Isolation of several human axonemal dynein heavy chain genes: genomic structure of the catalytic site, phylogenetic analysis and chromosomal assignment

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Received 10 June 1997

Abstract Dynein heavy chains (DHCs) are the main components of multisubunit motor ATPase complexes called dyneins. Axonemal dyneins provide the driving force for ciliary and flagellar motility. Recent molecular studies demonstrated that multiple DHC isoforms are produced by separate genes. We describe the isolation of five human axonemal DHC genes. Analysis of the human genomic clones revealed the existence of intronic sequences that were used to demonstrate that human axonemal DHC genes are located on different chromosomes. The cloned human DHC sequences were integrated into an evolutionary approach based on phylogenetic analysis. Tissue expression studies showed that these human axonemal DHCs are expressed in testis and/or trachea, two tissues with axonemal structures that can be altered in primary ciliary dyskinesia, making DHC genes strong candidates in the genesis of these human diseases.

Key words: Dynein; Cilium; Phylogeny; Primary ciliary dyskinesia; Human genomic DNA

1. Introduction

Dynein heavy chains (DHC) are high-molecular-weight (>400 kDa) microtubule-dependent motor ATPases responsible for many types of cell motility. Although they are functionally similar to kinesin and myosin as regards the general mechanism coupling the energy of ATP hydrolysis to the production of mechanical force, they are structurally distinct. DHCs belong to large multisubunit complexes (>1000 kDa), the dyneins, that contain DHCs (400–500 kDa), intermediate chains (45–110 kDa) and light chains (8–55 kDa) [1,2]. Two major classes of dyneins have been identified, the axonemal and cytoplasmic dyneins. The cytoplasmic dynein isoforms are involved in a relatively wide range of intracellular functions, including retrograde vesicle transport, positioning of some organelles (e.g. Golgi apparatus) and chromosome movements on the mitotic spindle [3]. In ciliary and flagellar axonemes, the axonemal dyneins are components of the outer and inner dynein arms attached to the peripheral microtubule doublets. While only two cytoplasmic DHC isoforms have been described in sea urchins, rats and humans, the composition of axonemal dyneins is more complex. Immunological studies and genetic analyses of *Chlamydomonas reinhardtii* mutants with defective flagellar motility suggested the existence of a multigene DHC family [4–9]. This hypothesis has been confirmed by the determination of full-length coding cDNA sequences (about 12000–15000 bp encoding 4000–5000 amino acids) and partial cDNA or genomic sequences encoding cytoplasmic and axonemal DHCs in various species. Regarding axonemal DHCs, three full-length coding sequences have been cloned: the sea urchin β DHC [10,11] and the *Chlamydomonas* β and γ DHCs [12,13]. In addition, several partial axonemal DHC sequences have been identified in *Chlamydomonas* [13,14] *P. tetraurelia* [15], *D. melanogaster* [16], sea urchins [17], rats [18,19] and very recently in humans [20]. Phylogenetic studies suggest that the DHC gene family comprises at least fifteen members, two cytoplasmic DHC genes and at least thirteen axonemal DHC genes [17]. Comparison of the predictive amino acid sequences deduced from complete coding sequences revealed highly conserved polypeptide domains. The central third of DHCs contains the catalytic domain of the protein which is the most highly conserved region with four P-loop consensus motifs involved in nucleotide binding (GXXXXGKT/S/Q) [21].

In humans, axonemal ultrastructural abnormalities have been described in several patients with a congenital respiratory disease known as primary ciliary dyskinesia, which includes Kartagener's syndrome [22]. As defects in axonemal DHCs could therefore underlie those genetic disorders, we designed a cloning strategy, based on the conservation of the first P-loop region (P1-loop), to explore the diversity of the human DHC gene family.

