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ORIGINAL ARTICLE ***Reproductive biology***

Ciliary function and motor protein composition of human fallopian tubes

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STUDY QUESTION: What is the motor protein composition and function of human fallopian tube (FT) cilia?

SUMMARY ANSWER: Although the motor protein composition and function of human FT cilia resemble that of respiratory cilia, females with primary ciliary dyskinesia (PCD) are not necessarily infertile.

WHAT IS KNOWN ALREADY: FTs are lined with multiple motile cilia, which show a 9 + 2 ultrastructure by transmission electron microscopy. Case reports suggest an increased incidence of subfertility and ectopic pregnancy in women with PCD, a disease characterized by dysfunction of motile cilia and flagella.

STUDY DESIGN, SIZE, DURATION: This study consisted of an observational laboratory study on human FT specimens from five healthy females recruited from April 2012 to December 2013 and a descriptive observational retrospective analysis of a clinical PCD database.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Human FT tissue was obtained from five healthy females after tubal ligation during caesarean delivery. Motor protein composition was assessed by immunofluorescence microscopy using antibodies against dynein arms and nexin–dynein regulatory complex subunits. Ciliary motility was analysed by high-speed video microscopy. A retrospective search of our database of PCD individuals was performed for information on conception and childbirth.

MAIN RESULTS AND THE ROLE OF CHANCE: The motor protein composition of human FT cilia was identical to that of respiratory cilia. FT cilia showed coordinated beating, resulting in a directed fluid flow towards the uterine cavity. We identified nine PCD individuals with severe dysfunction of respiratory cilia who gave birth to children after spontaneous conception. This suggests that ciliary beating is not the key motor of ovum transport.

LIMITATIONS, REASON FOR CAUTION: FT cilia of affected PCD females were not available for analysis. Thus, it remains to be proven that FT cilia indeed show the same defects as respiratory cilia in PCD individuals. Comprehensive epidemiological studies are needed to determine the extent of female (sub-) fertility in PCD.

WIDER IMPLICATIONS OF THE FINDINGS: Knowledge of the exact protein composition and function of FT cilia will contribute to a better understanding of cilia-generated fluid flow in female reproduction. These findings are important for subsequent studies of function and protein composition of FT cilia in PCD patients.

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Key words: oviduct / cilia / motor proteins / PCD

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Introduction

Cilia and flagella (sperm tails) are microtubule-based hair-like organelles that extend from the surface of almost all cell types of the human body (Fliegauf *et al.*, 2007). Most cell types have a single cilium (monocilium or primary cilium), which can be motile or immotile, whereas some cells build 200–300 multiple motile cilia (MMC).

MMC line the upper and lower airways, ependymal cells of the brain ventricles and the fallopian tubes (FTs) of the female reproductive system. Respiratory cilia are responsible for mucociliary clearance, an important mechanism to prevent chronic destructive airway disease, and ependymal cilia propel cerebrospinal fluid through the aqueduct (Ibañez-Tallon *et al.*, 2003). Several studies have documented that female FTs are lined with MMC (Afzelius and Eliasson, 1983; Lyons *et al.*, 2006). Human FTs are tubular seromucosal organs connecting the ovaries to the uterus. Anatomic structures include (from lateral to medial) the fimbria, infundibulum, ampulla and isthmus (Fig. 1A). Ciliated and secretory cells represent the main mucosal cell types. Ciliated cells are mostly found at the apex of the mucosal folds (Lyons *et al.*, 2006; Ezzati *et al.*, 2014). It is assumed that coordinated ciliary beating, in concert with muscle contractions, plays a role in human reproduction by directing the oocyte through the FT to the uterus (Lyons *et al.*, 2006). This process is modified by hormone levels as well as tubal mucus composition and can be impaired by infections, smoking and gynaecological disorders such as endometriosis (Ezzati *et al.*, 2014).

Primary ciliary dyskinesia (PCD) is an autosomal-recessive disorder of motile cilia leading to upper and lower airways disease due to defective respiratory ciliary function. It has been hypothesized that females with PCD may be affected by subfertility because the genetic defect also affects FT cilia (Afzelius and Eliasson, 1983; Halbert *et al.*, 1997; Lyons *et al.*, 2006).

