Mutations in *DNAH5* cause primary ciliary dyskinesia and randomization of left–right asymmetry

Published online: 14 January 2002, DOI: 10.1038/ng817

Primary ciliary dyskinesia (PCD, MIM 242650) is characterized by recurrent infections of the respiratory tract due to reduced mucociliary clearance and by sperm immobility. Half of the affected offspring have situs inversus (reversed organs), which results from randomization of left-right (LR) asymmetry[1]. We previously localized to chromosome 5p a PCD locus containing *DNAH5*, which encodes a protein highly similar to the *Chlamydomonas* γ-dynein heavy chain[2]. Here we characterize the full-length 14-kb transcript of *DNAH5*. Sequence analysis in individuals with PCD with randomization of LR asymmetry identified mutations resulting in non-functional DNAH5 proteins.

Starting with a 1.5-kb partial cDNA[2], we obtained a full-length cDNA for *DNAH5* by PCR amplification of overlapping cDNA fragments from testis and trachea libraries as well as from RACE analyses. We found that the composite transcript contains an open reading frame of 13,872 nt, encoding a protein with 4,624 amino-acid residues. The *DNAH5* genomic region comprises 79 exons and spans 250 kb. We found evidence of an alternative first exon by sequencing expressed-sequence tag (EST) clones (Web Fig. A).

Northern-blot analysis with a partial cDNA probe of the murine orthologous gene, *Dnahc5* (sequence identity of 91% to *DNAH5*), showed one strong band in lung and kidney and a weaker signal of the same size in brain, heart and testis, most likely corresponding to a 14-kb transcript (Web Fig. B). To determine the embryonic expression pattern of *Dnahc5*, we carried out *in situ* hybridization analysis on whole mouse embryos from 6.5 days *post coitum* (dpc) to 9.5 dpc (Web Note A). Expression was weak but clearly confined to the node from 7.0 dpc to 8.25 dpc (Fig. 1*a*).

The pedigrees of all families with PCD studied here were compatible with autosomal recessive inheritance. Using haplotype analyses with two intragenic polymorphic markers (Web Note A), we identified 25 families with PCD showing allele segregation compatible with linkage to *DNAH5*. Four families (F373, F658, F758 and UNC7) showed haplotypes suggesting homozygosity by descent. We searched for mutations by sequencing all exons of *DNAH5* in affected individuals of these 25 affected families. We identified four homozygous and six heterozygous mutations (Table 1 and Web Fig. C). We identified mutations on two alleles, explaining the recessive phenotype in six families with PCD. In two families, we detected only one mutation, leaving the mutation on the second allele unidentified. We confirmed cosegregation of the mutations with disease haplotypes by sequencing other available family members. Most mutations resulted in premature termination, predicting total or partial loss of the motor and the microtubule binding domain (Web Fig. A). Only two missense mutations within conserved domains were found. The eight families with PCD carrying *DNAH5* mutations contained 17 affected individuals. Seven of these affected individuals had Kartagener syndrome, which is defined by an associated complete *situs inversus*[1]. The occurrence of affected individuals with and without associated *situs inversus* within the same affected family (F373, UNC7, F661, UCL4 and UCL13) indicates randomization of LR asymmetry in individuals with PCD carrying *DNAH5* mutations (Web Fig. C). We carried out ultrastructural studies of respiratory cilia in six families and found defects of the outer dynein arms (ODA-phenotype; Fig. 1*b*,*c* and Web Fig. C). In five families with PCD, immotile cilia were noted by direct visualization of respiratory cilia (Web Fig. C).

Our results show that mutations in *DNAH5* cause PCD with randomization of LR asymmetry. This conclusion is supported by several findings. First, *DNAH5* maps to the PCD locus on chromosome 5p[2]. Second, expression of *Dnahc5* is present in the lung and node, explaining the disease phenotype. In addition, four homozygous mutations (including the consanguineous family used to map the locus) and six heterozygous mutations were found in affected members of eight families with PCD. Moreover, mutations in the *Chlamydomonas* ortholog are suspected to result in slow-swimming algae with a phenotype highly similar on the ultrastructural level[3]. Thus, the functional role of this gene seems to be evolutionarily conserved. This is also supported by the high degree of sequence conservation (40% identity, 59% similarity) between both proteins (Web Fig. D). The amino-terminal third of the protein forms a relatively short tail, which is important for binding of dynein light and intermediate chains[4]. Mutations in *DNAI1* and the orthologous gene of *Chlamydomonas*, which encode intermediate chains, also result in PCD or Kartagener syndrome with an ODA-phenotype[5–7]. This indicates the importance of DNAI1 and DNAH5 for the function of the outer arm dynein complex. The remaining residues of DNAH5 (>3000) form the globular motor domain. The dynein motor is organized into six AAA modules[4,8] and a