2. Materials and methods

2.1. Alignment of nucleotide sequences and primer design

Fifty-six nucleotide sequences encoding the P1-loop region of various species were aligned by using the ClustalW program [23] with default parameters. We analysed data released from the GenBank data base for cytoplasmic DHCs from rats [24,25], humans [26], *D. discoideum* [27], yeast [28,29], *E. nidulans* [30], *N. crassa* [31], *P. tetraurelia* [15], *C. elegans* [32], *D. melanogaster* [33] and sea urchins [17], and for axonemal DHCs from rats [18], *P. tetraurelia* [15], *C. reinhardtii* [12,13], *D. melanogaster* [16] and sea urchins [17]. The oligonucleotide primers for PCR were designed according to the amino acid sequences corresponding to the most conserved regions of the P1-loop domain among the cytoplasmic or axonemal DHC sequences, and to compensate for the potential mismatches between templates

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Abbreviations: DHC, dynein heavy chain

PII S0014-5793(97)00800-4

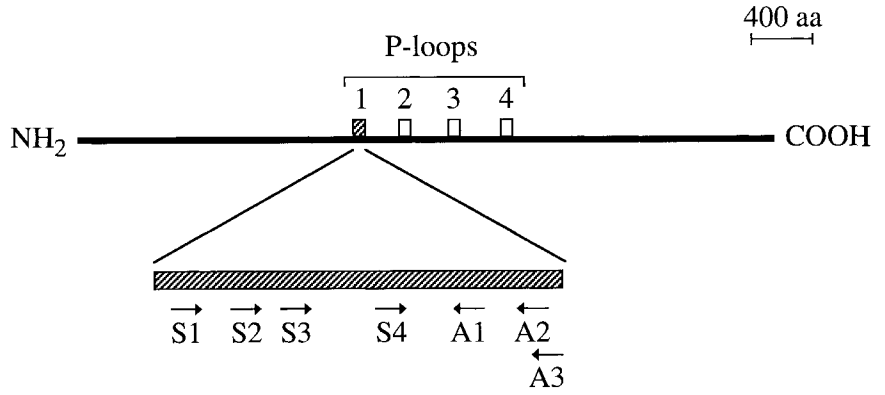

Fig. 1. Schematic representation of the seven primers synthesized for PCR amplification. The four centrally located P-loops are indicated by boxes 1–4 on a whole dynein heavy chain depicted by black bar, box 1 (hatched box) being the catalytic functional P1-loop. Arrows indicate the locations and directions corresponding to the primers (S1–S4 and A1–A3), whose sequences are described in Table 1.

and primers. Four sense (S1, S2, S3 and S4) and three antisense (A1, A2 and A3) degenerate or specific primers were synthesized; their positions and nucleotide sequences are shown in Fig. 1 and Table 1, respectively.

2.2. PCR conditions, subcloning, and sequencing

Human genomic DNA (500 ng) was diluted into a reaction volume of 50 μl containing 100 pmols of each primer, 200 mM each dNTP, 5 μl of 10×PCR buffer and 0.5 unit of Taq polymerase (ATGC Biotechnologies, Noisy-le-Grand, France). After initial denaturation for 5 min at 94°C, amplification conditions were 30 cycles of 1 min at 94°C, 1 min at 58°C and 1.5 min at 72°C, followed by a final extension for 7 min at 72°C. Amplification products were analysed by electrophoresis on a 1% agarose gel (Life Technologies) stained with ethidium bromide. PCR products were subcloned into the vector pTAg by using the LigATor cloning kit following the manufacturer's instructions (R and D systems Europe Ltd, Abingdon, UK). The plasmids were purified using the QIAquick PCR purification kit (Qiagen). Fluorescence sequencing was performed in a Perkin-Elmer Applied Biosystem Model 373A instrument using an ABI PRISM dye terminator cycle sequencing ready reaction kit with AmpliTaq FS DNA polymerase (Perkin Elmer). Each plasmid was sequenced on both strands with M13-20 and M13-RP sequencing primers.

