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Identification, tissue specific expression, and chromosomal localisation of several human dynein heavy chain genes

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Sliding between adjacent microtubules within the axonema gives rise to the motility of cilia and flagella. The driving force is produced by dynein complexes which are mainly composed of the axonemal dynein heavy chains. We used cells of human respiratory epithelium after *in vitro* ciliogenesis to clone cDNA fragments of nine dynein heavy chain genes, one of which had never been identified before. Dynein heavy chains are highly conserved from protozoa to human and the evolutionary ancestry of these dynein heavy chain cDNA fragments was deduced by phylogenetic analysis. These dynein heavy chain cDNAs are highly transcribed in human tissues containing axonema such as trachea, testis and brain, but not in adult heart or placenta. PAC clones containing dynein heavy chains were obtained and used to determine by FISH their chromosomal position in the human genome. They were mapped to 2p12–p11, 2q33, 3p21.2–p21.1, 13q14, 16p12 and 17p12. The chromosomal assignment of these dynein heavy chain genes which was confirmed by GeneBridge 4 radiation hybrid screening, will be extremely useful for linkage analysis efforts in patients with primary ciliary dyskinesia (PCD). *European Journal of Human Genetics* (2000) **8**, 923–932.

Keywords: chromosomal mapping; human; dynein; heavy chain; primary ciliary dyskinesia; evolution

Introduction

Immotile cilia syndrome (ICS) or primary ciliary dyskinesia (PCD) is an autosomal recessive disorder in humans impairing the beating of cilia and flagella. Ciliated epithelial cell lining is present in respiratory tract, paranasal sinuses, middle ear, efferent duct of testis, Fallopian tubes, brain and spinal cord. Patients with PCD have recurrent respiratory tract infections leading to bronchietactasis and often male infertility. About 50% of the patients have situs inversus and hence Kartagener syndrome. The motility of cilia and flagella is generated in the axoneme which is composed of more than 250 different proteins.[1] Electron microscopic studies on the ultrastructure of cilia in PCD patients revealed frequent abnormalities in the inner, outer or both dynein arms and in the central pair of microtubules.[2] Dynein is one of the major components of the axoneme. The A-tubule of the microtubular doublets is connected to outer and inner dynein arms at a regular distance and each dynein arm consists presumably of two to three heavy chains, two to four intermediate chains and several light chains.[3] Each dynein heavy chain molecule has a highly conserved putative ATP-binding motif (P-loop) suggesting a force generation during movement. The function of light and intermediate chains is poorly understood. They may have an important role in regulation, assembly of dynein complex and attachment of heavy chains to the A-tubule.[4,5] Recently, DNAI1, a dynein intermediate chain was implied in PCD since a patient with PCD was found to be a double heterozygote for mutations in this gene.[6]

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Numerous distinct dynein heavy chain genes have been isolated from *Paramecium*,[7] *Chlamydomonas*,[8,9] sea urchin,[10] rat,[11,12] mouse,[13] and human.[13–16] However, comparing the dynein heavy chain gene family in well characterised species such as sea urchin suggests that either mammals have less axonemal dynein heavy genes or that some mammal genes are still to be discovered.

As a first step, *in vitro* ciliogenesis of the ciliated epithelial cells (obtained by bronchial or nasal biopsy of patients) was obtained after sequential monolayer and suspension culture.[17,18] From the regenerated ciliated epithelial cells, we cloned and identified several dynein heavy chain cDNAs, one of which corresponds to a new human dynein heavy chain. The transcription of these dynein heavy chain cDNAs was studied in different human tissues including trachea, brain, testis, placenta and heart. Five of these genes and one pseudogene were localised in chromosomes by *in situ* fluorescence hybridisation (FISH) and by radiation hybrid mapping.

Materials and methods

Human epithelial cell culture

Human nasal epithelial cells were treated in a sequential monolayer suspension culture system described previously.[17,18] After about 4 weeks, stable aggregates, spheroids and vesicles were formed of mature epithelial cells with functional ciliary beating.

Primer design, RT-PCR amplification, cloning and sequencing of dynein heavy chain genes

Primers were designed around the first P-loop region according to amino acid sequences of already published dynein heavy chain genes; DNAHloop-1F,-2F,-3F,-4R (Table 1). During the course of this study, Neesen *et al*[13] reported several dynein heavy chain isoform from human testis. A primer pair was selected from their hdhc9 sequence: RT-DNAH5F and -5R (Table 1).

Total RNA was isolated from the cultured cells as already described.[19,20] RT-PCR was carried out with GeneAmp RT-PCR Kit (Perkin Elmer, Essonne, France) with 2 μg of RNA. PCR products were cloned in PCR2.1 vector of the TA-cloning Kit (Invitrogen, Gronigen, The Netherlands). White colonies were checked for correct insert size by either restriction digestion or by PCR with the parent primers. Clones were sequenced and sequences were screened with BLAST. Each clone with a strong homology to dynein heavy chain genes was sequenced again in both directions. Amino acid sequences were deduced and all sequences were aligned to make comparison among them.