FT cilia have a length of ~10 μm and a diameter of 0.25 μm (Satir, 1992). Transmission electron microscopy (TEM) analyses have demonstrated that cross sections of FT cilia share the same axonemal ultrastructure with other motile cilia such as respiratory and ependymal MMC or sperm flagella (Dirksen and Satir, 1972; Afzelius, 1976, 2004; Halbert *et al.*, 1976; Ibañez-Tallon *et al.*, 2003; Lyons *et al.*, 2006; Ezzati *et al.*, 2014). Nine peripheral doublet microtubules surround two singular central microtubules to form a 9 + 2 microtubular structure (Fig. 1C and D). Outer and inner dynein arms (ODAs and IDAs, respectively) contain motor proteins involved in ciliary beat generation. The nexin–dynein regulatory complex (N-DRC) connects the outer doublets, and the radial spokes connect the peripheral doublets with the central pair apparatus (Fig. 1C and D).

Although cilia exhibit the same ultrastructure by TEM, the composition of motor proteins that effectuate ciliary beat generation differs among species. As an example, the ODA of *Chlamydomonas reinhardtii* is a three-headed

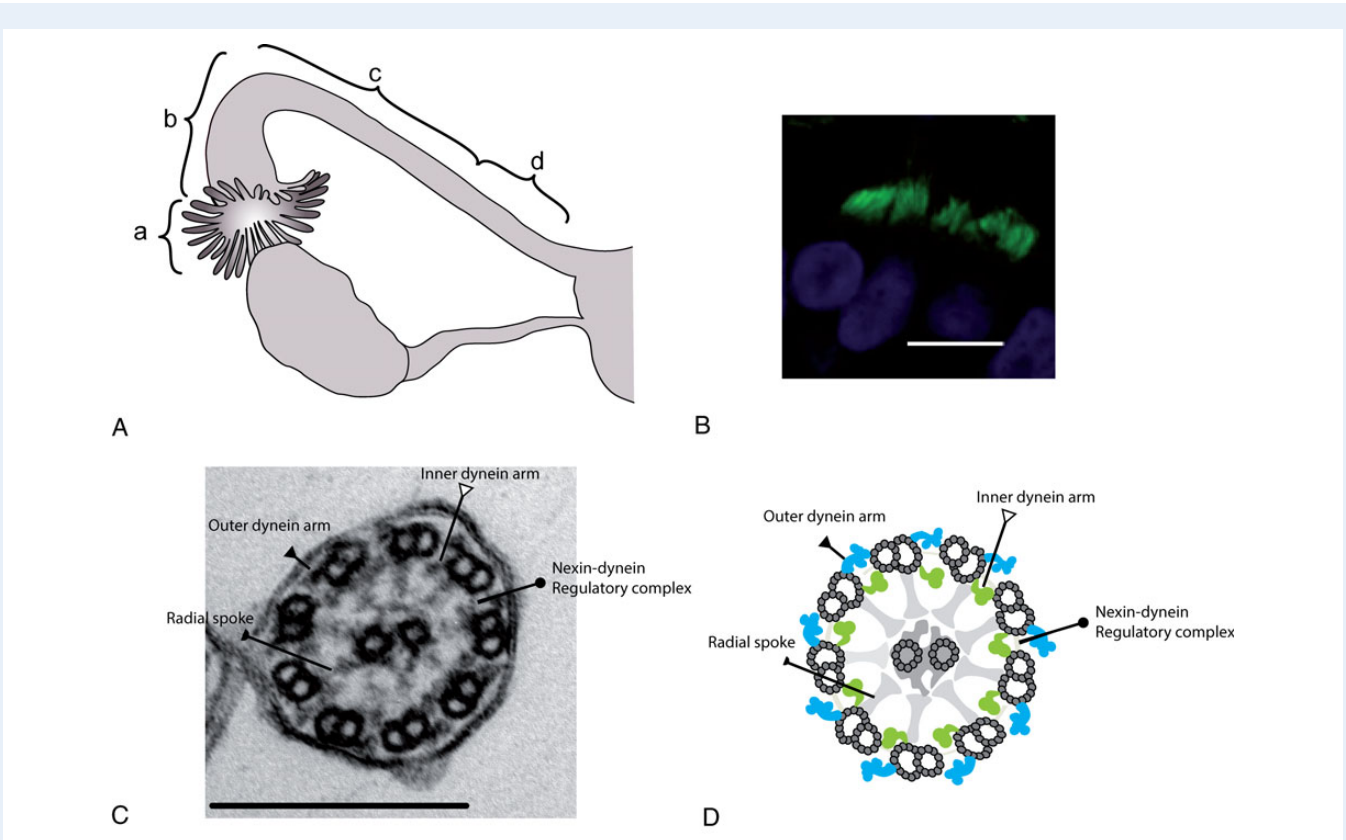

Figure 1 (**A**) Anatomy of the human fallopian tube (FT), presenting the distinct segments: (a) fimbria, (b) infundibulum, (c) ampulla and (d) isthmus. (**B**) FT multiciliated cells from a healthy control person stained with an antibody directed against acetylated α-tubulin (green) as a ciliary marker. Nuclei were stained with Hoechst33342 (blue). Scale bar equals 10 μm. (**C**) TEM cross section of a porcine FT cilium showing an axonemal composition resembling that of human respiratory cilia with a 9 + 2 microtubular structure, outer dynein arms, inner dynein arms, nexin–dynein regulatory complex and radial spokes. Scale bar equals 200 nm. (**D**) Schematic of a ciliary cross section indicating ultrastructural components.

