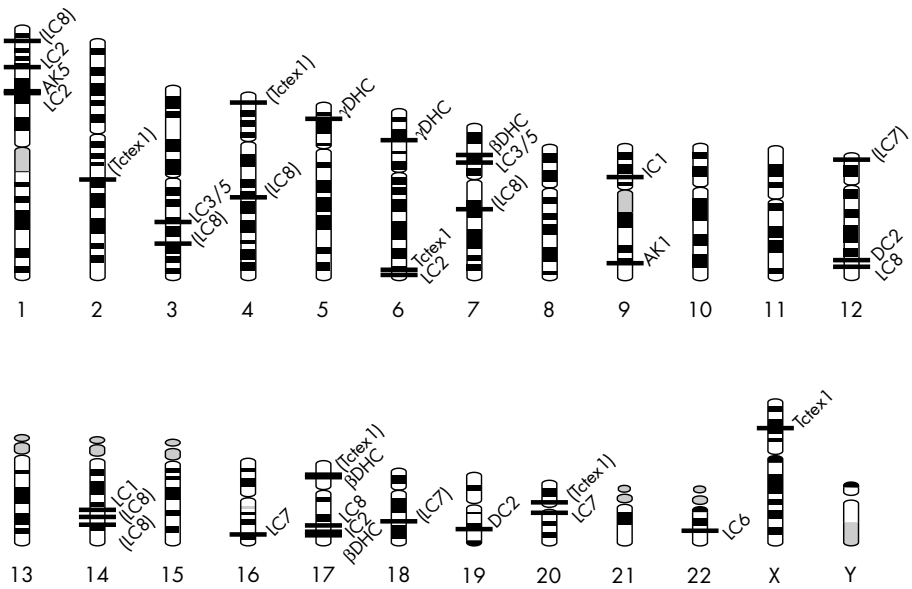

Figure 4 Chromosomal ideograms showing the approximate positions of all loci encoding potential outer dynein arm subunits. Parentheses indicate that the genes at these positions are not expressed and many are likely to be pseudogenes.

Table 13 Outer arm dynein genes screened in human PCD populations

Gene product	Position	Outcome	Reference
Heavy chains			
HCβ DNAH9	17p12	No mutations found	58
HCβ DNAH11	7p21	PCD-causing mutation	5
HCγ DNAH5	5p15	PCD-causing mutations	4
Intermediate chains			
IC1 DNAI1	9p21-p13	PCD-causing mutations	3
		PCD-causing mutations	54
		PCD-causing mutations	55
IC2 DNAI2	17q25	No mutations found	25
		No mutations found	26
Light chains			
LC2 TCTE3	6q27	No mutations found	59
LC8 DLC2	17q23.2	No mutations found	26

will be of interest to determine if the multiple LC homologues in humans represent evolutionary divergence and specialisation for use in different types of dynein, or if they function in different isoforms of outer arm dynein.

Outer dynein arm genes and PCD loci

Including the multiple human homologues of several *Chlamydomonas* outer dynein arm subunits, as well as two possible homologues each of DC2, the outer arm associated AK, and Tctex1, a total of 24 human genes that are potentially involved in outer arm assembly have been identified and mapped on the human genome (fig 4). These genes are good candidates to be screened for nucleotide changes in PCD patients with defects in the outer dynein arms. Three of these genes have already been shown to be defective in PCD patients (table 13). Pennarun *et al*[3] sequenced exons from the human homologue (NCBI GeneID 27019 at 9p13-21) of the *Chlamydomonas* IC1 gene and identified two loss-of-function mutations in a PCD patient lacking outer dynein arms; further studies have led to the identification of additional mutations in this gene in affected individuals.[54 55] Omran *et al*,[56] studying a large family with PCD and absence of outer arms, mapped the PCD gene to a region (5p15-p14) containing a gene encoding one of the homologues of *Chlamydomonas* DHCγ. Olbrich *et al*[4] then sequenced all exons of the candidate gene (NCBI GeneID 1767) in several affected individuals and found several mutations, most of which resulted in premature termination of the HC motor domain. A genome-wide scan of 70 affected individuals from 31 families identified several ''suggestive'' PCD loci[57] (table 14), including one at chromosome 7p15.3-21 in families with dynein arm deficiencies; this region contains a gene encoding one of the outer dynein arm β HC isoforms (NCBI GeneID 8701), which Bartoloni *et al*[5] subsequently showed to contain a nonsense mutation on both chromosomes in a patient with probable Kartagener syndrome but no obvious ultrastructural defects in the axoneme. Similar screens have been carried out on the genes encoding IC2 (GeneID 64446 on 17q25),[25 26] another isoform of the outer dynein arm β HC (GeneID 1765 on 17p13),[58] an isoform of TCTEX1 (GeneID 6991 on 6q27),[59] and an isoform of LC8 (GeneID 140735 on 17q23.2),[26] but did not identify mutations in these genes (table 13); however, failure to find mutations does not exclude the genes from consideration as candidate PCD genes in other PCD families. The work presented here identifies a number of additional candidate genes that should be considered in screening for potential PCD genes. Focusing on homologues that show outer dynein arm assembly phenotypes in *Chlamydomonas* (table 1) is likely to increase the success rate.