2.3. Chromosomal location by somatic cell hybrid analysis

The chromosomal location of the cloned products was determined by using DNA from NIGMS human X rodent somatic cell hybrid mapping panel 2 (Coriell Institute for Medical Research, Camden, NJ) as template. PCR was performed as described above with primers located in intronic sequences of the different cloned products (Table 1). Amplification products were analysed by electrophoresis on 4% agarose gels (2% agarose/2%Nusieve) (Life Technologies and FMC BioProducts, Rockland, ME) stained with ethidium bromide.

2.4. Construction of a phylogenetic tree

The deduced amino acid sequences of the P1-loop region from yeast [28], sea urchins [10,17], rats [18,24] and humans [20,26] were aligned with the ClustalW program using the default parameters [23]. This alignment was performed using truncated sequences of 43 amino acids from 1917 to 1959, referring to rat cytoplasmic amino acid DHC sequence [24]. Phylogenetic tree was constructed by the distance matrix method and neighbor-joining (NJ) method [34]. The yeast cytoplasmic DHC was arbitrarily used to root the tree based on UPGMA analysis. Figures were drawn using the NJ-plot program.

2.5. Reverse transcriptase-PCR analysis

Total RNA from several human tissues including adult trachea,

Table 1
Sequences of oligonucleotide primers used for amplification reactions

Primer[a]	Sequence	Specificity
S1	5′-CTSAAYCTTGGWGGWGCHCCNG-3′	axonemal DHC
S2	5′-GGNAARACNGAGACCACCAARGAT-3′	axonemal DHC
S3	5′-ACCACCAARGATYTKGSCAARGC-3′	axonemal DHC
S4	5′-CGGTTTGTTTTAGTTTTCAAC-3′	cytoplasmic DHC
A1	5′-CGGTTGAACTCGTCAAAGCA-3′	cytoplasmic/axonemal DHC
A2	5′-GGATTCATGGTGATGAAGAT-3′	cytoplasmic/axonemal DHC
A3	5′-DCSDCCDGCRTAICCDGGRTTCAT-3′	axonemal DHC
Se1	5′-CTCTGACCAGCTCGACTTCATGGC-3′	exonic Dnahc1
Se2	5′-CTGGGCATATATGTCATTGTGGTC-3′	exonic Dnahc2
Se3	5′-GACTTGGCTAAAGCTCTTGCTGTA-3′	exonic Dnahc3
Se4	5′-GCAAGACAGAAACCACCAAAGATT-3′	exonic Dnahc3-b
Se5	5′-CGTGCCCTTGGCATGATGGTCTAT-3′	exonic Dnahc11
Si1	5′-GCAGCAGCAGCGGGTGAGCC-3′	intronic Dnahc1
Ai2	5′-GTGGGGAIAGGCTGACTTGC-3′	intronic Dnahc1
Si3	5′-GGTCAGTATCCTGCCACCCT-3′	intronic Dnahc2
Ai4	5′-CAGGCAGAGTCACTGGGGAG-3′	intronic Dnahc2
Si5	5′-GGGAAAGGTAGTTAAATTGC-3′	intronic Dnahc3
Ai6	5′-TTAAAAAACTAAGGACAAAG-3′	intronic Dnahc3
Si7	5′-CAGGTAAGCAAATGCCATCT-3′	intronic Dnahc3-b
Ai8	5′-ACCACACACTAGAAAGAGGG-3′	intronic Dnahc3-b
Si9	5′-AATGGACTACAAAGTAAGTT-3′	intronic Dnahc11
Ai10	5′-TATTGCCTATGGACTAAATG-3′	intronic Dnahc11
Si11	5′-TTTTAGTGCCTGTGTCTTGG-3′	intronic Dnchc1
Ai12	5′-GACAAAATAICCACGTGCAA-3′	intronic Dnchc1

[a]Primers are designated with a first letter (S or A) referring to their orientations (sense or antisense, respectively).