structure containing three dynein heavy chains, whereas the human ODA only consists of two heads (O'Toole *et al.*, 2012). In humans, the motor protein composition of the ciliary axoneme of sperm tails differs from that of motile respiratory cilia (Fliegauf *et al.*, 2005). In human FT cilia, the ciliary motor protein composition has not yet been studied. This prompted us to analyse in detail the motor protein composition of human FT cilia and the consequent ciliary function. Furthermore, we searched our PCD database for information on conception and childbirth in PCD females with severe respiratory disease.

Materials and Methods

Subjects

FT tissue was obtained from five women who underwent sterilization during caesarean delivery. For comparative analyses, respiratory epithelium cell samples were taken from healthy controls as described previously (Raidt *et al.*, 2014). The mean age was similar in both groups (FT: 34.6 years versus respiratory epithelium controls: 32.2 years). Written informed consent to participate in this study was obtained from each individual prior to sampling

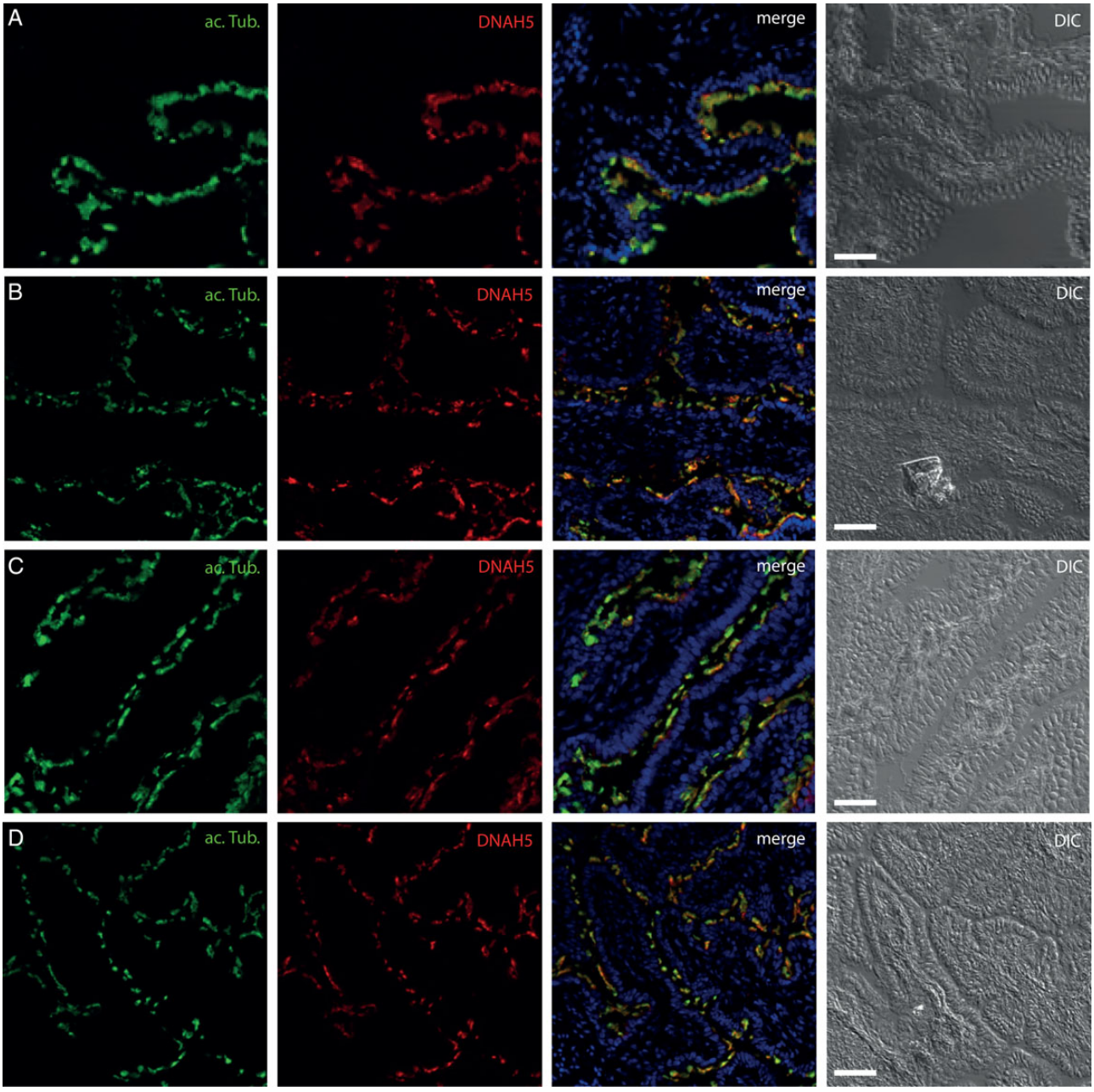

Figure 2 Cryosections of human fallopian tubes (FT) from healthy control persons were double labelled with antibodies directed against acetylated tubulin (green) and DNAH5 (red). Both proteins co-localize (yellow) along the ciliary axoneme. Nuclei were stained with Hoechst33342 (blue). Distinct segments of the human FT were stained: (**A**) fimbria, (**B**) infundibulum, (**C**) ampulla and (**D**) isthmus. Multiciliated cells were present in all FT segments. DIC represents differential interference contrast. Scale bar equals 50 μm.

FT tissue or respiratory cells. The study was approved by the Ethics Committee of the Westphalian Wilhelms University, Muenster, Germany.

High-resolution immunofluorescence microscopy analysis

Human FT cryosections were spread onto glass slides, air-dried and stored at −80°C before use. Representative sections were prepared from each segment of the FT in order to visualize the distribution and composition of motile cilia. In addition, we studied single-ciliated FT cells obtained by brush biopsy of fimbria and the infundibulum as well as respiratory cilia after nasal sampling.