After several reports, the nomenclature system of dynein heavy chain genes by different groups is confusing and the homologue of each isoform in mammals and in human is difficult to follow. Here, we followed the nomenclature suggested by the HUGO Nomenclature Committee for naming genes. The name for human axonemal dynein heavy chain gene is DNAH (DN for dynein, A for axonal and H for heavy chain). A prefix in parenthesis is given to specify species: HSA for *Homo sapiens*, RNO for *Rattus norvigecus* and

Table 1 Primers used in this study

DNAHLoop-1F	TAYGGNTTYGARTAYYTNGG	DNAHLoop-2F	GTNCRRACNNNCYTNACNGA
DNAHLoop-3F	ACNGGNAARACNGARACNAC	DNAHLoop-4R	CCNGGRTTCATNGTDATRAA
RT-DNAH1F	AACTGAGACCACCAAAGAC	RT-DNAH1R	CCTTCTGGATGGTGGTGATC
RT-DNAH3F	GCAAGACAGAAACCACCAAAGATT	RT-DNAH3R	ATGGCTTGTTGGATGCTGAGG
RT-DNAH5F	CACAAAAGACATGGGAAG	RT-DNAH5R	TGCTGCCACTGATAATAC
RT-DNAH6F	GCAAAAGCTCTTGCCATCCAGT	RT-DNAH6R	CATCACCAACTTTATTTCCC
RT-DNAH7F	GATGGGTTGGATTATTTGG	RT-DNAH7R	TGGGGTCAAGTTTTAGTTC
RT-DNAH9F	ATCCTGGTCTATGTGTTC	RT-DNAH9R	AGATACCGACAGAAGGATTC
RT-DNAH10F	TGGGGAAGATTTTCTCTGG	RT-DNAH10R	CGGGTTCATTGTGATAAAG
RT-DNAH11F	CGTGCCCTTGGCATGATGGTCTAT	RT-DNAH11R	TCCAACTGATGGCTTGAGTGTG
DNAH-2F	GAAGAGCCTAATTTCTCC	DNAH-1Rbis	ATCTCCACACCCTCAAAC
DNAH-6Fbis	GTGTGTGGTCTTTACTG		
DNAH-9iF	GGATGGAGAATGGCTTCAAC	DNAH-9iR	CAAGAGGACATAGACTTCCGC
DNAH-12F	GACTTGGCTAAAGCTCTTGCTGTA		
DNAH-14F	CCTTAGGCAAACATTGTG		
RH-DNAH1F	TGGGCTCTGAACACATGTGC	RH-DNAH1R	GTGAGTATGGCAGATTTGAGG
RH-DNAH3F	AGTGTGTGGGTATCATTCAGG	RH-DNAH3R	CTTGGCTAAGCAGGTAAGC
RH-DNAH6F	AACATGAGTGCAGTGCCAGC	RH-DNAH6R	AGAGCTGCTGGAGAAGCTTG
RH-DNAH7F	CTTGTCAGTCCATGAGCTTC	RH-DNAH7R	CTCGCAACACACAATCTACC
RH-DNAH10F	TCCAATCTTTGGTAGACACC	RH-DNAH10R	TTACAGAGGCAGGTGTGACC

DNAHLoop primers are degenerated primers used to clone dynein cDNA; RT-DNAH primers were used for specific RT-PCR amplifications; RH-DNAH primers were used for radiation hybrid mapping.

Table 2 Dynein nomenclature

Maiti et al[a] *Human*	*Vaughan* et al[14] *Human*	*Chapelin* et al[16] *Human*	*Neesen* et al[13] *Human*	*Tanaka* et al[11] *Rat*	*Gibbons* et al[10] *Sea urchin*	*HUGO in*	*Chrom. position Human*
DNAH1	*Dnahc1*	*Dnahc1*	*hdhc7*	*Dlp1*	*Tgdyh6*	*DNAH1*	3p21–21.3
–	*Dnahc2*	*Dnahc2*	–	*Dlp2*	*Tgdyh5c*	*DNAH2*	
DNAH3	*Dnahc3*	*Dnahc3b*	*hdhc8*	*Dlp3*	*Tgdyh7b*	*DNAH3*	16p12
DNAH5	*Dnahc5*	–	–	*Dlp5*	*Tgdyh3b*	*DNAH5*	
DNAH6	*Dnahc6*	–	–	*Dlp6*	*Tgdyh5a*	*DNAH6*	2p12
DNAH7	*Dnahc7*	–	*hdhc2*	*Dlp7*	*Tgdyh7a*	*DNAH7*	
DNAH8	*Dnahc8*	–	*hdhc9*	*Dlp8*	*Tgdyh3a*	*DNAH8*	6p21
DNAH9	*Dnahc9*	–	*hdhc1*	*Dlp9a*	*Tgdyh2*	*DNAH9/ DNAH17L*	17p12
DNAH10	–	–	–	*Dlp10*	*Tgdyh4*	–	13q14
DNAH11	*Dnahc11*	*Dnahc11*	*hdhc4*	*Dlp11*	*Tgdyh1*	*DNAH11*	7p15
–	*Dnahc12*	*Dnahc3*	–	*Dlp12*	*Tgdyh7c*	*DNAH12*	
–	*Dnahc14*	–	–	–	*Tgdyh5b*	*DNAH14*	
Pseudogene of *DNAH7* (*DNAH7p*)	–	–	–	–	–	–	2q33

[a]this study; *note:* assignment of isoform with sea urchin and rat was done by amino acid identity in the P-loop region of amino acid 1 to 101 of Figure 1; HUGO-NC: current HUGO nomenclature.