testis, liver and lung was obtained from Clontech. Reverse transcription was performed using 10 μg of RNA with Superscript II reverse transcriptase (Life Technologies) and 100 ng of hexanucleotides (Pharmacia). One-sixth of this material was used as a cDNA template in PCR assays with different sets of primers: a specific primer (corresponding to the exonic sequences, Table 1) and a degenerated primer (used in the cloning procedure, Table 1). To ensure that cDNA and not genomic DNA was amplified, each amplification was performed using a primer set that brackets at least one intronic sequence. Amplification of the Dnchcl, Dnahcl, Dnahc2, Dnahc3, Dnahc3-b and Dnahcl1 transcripts was performed using the primer sets S4/A2, Sel/A3, Se2/A2, Se3/A3, Se4/A1 and Se5/A1, respectively. To check the integrity of RNAs, an RT-PCR run was performed with a primer set specific for $\beta_2$microglobulin. Amplification products were analysed by electrophoresis on 4% agarose gels (2% agarose/2%Nusieve) (Life Technologies and FMC BioProducts, Rockland, ME) stained with ethidium bromide.

3. Results

3.1. Isolation of human DHC genomic clones

Amplification of human genomic DNA with primers designed according to the sequences of conserved DHC subdomains, i.e. encoding the P1-loop region, gave rise to PCR products which sizes were larger than expected for coding sequence, a result pointing to the presence of intronic sequences. These PCR products were systematically subcloned. Sequence analysis showed that thirty independent clones contained a sequence encoding a consensus motif of the DHC P1-loop region. Screening for splice donor and acceptor sites was performed to locate potential intronic sequences, based on splice junction consensus sequences [35]. We also verified that the reading frame across exon boundaries was as expected; this was indeed the case for all the genomic clones. Sequence analysis revealed the existence of six different clones containing at least one intron. The insert locations of the introns are variable (Fig. 2).

3.2. Analysis of the nucleotide and deduced amino acid sequences of the coding sequence of the six genomic clones

We aligned the nucleotide and deduced amino acid sequences of the coding sequence of the six human clones isolated in this study with cytoplasmic and axonemal human DHC cDNA sequences. The coding sequences of three of these six clones are identical to DHC sequences recently reported: one is identical to the Dnchcl cytoplasmic DHC [20,26,36], two are identical to the Dnahcl and Dnahc3 axonemal DHCs (Dnahcl [20], DHC3 [36] which is identical to Dnahc3 [20]). The three remaining clones correspond to new human DHC sequences and were designated Dnahc2, Dnahc3-b and Dnahcl1 with respect to their homology with rat DHC sequences, i.e. DLP2, DLP3 and DLP11 sequences, respectively [18]. As human Dnahc3, previously cloned and named by Vaughan et al. [20], is actually more homologous to rat DLP12 than to rat DLP3, the human clone isolated in the present study, that indeed corresponds to rat DLP3 (Figs. 2 and 3), was designated Dnahc3-b.