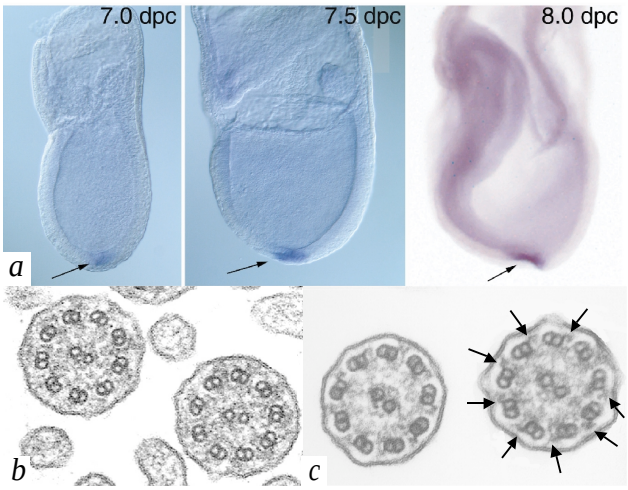

Fig. 1 Early embryonic expression of murine *Dnahc5* and axonemal phenotype resulting from *DNAH5* mutations. ***a***, Whole-mount *in situ* hybridization analysis of murine *Dnahc5* orthologous to human *DNAH5* in early–gastrulation stage mouse embryos. *Dnahc5* expression is restricted to the node (arrow) in embryos at 7.0–8.0 dpc. ***b***, Electron micrograph of cross-sections of respiratory cilia (original magnification ×88,000) from an affected member of family F658. Absence of outer dynein arms is observed on all peripheral doublets. ***c***, Electron micrograph of cross-sections of respiratory cilia (original magnification ×154,000) from a healthy individual for comparison. The location of outer dynein arms are indicated by arrows in the right axoneme.

Table 1 • Mutations in *DNAH5*

Family	Origin	Exon	DNA change[a]	Protein change
373	Lebanon	[34] + [34]	[5563insA]	[1855NfsX5]
658	Germany	[50] + [50]	[8440delGAACCAAA]	[2814fsX1]
661	Germany	[28] + [53]	[4361G→A][b] + [8910+8911delAT→insG]	[R1454Q] + [2970SfsX7]
758	Germany	[62] + [62]	[10555G→C][b]	[G3519R]
UNC-7	USA	[75] + [75]	[IVS74-1G→C]	splice-site mutation
UCL-4	England	[28] + [?]	[4360C→T] + [?]	[R1454X] + [?]
UCL-13	England	[48] + [?]	[7915C→T] + [?]	[R2639X] + [?]
UCL-16	Scotland	[14] + [32]	[1828C→T] + [5130insA]	[Q610X] +[R1711TfsX36]

[a]Base 1 corresponds to the first base of the initiation codon in the cDNA sequence. [b]Not found in 200 control chromosomes. [?] represents unknown change.

microtubule-binding domain, which is predicted to form an extended hairpin-like stalk of anti-parallel coiled-coils[9] (Web Fig. A).

Recent studies have shown that monocilia of nodal cells produce an overall leftward flow of extracellular fluid[10,11]. Disturbances in nodal flow, due to absent nodal cilia in *Kif3B*$^{-/-}$, *Kif3A*$^{-/-}$ and *Tg737*$^{\Delta 2\text{-}3\beta Gal}$ mice[10–12] and immotile cilia in *lrd*^{iv} mutant mice[13], were associated with randomization of LR patterning. It has therefore been suggested that the cilia-driven nodal flow creates an asymmetric gradient of a LR morphogen that starts the LR signaling cascade. However, *Kif3B*$^{-/-}$, *Kif3A*$^{-/-}$ and *Tg737*$^{\Delta 2\text{-}3\beta Gal}$ mice have severe developmental defects resulting in early embryonic death[10–12], suggesting that other gene functions might also account for the randomization of LR patterning in these mutant mice. Mutations in *DNAH5*, which cause randomization of LR asymmetry, and expression of *Dnahc5* in the node also support the nodal-flow hypothesis. Notably, mutation of lrd (encoding an axonemal dynein heavy chain) also affects nodal ciliary function[13]. Based on our results, DNAH5 is the first recognized axonemal dynein heavy chain that is essential for nodal and respiratory cilia function. Expression of *DNAH5* in brain and kidney is explained by the presence of ciliated cells lining the ventricles in brain and in kidney tubules[14]. A potential ciliary function in these organs is supported by *Hfh4* mutants showing hydrocephalus in addition to random LR asymmetry, as well as *Tg737*^{orpk} mice developing cystic kidneys and hydrocephalus[14,15].