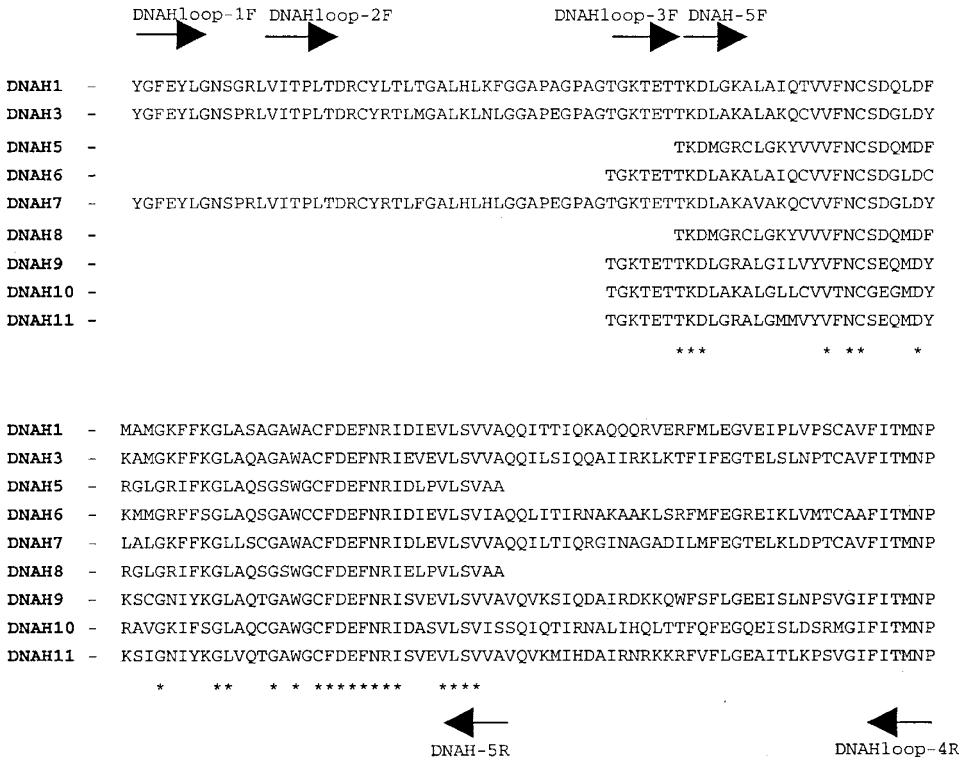

Figure 1 Alignment of the deduced amino sequences and position of the different primers for picking these cDNAs around the P-loop region of the dynein heavy chain gene. This part of the gene is highly conserved. DNAHloop-1F, DNAHloop-2F, DNAHloop-3F and DNAH-5F are sense strand primers. DNAHloop-4R and DNAH-5R are antisense primers. All dynein heavy chain cDNAs were cloned with primers 1, 2, 3, 4 except *DNAH5* and -8 which were cloned with specific non-degenerated primers DNAH-5F and DNAH-5R. Arrows indicate the positions of primers, see Materials and methods for sequence: * indicates the conserved amino acids in all represented genes.

TGR for *Tripneustes gratilla*. In rat and human, these names bear Arabic numerals for each dynein heavy chain gene according to Tanaka *et al*[11] and Vaughan *et al*.[14] In other organisms each dynein gene bears its original identifying number and letter, as for example, *Tripneustes gratilla* DYH5a becomes (TGR)DNAH5a. Table 2 summarises old and new names.

Phylogenic analysis of dynein heavy chain genes

Amino acid sequences of sea urchin, rat, mouse and human sequences were taken from databases and aligned by CLUSTALW. The phylogenetic tree of the dynein heavy chain genes were constructed by the program PHYLIP in neighbour-joining method with Poisson correction, global gap removal and 1000 bootstrap replicates.[21] Forty-five amino acid positions for each clone were taken into account.

Expression of dynein heavy chain genes in different tissues

For axonemal dynein heavy chain gene expression, 2 μg of total RNA from human brain, testis and trachea (Invitrogen) and from adult heart was reverse transcribed with random hexamer as specified in Perkin-Elmer RT-PCT Kit. An aliquot of the reaction was further PCR amplified with isoform specific primers (Table 1). Annealing temperatures were: DNAH1 (54°C), -3 (56°C), -5 (55°C), -6 (52°C), -7 (53°C), -9 (56°C), -10 (53°C) and -11 (58°C).

Hybridisation of PAC library and identification of dynein heavy chain genes

A clone of each dynein isoform was individually amplified and radiolabelled. Radioactive PCR products were then pooled to get the 'pooled dynein probe' which was used to screen PAC filters. Thirty positive PAC clones were obtained from Lawrence Livermore National Laboratory[22] and checked again with the pooled dynein probe. Specific upstream primers were designed, pooled and used with DNAHpool-4R in an attempt to amplify dynein fragments for PAC clones. Upstream specific primers are: RT-DNAH1F; DNAH-2F,[14] RT-DNAH3F, RT-DNAH5F, DNAH-6Fbis, RT-DNAH7F, RT-DNAH9F, RT-DNAH10F, RT-DNAH11F, RT-DNAH12F,[16] RT-DNAH14F.[14] Three PAC clones amplified a 276 bp fragment that after cloning and sequencing of several clones corresponded to (HSA)DNAH3. Primers derived from (HSA)DNAH1 cDNA sequence were used for PCR amplification of each PAC clone DNA. Primers were RT-DNAH1F and DNAH-1Rbis. An amplimer product (1182 bp) was obtained from 5 PAC clones and sequenced. Their sequence identity with the (HSA)DNAH1 mRNA confirmed that these clones contained the (HSA)*DNHA1* gene. Primers RT-DNHA7F and -7R amplified a 255 bp product from PAC P0764. Subsequent sequencing revealed a 54 bp intronic sequence included between two exons. We noted that the 5' exonic sequence had an A insertion just before the normal GT donor site, suggesting that it might be a pseudogene.