3.3. Chromosomal location

To define the chromosomal distribution of the identified human DHC genes, we took advantage of the intronic nucleotide sequences. For each of the six clones, we designed a primer set located in an intron (Table 1). These primer sets were used to screen a panel of 24 hybrid somatic cell lines (humanÊXÊrodent), each of them retaining one of the 24 human chromosomes. The Dnchcl, Dnahcl, Dnahc2, Dnahc3 and Dnahcl1 genes were found to be located on chromosomes 14, 3, 17, 3 and 7, respectively (data not shown). Regarding the Dnahc3-b gene, an amplification product of expected size and sequence was detected in two somatic hybrid cell lines, corresponding to chromosomes 14 and 16 (data not shown).

```
                                      1                                                         60
Dnchcl   [S4/A2]*                     ...................................DETFDFQAMGRIFVGLCQVGAWGCF
Dnahcl   [S2/A3]*                     .....................LGKALAIQTVVFNCSDQLDFMAMGKFFKGLASAGAWACF
Dnahc2   [S3/A2]                      .........................LGIYVIVVNCSEGLDYKSMGRMYSGLAQTGAWGCF
Dnahc3   [S1/A3]*                     ........GPAGTGKTETTKDLAKALAVQCVVFNCSDGLDYLAMGKFFKGLASSGAWACF
Dnahc3-b [S1/A1]                      ........GPAGTGKTETTKDLAKALAKQCVVFNCSDGLDYKAMGKFFKGLAQAGAWA..
Dnahcl1  [S2/A1]                      .....................LGRALGMMVYVFNCSEQMDYKSIGNIYKGLVQTGAWG..
Dnchc2   (DHC2, Vaisberg 1996)        MGLGGNPYGPAGTGKTESVKALGGLLGRQVLVFNCDEGIDVKSMGRIFVGLVKCGAWGCF
Dnahc5   (Vaughan 1994)               MSMGGAPAGPAGTGKTETTKDMGRCLGKDVVVFNCSDQMDFRGLGRIFKGLAQSGAWGCF
Dnahc6   (Vaughan 1994)               LDLGGAPAGPAGTGKTETTKDLAKALAIQCVVFNCSDGLDYKMMGRFFSGLAQSGAWGCF
Dnahc9   (Vaughan 1994)               ..........AGTGKTETTKDLGRALGILVYVFNCSEQMDYKSCGNIYKGLAQTGAWGCF
Dnahc14  (Vaughan 1994)               .........PAGTGKTETVKDLAKSLGKHCVVFNCFEDLDYKIVRKFFFGLVQSGALGCF

                                      61                                                       120
Dnchcl   [S4/A2]*                     DEFNRLEERMLSAVSQQVQCIQEALREHSNPNYDKTSAPITCELLNKQVKVSPDMA....
Dnahcl   [S2/A3]*                     DEFNRIDIEVLSVVSQQITTIQKAQQQRVE----------RFMFEGVEIPLVPSCAVFTT
Dnahc2   [S3/A2]                      DEFNRINIEVLSVVAHQILCILSALAAGLT----------HFHFDGFEINLVWSCG....
Dnahc3   [S1/A3]*                     DEFNRIELEVLSVVAQQILCIQRAIQQKLV----------VFVFEGTELKLNPNCFVAIT
Dnahc3-b [S1/A1]                      ............................................................
Dnahcl1  [S2/A1]                      ............................................................
Dnchc2   (DHC2, Vaisberg 1996)        DEFNRLEESVLSAVSMQIQTIQDALKNHRT----------VCELLGKEVEVNSNSGIFIT
Dnahc5   (Vaughan 1994)               DEF.........................................................
Dnahc6   (Vaughan 1994)               DEFN........................................................
Dnahc9   (Vaughan 1994)               DEFN........................................................
Dnahc14  (Vaughan 1994)               DEFN........................................................