Table 14 PCD loci

Defect*	Map position	Gene (if known)	Reference
Identified by candidate gene approach			
ODA	9p21-p13	IC1 DNAI1	3, 54, 55
ODA	5p15	HCγ DNAH5	4
Normal arms, situs inversus	7p21	HCβ DNAH11	5
Based on familial studies			
ODA	5p15	HCγ DNAH5	56
ODA	16p12.1-12.2		60
ODA	19q13-19qter		61
IDA	15q13.1-15.1		60
IDA	X		62
ND	7		66
Based on genome-wide scan			57
Defect†	Suggestive loci		
All PCD	4q32		
All PCD	5p14		
All PCD, SI, DAD	8q22		
All PCD, DAD	16pter		
SI, DAD	19q13		
	Potential loci		
All PCD	3p		
SI	3p		
DAD	7p		
All PCD	10p		
SI	11q		
SI	13q		
All PCD	15q		
SI, DAD	17q		

*IDA, inner dynein arm deficiency; ODA, outer dynein arm deficiency; ND, not determined.
†Results for all 31 PCD families (All PCD) and subgroups of PCD families with situs inversus (SI) or outer and/or inner dynein arm deficiency (DAD).[57]

In addition to the three PCD genes noted above, numerous other potential PCD loci have been identified (table 14). The use of large families and isolated populations has identified PCD loci at 15q13-q15, 16p12,[60] 19q13-qter[61] and X.[62] The loci on chromosomes 16 and 19 are associated with outer dynein arm defects, whereas the patients with mutations on chromosomes 15 and X have defects in inner dynein arms. Our work has not identified any outer dynein arm genes at 16p12 that would be candidates for a PCD gene. An outer dynein arm docking complex protein is encoded at 19q13 but apparently lies just outside of the critical region (H Mitchison, personal communication). The locus on the X chromosome was found in a large Polish family that had retinitis pigmentosa associated with PCD.[62] Interestingly, there is an isoform of TCTEX1 encoded on the X chromosome. Since *Chlamydomonas* Tctex1 is a subunit of inner arm dynein[31] and a TCTEX1 subunit has been implicated in rhodopsin transport in retina,[63] this gene should be considered a potential candidate in these patients.

The genome wide scan of Blouin *et al*[57] identified ''suggestive'' PCD loci on 4q, 5p, 8q, 16p, and 19q with ''potential, interesting'' loci at 3p, 7p, 10p, 11q, 13q, 15q, and 17q; about half of the families in this study had defects in outer and/or inner dynein arms, whereas the other half had other abnormalities in ciliary structure or no apparent abnormalities. As noted above, the β HC, IC2, and LC8 genes on chromosome 17q subsequently were examined in some of these families and other PCD patients, and no mutations were found. The locus at 3p was identified by linkage analysis using all PCD families; therefore, the inner dynein arm HC genes *DNAH1* and *DNAH12* at 3p21 and 3p21.1 should be considered candidates for this potential PCD locus. Blouin *et al*[57] identified 16pter as a suggestive PCD locus based on analysis of families with outer and/or inner dynein arm deficiencies; although no outer dynein arm genes map to

chromosome 16, the inner arm HC gene *DNAH3* at 16p12 may be a candidate for this potential PCD locus if the defect involves loss of inner dynein arms.

There are potential PCD loci that do not correlate with chromosomal regions containing presumed outer dynein arm genes. However, there are additional, uncharacterised genes necessary for outer arm assembly in *Chlamydomonas*[51]; cloning and sequencing of these genes probably will provide additional candidate PCD genes in humans, some of which may map to the PCD loci that are not currently correlated with outer dynein arm genes. In addition, some of the PCD families used in the linkage studies of Blouin *et al*[57] had defects in axonemal substructures other than the outer dynein arm; an analysis of *Chlamydomonas* radial spoke proteins (Yang *et al*, in preparation) and a proteomic analysis of the *Chlamydomonas* flagellum[7] are likely to provide candidate genes for some of these PCD loci.

In summary, we have used information from the model organism *Chlamydomonas*, in which the flagellar outer dynein arm has been extensively characterised, to identify a total of 24 genes that are predicted to encode subunits of this important motor in humans. Fifteen closely related genes that are likely to encode human inner dynein arm subunits are also identified. The resulting genes are excellent candidates for PCD disease genes.

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ELECTRONIC-DATABASE INFORMATION

Supplemental material is available at http://www.jmedgenet.com/supplemental. The following URLs have also been mentioned in this study: HUGO Gene Nomenclature Committee, http://www.gene.ucl.ac.uk/nomenclature/; NCBI BLAST server, http://www.ncbi.nlm.nih.gov/BLAST/; NCBI human genome BLAST server, http://www.ncbi.nlm.nih.gov/genome/seq/HsBlast.html; Golden Path web server at the University of California at Santa Cruz, http://genome.ucsc.edu.

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