(HSA)*DNAH6* and (HSA)*DNAH10* containing PACs were identified by *Hind*III restriction digestion, Southern blotting and hybridisation with the pooled dynein probe. The 1.1 kb (DNAH6) and 2.1 kb (DNAH10) positive bands were purified, cloned, and sequences were obtained with respective gene-specific upstream primer (RT-DNAH6R and RT-DNAH10F). When (HSA)DNAH9 sequence was subjected to BLAST search, it gave a strong homology to some portion of a genomic fragment (EMBL: AC005701). Base to base comparison demonstrated an intronic sequence between the two exonic sequences. Primers were designed from the intronic sequence: DNAH9i-F and -R (Table 1 and an amplification product of the expected size (249 bp) was obtained from PAC P18162. The sequence of this fragment was exactly homologous to the genomic fragment demonstrating that PAC P18162 contained the *DNAH9* gene. We were unable to identify PAC clones that contained the p-loop region of (HSA)DNAH2, -5, -7, -8 and -11.

Fluorescence *in situ* hybridisation of PAC

FISH of PAC clones was done by standard protocol. PAC clone DNA was biotinylated by nick translation with biotin-16-UTP, according to the manufacturer's protocol (Roche Diagnostics, Meylan, Isève, France). Hybridisation of chromosome spreads was performed according to standard protocol.[23,24] A total of 20 metaphase cells was analysed for each PAC clone. PAC clones H2110, L1135, J1436, P0764, P18162, and L2158 were used to map *DNAH1*, *3*, *6*, *7* pseudogene, *9* and *10*, respectively.

GeneBridge 4 radiation hybrid screening

Radiation hybrids (GeneBridge 4) were obtained from HGMP-RC. Primer sequences were derived from PAC genome sequences (Table 1). Positives and negatives were scored with RHyME (Radiation Hybrid Mapping Environment) program with the 'all chromosomes' option.

Results

Cloning, identification, and phylogeny of dynein heavy chain genes

To identify the dynein heavy chain genes, we aligned the *Chlamydomonas*, sea urchin and rat dynein heavy chain genes from databases and designed for RT-PCR (Figure 1) four degenerated primers around the first P-loop region. Total cellular RNA was isolated from human regenerated ciliated epithelial cells and RT-PCR was performed with this set of primers by turn. The expected size bands were directly cloned in *E. coli* vector. Insert size was checked by PCR and sequenced. Then DNA sequence was analysed by BLAST to identify dynein heavy chain genes. Out of almost 400 sequenced clones, 62 showed a striking homology with dynein heavy chain genes. These 62 clones were further resolved in nine different isoforms (Figure 1). It is interesting that the primer pair DNAHloop-3F, DNAHloop-4R was highly

dynein heavy chain specific since 25 out of 27 sequenced clones contained one of seven different dynein isoforms. Moreover, these seven dynein heavy chain isoforms contain the TGKTETT amino acid sequence specific to axonemal P-loop[12] which suggests that they actually are axonemal dyneins. Nevertheless, this primer pair failed to amplify *(HSA) DNAH5* and *-8* which were only amplified by a pair of non-degenerated primers (RT-DNAH5F and -5R).[13] These two dynein heavy chains differ only by one amino acid in this highly conserved region (Figure 1).

During the course of this study, Neesen *et al*[13] and Chapelin *et al*[16] reported several dynein heavy chain isoforms from human testis and genomic DNA. After comparison, we report here one new isoform (HSA)DNAH10 which had not been isolated in human.

A phylogenetic tree (Figure 2) was constructed by taking amino acid sequences of the dynein heavy chain genes of different organisms: sea urchin, mouse, rat, and human. In the evolutionary tree, cytoplasmic and axonemal dyneins are distinctly separated and their evolutionary origin can be traced. Putative outer and inner arm dyneins are distinctly clustered and in most cases, although not all, their respective homologues have been isolated in all these organisms. (TGR)DNCH1b is the sea urchin DYH1B which is homologous[25] to human DYH1B (DNCH2). Human cytoplasmic dynein (HSA)DNCH1 and (HSA)DNCH2 were isolated from non-axonema containing HELA cells[15] and clustered with rat cytoplasmic homologues, (RNO)Dnch1 and (RNO)Dnah4, respectively. The later was isolated from rat brain but was assigned as a cytoplasmic dynein.[11] (HSA)DNAH12 also was isolated from HELA cells although this isoform has the axonemal P-loop motif (TGKTETT) and according to the phylogenetic tree this dynein is more probably axonemal than cytoplasmic dynein.[15] (HSA)DNAH10, the newly reported dynein heavy chain, clusters with (MMU)Dnah4, (RNO)Dnha10 and (TGR)DNAH4 in the group of potential inner arm dyneins. (TGR)DNAH5A and (HSA)DNAH14 have no rat and mouse counterparts suggesting that these isoforms have not been yet cloned in these mammals.