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Fig. 2. Alignment of the deduced amino acid sequences of the cloned human dynein heavy chains with other members of the human DHC family. The underlined amino acid residues mark the locations of introns. Primer sets used to generate the amplification products are indicated in square brackets. Sequence references are indicated in parentheses. Asterisks indicate clones that have been previously described.

3.4. Construction of a phylogenetic tree

We aligned the deduced amino acid sequences of the yeast cytoplasmic DHC [28], thirteen sea urchin DHCs [17] and thirteen rat DHCs [20,24] with all cloned human DHCs, i.e. two cytoplasmic DHCs (Dnchc1 and Dnchc2, [20,36]), six axonemal DHCs just recently reported (Dnahc1, Dnahc3, Dnahc5, Dnahc6, Dnahc9 and Dnahc14, [20]) and the three new DHC products cloned in this study (Dnahc2, Dnahc3-b and Dnahc11). To integrate the human DHC genes into an evolutionary approach, we used this alignment to generate a phylogenetic tree with the neighbor-joining method (Fig. 3).

According to previous studies [14,17,18], phylogenetic data indicate that DHC sequences can be divided into two main groups, the cytoplasmic and axonemal DHCs. The former consists of two subtypes (Dnchc1 and Dnchc2 in humans), while the latter contains outer and inner dynein arm DHCs. All the human DHC genes identified in this study fit into one of these subgroups. Dnchc1 belongs to the cytoplasmic DHC group, Dnahc11 to the axonemal outer dynein arm DHC group, and Dnahc1, Dnahc2, Dnahc3 and Dnahc3-b to the putative axonemal inner dynein arm DHC group.

3.5. Tissue distribution of human DHCs

The expression of the six identified DHC genes was analysed in various human tissues by means of reverse-transcription PCR on total RNA from trachea, testis, liver and lung.

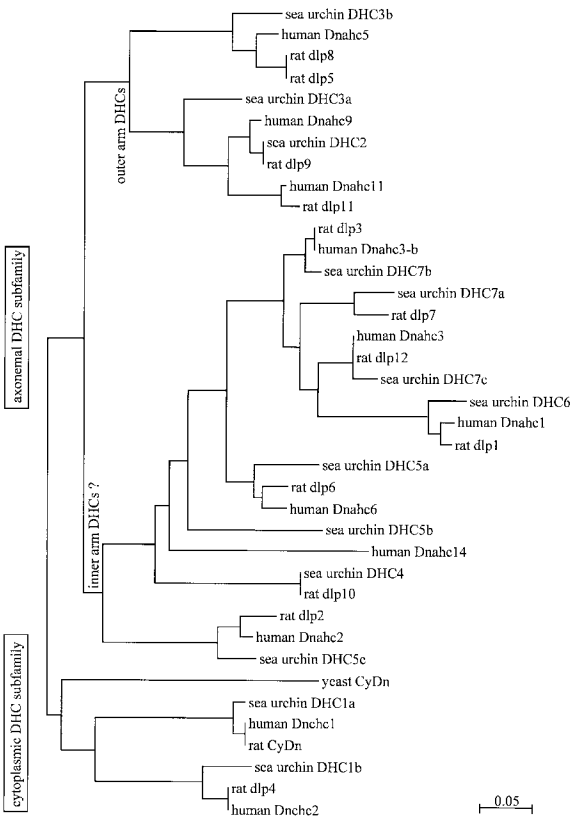

Fig. 3. Phylogenetic tree of the predictive amino acid sequences in the P1-loop region of DHC genes from humans, rats, sea urchins and yeast. The different classes of dynein heavy chain are indicated on the left, cytoplasmic DHCs and axonemal DHCs including outer and putative inner arm DHCs. The scale indicates the number of amino acid differences per residue. The yeast cytoplasmic DHC (yeast CyDn) was arbitrarily used to root the tree based on UPGMA analysis.

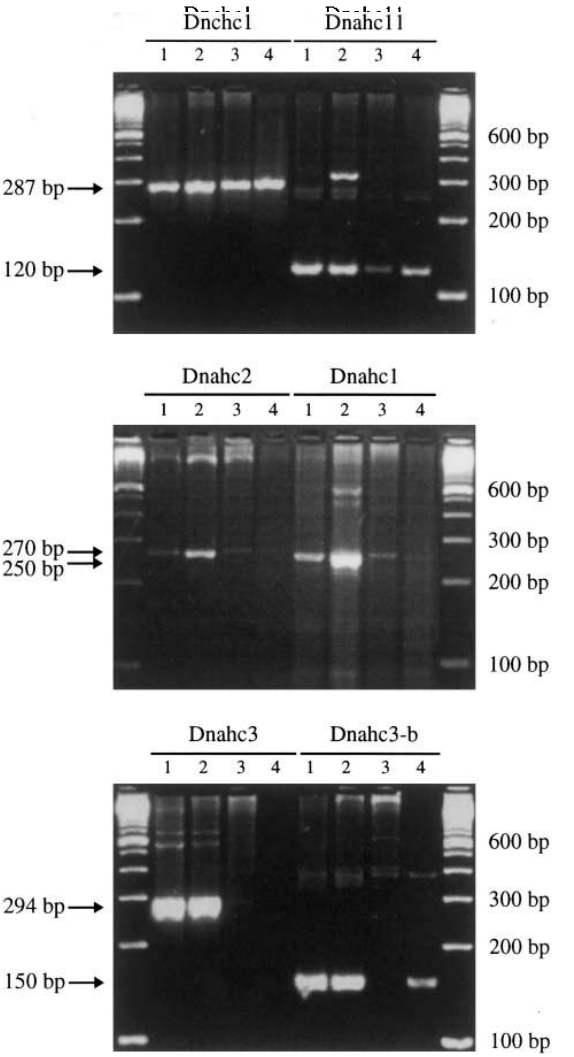

Fig. 4. Expression of human DHC genes (Dnchc1, Dnahc1, Dnahc2, Dnahc3, Dnahc3-b and Dnahc11) in various tissues. The figure shows 4% agarose gels loaded with the reverse-transcription PCR products specific for each human DHC. Numbers on the left indicate the length of the PCR products (which are as expected), and numbers on the right indicate the length of the 100-bp ladder (Life Technologies). Lanes: 1, trachea; 2, testis; 3, liver; 4, lung.

The integrity of RNAs was checked using an RT-PCR run performed with a primer set specific for $\beta_2$microglobulin (data not shown). Three independent experiments revealed that the DHC genes are mainly expressed in specific tissues. The Dnchc1 gene is expressed in all tested tissues, a result in agreement with the ubiquitous expression of DHCs belonging to the cytoplasmic group; the other DHCs (i.e. Dnahc1, Dnahc2, Dnahc3, Dnahc3-b and Dnahc11) are mostly expressed in trachea and testis (Fig. 4).