Note: Supplementary information is available on the Nature Genetics web site (http://genetics.nature.com/supplementary_info/).

Acknowledgments

We thank the affected individuals and their families for their participation in this study. We thank the German patient support group 'Kartagener Syndrom und Primäre Ciliäre Dyskinesie e.V.' and the UK PCD support group for their cooperation. We are grateful to S. Lehrack, R. Melkaoui, M. Petry and K. Rak for technical assistance. We also wish to thank H. Felix for the contribution of electron microscopic photographs and S.E. Antonarakis, J. L. C. Blouin, L. Bartoloni, A. Bush, E. Byrne, T. Willems and M. Jorissen for the contribution of DNA samples and clinical data. This work was supported by grants from the Deutsche Forschungsgemeinschaft (to H.O.), the B. Braun-Stiftung (to H.O.), the Wissenschaftliche Gesellschaft Freiburg (to H.O.), the US National Institutes of Health, the Medical Research Council of the UK, the Wellcome Trust, and Action Research.

Heike Olbrich[1]*, Karsten Häffner[1]*, Andreas Kispert[2], Alexander Völkel[1], Andreas Volz[1], Gürsel Sasmaz[1], Richard Reinhardt[3], Steffen Hennig[3], Hans Lehrach[3], Nikolaus Konietzko[4], Maimoona Zariwala[5], Peadar G. Noone[5], Michael Knowles[5], Hannah M. Mitchison[6], Maggie Meeks[6], Eddie M.K. Chung[6], Friedhelm Hildebrandt[1], Ralf Sudbrak[3] & Heymut Omran[1]

**These authors contributed equally to this work.*

[1]Department of Pediatrics and Adolescent Medicine, Albert Ludwigs University, 79106 Freiburg, Germany. [2]Medizinische Hochschule Hannover, Germany. [3] Max-Planck Institute for Molecular Genetics, Berlin, Germany. [4]Ruhrland-Klinik, Essen, Germany. [5]University of North Carolina at Chapel Hill, North Carolina, USA. [6]Department of Paediatrics and Child Health, Royal Free and University College London, UK. Correspondence should be addressed to H.O. (e-mail: omran@kikli.ukl.uni-freiburg.de).

Received 23 August; accepted 28 November 2001.

1. Afzelius, B.A. & Mossberg, B. *The Metabolic and Molecular Bases of Inherited Disease* (eds Scriver, C.R., Beaudet, A.L. & Sly, W.S.) 3943–3954 (McGraw-Hill, New York, 1995).
2. Omran, H. *et al. Am. J. Resp. Cell Mol. Biol.* **23**, 669–702 (2000).
3. Wilkerson, C.G., King, S.M. & Witman, G.B. *J. Cell Sci.* **107**, 497–506 (1994).
4. Asai, J. & Koonce, M.P. *Trends Cell Biol.* **11**, 196–202 (2001).
5. Pennarun, G. *et al. Am. J. Hum. Genet.* **65**, 1508–1519 (1999).
6. Guichard, C. *et al. Am. J. Hum. Genet.* **68**, 1030–1035 (2001).
7. Wilkerson, C.G., King, S.M., Koutoulis, A., Pazour, G.J. & Witman, G.B. *J. Cell Biol.* **129**, 169–178 (1995).
8. Mocz, G. & Gibbons, R. *Structure* **9**, 93–103 (2001).
9. Gee, M.A., Heuser, J.E. & Vallee, R.B. *Nature* **390**, 636–639 (1997).
10. Nonaka, S. *et al. Cell* **95**, 829–837 (1998).
11. Takeda, S. *et al. J. Cell Biol.* **145**, 825–836 (1999).
12. Murcia, N.S. *et al. Development* **127**, 2347–2355 (2000).
13. Supp, D.M., Witte, D.P., Potter, S.S. & Brueckner, M. *Nature* **389**, 963–966 (1997).
14. Taulman, P.D., Haycraft, C.J., Balkovetz, D.F. & Yoder, B.K. *Mol. Biol. Cell* **12**, 589–599 (2001).
15. Chen, J., Knowles, H.J., Hebert, J.L. & Hackett, B.P. *J. Clin. Invest.* **102**, 1077–1082 (1998).