Expression of dynein heavy chain genes in different human tissues

In our cloning strategy, we observed that 25 out of 27 clones arising from the RT-PCR product of a particular pair of primers (DNAHloop-3F and DNAHloop-4R) were dynein heavy chain genes and consisted of altogether seven different isoforms. Therefore, these primer pairs were regarded as axonemal dynein specific primers. To test the expression pattern of the dynein genes, we designed gene-specific primers (Table 1) and performed RT-PCR experiments for each dynein gene using RNA from brain, testis, trachea and heart. It can be deduced from Figure 3 that each gene is expressed in ciliated tissues but not in adult heart. The two bands that were amplified with RT-DNAH7F and -7R (Figure 3) were cloned and sequenced. The shorter band (201 bp) was

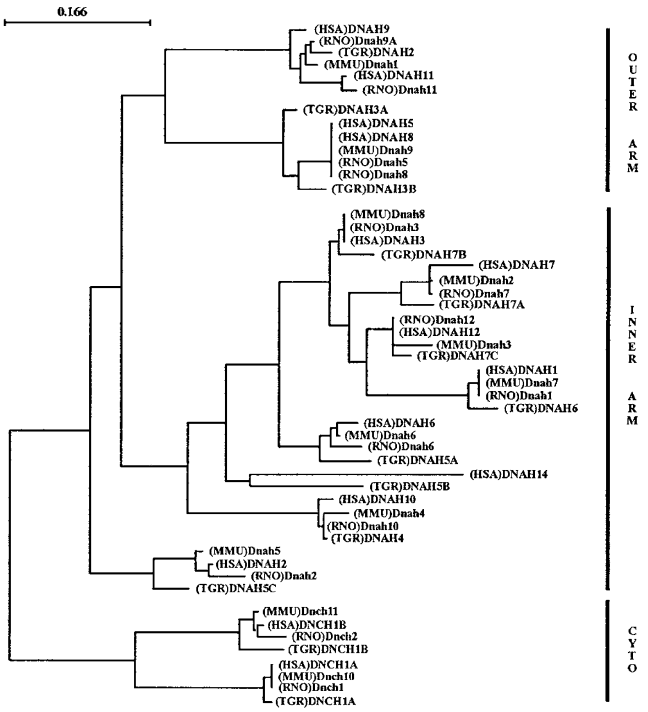

Figure 2 Phylogenetic tree of the dynein heavy chain amino acid sequences. Sequences of *Tripneustes gratilla* (TGR), *Rattus norvegicus* (RNO), *Mus musculus* (MMU) and human (HSA) were obtained from EMBL and each gene is named according to the rules of the HUGO nomenclature committee: (HSA)*DNAH1* (HSA1320833); (HSA)*DNAH2* (U83570); (HSA)*DNAH3* (HSA132085 and Z83805); (HSA)*DNAH5* (HSA132090 and U61738); (HSA)*DNAH6* (HSA132086 and U61736); (HSA)*DNAH7* (HSA132084 and Z83801); (HSA)*DNAH8* (HSA132091 and Z83806); (HSA)*DNAH9* (HSA132088); (HSA)*DNAH10* (HSA132089); (HSA)*DNAH11* (HSA132087); (HSA)*DNAH12* (Z83802); (HSA)*DNAH114* (U61741); (HSA)*DNCH1A* (L23958) and (HSA)*DNCH1B* (HSU20552). (MMU)*Dnah1* (MMMDHC1); (MMU)*Dnah2* (MMMDHC2); (MMU)*Dnah3* (MMDHC3); (MMU)*Dnah4* (MMDHC4); (MMU)*Dnah5* (MMDHC5); (MMU)*Dnah6* (MMDHC6); (MMU)*Dnah7* (MMMDHC7); (MMU)*Dnah8* (MMMDHC8); (MMU)*Dnah9* (MMMDHC9); (MMU)*Dnch10* (MMMDHC10) and (MMU)*Dnch11* (MMMDHC11). (RNO)*Dnah1* (RNDLP1A); (RNO)*Dnah2* (RNDLP2B); (RNO)*Dnah3* (RNDLP3C); (RNO)*Dnah5* (RNDLP5E); (RNO)*Dnah6* (RNDLP6F); (RNO)*Dnah7* (RNDLP7G and RN32182); (RNO)*Dnah8* (RNDLP8H); (RNO)*Dnah9A* (RNDLP9I); (RNO)*Dnah10* (RNDLP10K); (RNO)*Dnah11* (RNDLP11L); (RNO)*Dnah12* (RNDLP12M); (RNO)*Dnch1* (RNU61742) and (RNO)*Dnch2* (RNU61748). (TGR)*DNAH2* (TGDYH2); (TGR)*DNAH3A* (TGYH3A); (TGR)*DNAH3B* (TGYH3B); (TGR)*DNAH3C* (TGYH3C); (TGR)*DNAH4* (TGYH4); (TGR)*DNAH5A* (TGYH5A); (TGR)*DNAH5B* (TGYH5B); (TGR)*DNAH5C* (TGYH5C); (TGR)*DNAH6* (TGYH6); (TGR)*DNAH7A* (TGYH7A); (TGR)*DNAH7B* (TGYN7B); (TGR)*DNAH7C* (TGYN7C); (TGR)*DNAH7D* (TGYN7D); (TGR)*DNCH1A* (TGDYH1A) and (TGR)*DNCH1B* (TGYH1B). The putative outer, inner arm and cytoplasmic dyneins are clustered in the tree. The scale of branch length indicates the mean number of residue changes per site.