4. Discussion

We used a molecular approach to identify members of the DHC gene family in humans. Biochemical, immunological and genetic analyses suggested that cytoplasmic and axonemal dyneins are composed of different dynein heavy chains. Recently, molecular genetic studies confirmed this hypothesis by the cloning of a DHC multigene family in various species

revealing that the DHC gene family contains at least fifteen members (two cytoplasmic and thirteen axonemal DHC genes).

To isolate human DHC genes, we first aligned 56 nucleotide sequences encoding the P1-loop region from different species except the human axonemal DHC sequences that have been reported during the course of this study [20]. This sequence alignment allowed us to choose seven degenerated or specific oligonucleotide primers bracketing this highly conserved region. As the exonic sequences encoding the P1-loop of the different DHCs are characterized by a very high degree of nucleotide conservation, we used genomic DNA as a template in the PCR assays to discriminate the different DHC genes and to obtain an equal representation of the different DHC sequences. Indeed, genomic DNA allowed us to clone potential intronic sequences that could be used to distinguish the different genes. In addition, the use of such a template precludes any selection bias that could occur with a cDNA template. Furthermore, when studying DHC mRNA expression, the existence of intronic sequences provides a valuable control to ensure (i) the absence of genomic DNA contamination and (ii) the detection of mature mRNA transcripts. Using this strategy, we identified six human DHC genes. Three clones correspond to human DHC just recently reported: a human cytoplasmic DHC (Dnchc1) and two axonemal DHCs (Dnahc1 and Dnahc3) [20,26,36]. The remaining three clones (i.e. Dnahc2, Dnahc3-b and Dnahc11) identified new human axonemal DHC sequences. In summary, our results raise the number of known human DHC sequences to eleven, among which two are cytoplasmic whereas nine belong to the axonemal DHC group.

We constructed a phylogenetic tree with sea urchin, rat and human DHC sequences. We did not integrate the rat Dnahc13 sequence reported by Vaughan et al. [20] in this phylogenetic analysis, as careful examination of the corresponding nucleotide sequence revealed that it is identical to the rat DLP1 cDNA sequence isolated by Tanaka et al. [18], except for a 106-bp deletion that probably reveals the existence of an alternatively spliced exon. Such a hypothesis is further supported by the presence, in the genomic sequence encoding human Dnahc1 (homologous to rat DLP1), of splice consensus sequences that flank this 106-bp region (the sequences of acceptor and donor splice sites are ACTCAG and GTGAGC, respectively). As expected, in the human Dnahc1 clone, this exon splicing event would results in a frameshift leading to a premature stop codon 23 residues downstream of the 106-bp exon; and, if translated, this mRNA would lead to the synthesis of a new DHC protein whose physiological significance is unknown.