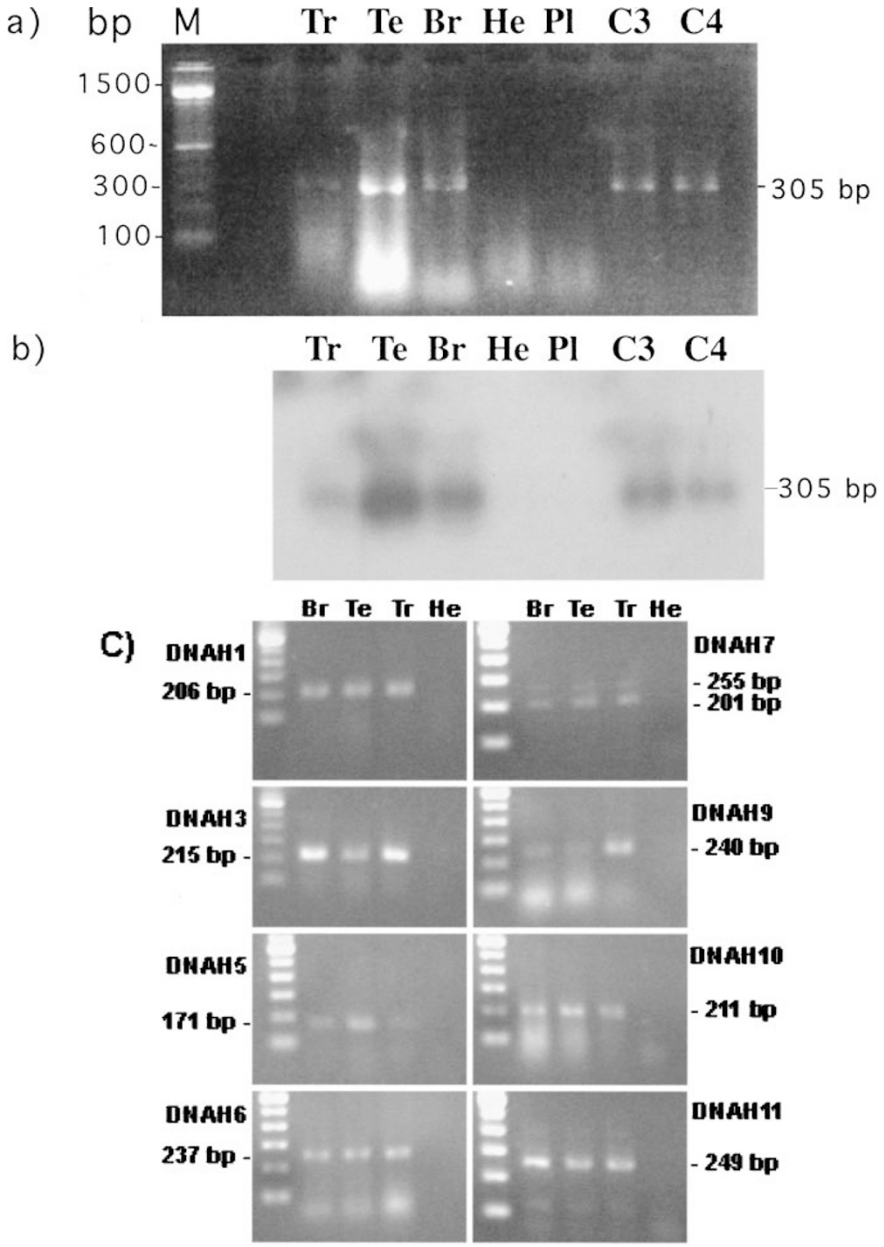

Figure 3 Expression of dynein heavy chain genes: **a)** RT-PCR is carried out with the axonema dynein specific DNAHloop-3F and DNAHloop-4R in different human tissue total RNAs. Lane M, 100 bp marker. Tr, Trachea; Te, Testis; Br, Brain; He, Heart; Pl, Placenta; C3 and C4, *in vitro* cultured ciliated epithelial cells with matured cilia. The major band which contains seven dynein isoforms (305 bp) is observed only in axonema containing tissue and ciliated epithelial cells but not in adult heart or placenta. **b)** The blot of this gel was hybridized with the pooled dynein probe and positive bands were observed only in axonema containing tissues, **c)** Gene-specific primers (Table 1) were used to perform RT-PCR analysis of each dynein gene using RNA from brain (Br), testis (Te), trachea (Tr) and adult heart (He). Left lane: a 100 bp ladder. The expected amplification products were obtained except for DNAH-7F/R which produced two different bands. These two bands (255 and 201 bp) were gel purified and sequenced. Note also that the 171 bp band corresponds probably to both DNAH5 and DNAH8 since these isoform sequences were too identical in this region to design specific primers.

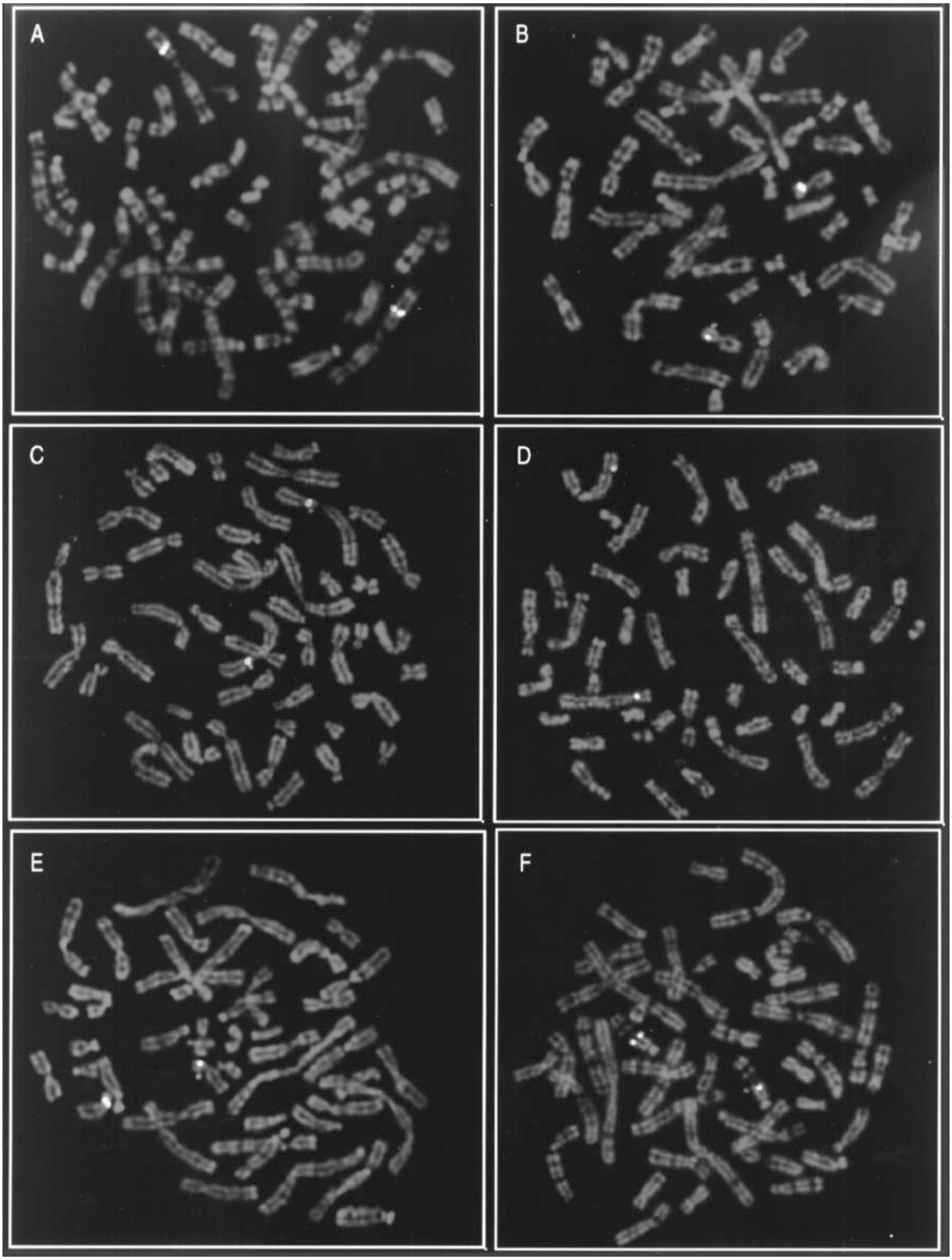

Figure 4 FISH mapping of PACs carrying the dynein heavy chain genes on human metaphase chromosomes. **A** PAC H2110 (*DNAH1* gene), **B** PAC L1135 (*DNAH3* gene), **C** PAC J1436 (*DNAH6*), **D** PAC P0764 (*DNAH7p*), **E** PAC P18162 (*DNAH9*), **F** PAC L2158 (*DNAH10*).

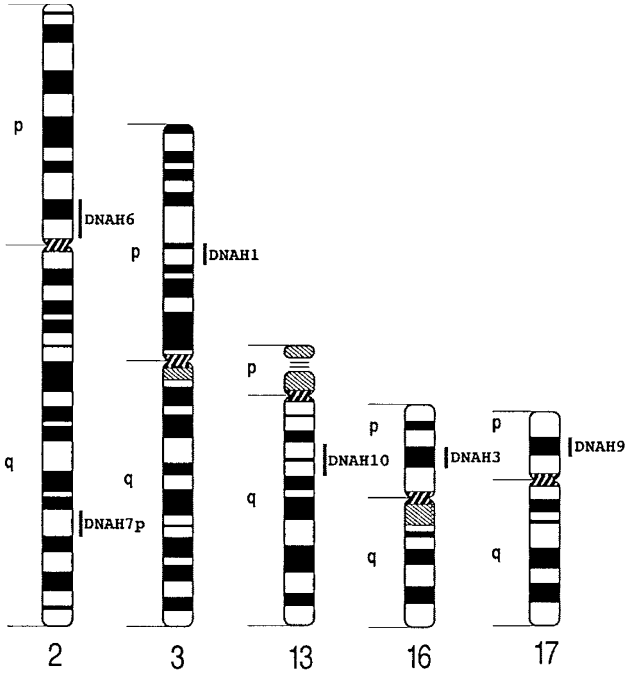

Figure 5 Chromosomal position of the dynein heavy chain genes are shown on the respective chromosome ideograms.

a normal spliced product, whereas the other (255 bp) was an unprocessed product. Since PAC clone P0764 amplified with the RT-DNAH7F and -7R primer pair a fragment with an A insertion before the normal GT donor site as the 255 bp unprocessed product, we suspected that P0764 contains a *DNAH7* pseudogene so that the human genome contains a *DNAH7* gene and a pseudogene.

A rough quantitative estimation of dynein isoform transcription in ciliated epithelial cells could be deducted from the number of clones picked up during our screening. Among the seven dyneins that were amplified by DNAHloop-3F and DNAHloop-4R, DNAH1 and DNAH7, 2 putative inner arm dyneins and DNAH9, a putative outer arm dynein were the most abundant isoforms in ciliated cells of upper airways of the human body.

Chromosomal localisation of dynein heavy chain genes

In order to map human dynein heavy chain genes, we screened the PAC human genomic library on filters obtained from Lawrence Livermore National Library (LLNL) with a 'pooled dynein probe' composed of the seven dynein cDNAs. We obtained 30 positive PACs that were scrutinised for dynein heavy chain isoform content by

(1) hybridisation with dynein heavy chain probe,

(2) PCR amplification with isoform specific primers and

(3) PAC DNA restriction digestion, Southern blotting, and hybridisation with pooled dynein probe.