According to previous studies, distribution of sequences along the tree indicated that DHCs form three main clusters, i.e. cytoplasmic DHCs and axonemal outer and inner arm DHCs. Different subgroups can be distinguished among the outer and inner dynein arm DHCs, as previously described [14,17,18,37]. According to this DHC distribution, the Dnchc1 and Dnchc2 genes encode cytoplasmic DHCs; the Dnahc5, Dnahc9 and Dnahc11 genes are members of the outer dynein arm DHC group, whereas the Dnahc1, Dnahc2, Dnahc3, Dnahc3-b, Dnahc6 and Dnahc14 genes belong to the inner dynein arm DHC group. Considering species representation in each subgroup, we speculate that there are at least two cytoplasmic DHCs, four axonemal outer arm DHCs and nine axonemal inner arm DHCs in mammals, raising the number of mammalian DHC genes to at least fifteen. This speculation is also supported by recent data obtained in *Chlamydomonas* [14]. Therefore, it is highly likely that at least three human axonemal DHC genes remain to be found (one outer and two inner arm DHCs).

It appeared that the human DHC genes were spread throughout the genome, Dnchc1 and Dnahc2 being located on chromosome 14 and 17, respectively, whereas the Dnahc1 and Dnahc3 genes are located on the same chromosome (chromosome 3). The chromosomal location of Dnahc2 (chromosome 17) is in agreement with that of its rat counterpart on a mouse chromosomal region which is syntenic to human chromosome 17 [20]. Similarly, Dnahc1 and Dnahc3 genes have been located on mouse chromosome 14 [20] in a region syntenic to human chromosome 3p21-p14, a result consistent with our human chromosomal assignment results. In addition, we mapped Dnahc11 to human chromosome 7. The double location of Dnahc3-b on human chromosome 14 and 16 suggests the existence of two axonemal DHC genes homologous. Such gene spreading could be due to early duplications of a common ancestral DHC gene, as suggested by phylogenetic analysis [37].

We also analysed the tissue expression of the six human DHCs identified in this study. These experiments showed that all products except one are mainly expressed in trachea and/or testis, while the remaining one (Dnchc1) is expressed in all the tissues tested (trachea, testis, liver and lung). The expression of Dnahc1, Dnahc2, Dnahc3, Dnahc3-b and Dnahc11, is stronger in trachea and testis (two tissues containing axonemal structures) than in other tested tissues. These expression patterns are therefore in agreement with the phylogenetic data that are based on sequence analysis and support the fact that the Dnchc1 gene encodes a cytoplasmic DHC, while the Dnahc1, Dnahc2, Dnahc3, Dnahc3-b and Dnahc11 genes encode axonemal DHCs.

Finally, the present results will serve to test the potential involvement of DHC genes in the primary ciliary dyskinesia phenotypes documented in humans.

Acknowledgements: This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM), Assistance Publique/Hôpitaux de Paris (CRC) and the Groupe de Recherche et d'Étude du Génome (GREG).

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