PCR products and dynein positive bands were cloned and partially sequenced. Chromosomal assignment of selected PAC clones was done by fluorescent *in situ* hybridisation: *DNAH1*, which was present in five PACs maps to 3p21.2–p21.1 (Figure 4) as expected by the syntenic region of mouse.[13,14] *DNAH3* – the homologue of rat DLP3 – (11) maps to 16p12. *DNAH6* containing PACs maps to 2p12–p11. *DNAH10* containing PACs maps to 13q14. *DNAH9* maps to 17p12 and the pseudogene of *DNAH7* to 2q33. Figure 5 summarises *DNAH* gene positions on chromosome ideograms.

FISH mapping was confirmed with primers amplifying from each dynein isoform (Table 3) except for *DNAH9* which was independently mapped by FISH and radiation hybrid to 17p12 by Bartoloni *et al*.[26]

Discussion

We report here the isolation of dynein heavy chain genes from ciliated epithelium after *in vitro* ciliogenesis of the upper respiratory tract. Although the assembled dyneins are one of the major components for ciliary assembly and motility, it is unclear how many dynein heavy chain genes are involved in the process. We cloned here nine dynein heavy chain genes and their occurrence in axonema-containing tissues suggests that they are an integral part of the ciliary assembly or motility. In the course of this study, several groups reported the identification of dynein heavy chain genes from different human tissues such as lung,[14] testis[13] and genomic DNA.[16] Comparing these sequences with ours, a new isoform has been isolated: (HSA)DNAH10, the homologue of which was already isolated in rat[11] but not from human testis and lung. According to the phylogenic tree, (HSA)DNAH10 is presumably a component of the inner dynein arm. The precise number of axonemal dynein heavy chain genes in mammals

Table 3 Results of RhyME analysis for mapping *DNAH* with radiation hybrids

Dynein	*AFM marker*	*D number*	*Lod score*	*Theta*	*Distance (CentiRad)*	*Map position*	*PAC by FISH*
DNAH1	AFM287yd9	D3S1588	3.17	0.572	314.1	3p21.2–p21.1	3p21.2–p21.1
DNAH3	AFM220xb10	D16S417	4.65	0.491	299.9	16p12.3	16p12
DNAH6	AFM333vh5	D2S388	11.07	0.263	562.3	2p12–p11.2	2p12–p11
DNAH7p[a]	AFM058ye3	D2S115	5.13	0.407	1173.0	2q32–q33	2q33
DNAH10	AFM210wa5	D13S168	6.43	0.357	322.2	13q14.3	13q14

[a]p=pseudogene

and in human is not known but there is a human homologue to each sea urchin isoform suggesting that all human axonemal isoforms have been cloned if (TGR)DNAH3C and (TGR)DNAH7D are considered as alternative spliced cDNAs. Apart from two cytoplasmic dynein heavy chain genes *DNCH1* and *DNCH2* (both have P-loop signature TGKTESV.[7,12]) and the ruling out of Dnahc13,[14,16] altogether 12 putative axonemal dynein heavy chain genes (with P-loop TGKTETT) have been isolated from human (*DNAH1, DNAH2, DNAH3, DNAH5, DNAH6, DNAH7, DNAH8, DNAH9, DNAH10, DNAH11, DNAH12* and *DNAH14*). In phylogenic analysis, cytoplasmic and axonemal dynein heavy chains are distinctly separate and remain their nearest neighbours. *DNAH11, DNAH9, DNAH5* and *DNAH8* and their respective homologues in other organisms are considered to be potential outer arm dynein heavy chain genes. Similarly, *DNAH1, DNAH2, DNAH3, DNAH6, DNAH7, DNAH10, DNAH12* and *DNAH14* and their homologues are regarded as potential inner arm dynein heavy chain genes. Among these *DNAH12*, which has also been isolated[15] from HELA cells, a non-axonema containing tissue could be taking part in organellar transport and ciliary assembly as speculated for *DHC1B* [(RNO) *Dnah4*), (RNO)*Dnhc2*] in rat.[11,25]

In our experiments, we were unable to clone *DNAH5* and *DNAH8* cDNA fragments by common degenerated primers and ultimately cloned them by specific primers. The amino acid sequence of *DNAH8* in the downstream common primer region is FITMNPG in human[13] as in other dynein heavy chain genes but different in mouse and rat: FLTMNPG.[11] By contrast the primer region sequence of DNAH5 has not been isolated in human but isoform 5 in mouse and rat is FLTMNPG. It is possible that the correct human sequence of these two genes is FLTMNPG instead of FITMNPG which may prevent transcription and subsequent amplification of these genes with the degenerated primer we selected (DNAHloop-4R).

Chromosomal assignment of these dynein genes is important for the pathogenicity of primary ciliary dyskinesia (PCD) in which ultrastructural abnormalities of inner and outer dynein arm is frequent. *DNAH1* is mapped as expected to 3p21.2–p21.1, but *DNAH12* which was also mapped to chromosome 3[16] by somatic cell hybrids and was predicted to be mapped to this region by mouse synteny,[14] was not evidenced in the five PACs hybridising to 3p21.2–p21.1. However, we cannot rule out that *DNAH1* and *DNAH12* are actually clustered and that we were unable to identify *DNAH12* in the PAC clones containing *DNAH1*. By mouse synteny, *DNAH6* was predicted in two regions, 2p12–p11 and 6p21.3[14] but was mapped only to the 2p12 region (Figure 5).

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