Cells and tissue were treated and incubated by primary and secondary antibodies as reported previously (Omran and Loges, 2009; Hjeij *et al.*, 2014). As markers of the ciliary axoneme, monoclonal mouse anti-acetylated α-tubulin antibody (Sigma-Aldrich, Taufkirchen, Germany) or polyclonal rabbit anti-α/ß tubulin antibody (Cell Signaling Technology, New England Biolabs, Frankfurt am Main, Germany) was used. Polyclonal rabbit antibodies directed against DNAH5, DNAL11, DNAH9, CCDC39 and LRRC48, as well as monoclonal mouse anti-DNAI2 antibody, were reported previously (Fliegauf *et al.*, 2005; Zariwala *et al.*, 2006; Loges *et al.*, 2008, 2009; Merveille *et al.*, 2011; Wirschell *et al.*, 2013). Anti-DNAI1 polyclonal rabbit antibody was obtained from Sigma-Aldrich. Anti-mouse Alexa Fluor 488 and anti-rabbit Alexa Fluor 546 (Life Technologies, Darmstadt, Germany) were used as secondary antibodies. Nuclei were stained with Hoechst 33 342 (Sigma-Aldrich). Immunofluorescence (IF) images were captured with a Zeiss Apotome Axiovert 200 and processed with AxioVision v.4.8 (Carl Zeiss AG, Göttingen, Germany) and Adobe Creative Suite 4 (Adobe Systems, San Jose, CA, USA).

High-speed video microscopy analysis

Directly after surgical removal, human FT tissues were suspended in RPMI 1640 medium. High-speed video microscopy analysis (HVMA) was performed both in ciliated cell strips, which were obtained by brushing the fimbria and infundibulum with a cytology brush (Engelbrecht Medicine and Laboratory Technology, Edermünde, Germany), and in whole tissue sections of all segments of longitudinally sliced oviducts. HVMA in respiratory epithelial cells was performed as previously described (Raidt *et al.*, 2014). Respiratory cells were obtained from healthy non-pregnant volunteers at a random time of menstrual cycle. Sisson-Ammons video analysis (SAVA) was used for the analysis of ciliary beat pattern and frequency (Sisson *et al.*, 2003).

Cilia-driven transport analysis

Transport function of human FT cilia was visualized using particles of 2 μm size (2% FluoSpheres Fluorescent Microspheres, Life Technologies). Videos were recorded with Hokawo 2.6 imaging software at 10× magnification with an ORCA.flash 4.0 Digital Camera (Hamamatsu Photonics, Herrsching, Germany) connected to a PolyVar MET microscope (Reichert-Jung, Vienna, Austria). Particle transport was analysed using Fiji (Schindelin *et al.*, 2012).

Results

Spatial distribution of MMC in human FTs

Distribution of ciliated cells along the human FT was analysed by IF. Staining of sections from fimbria, infundibulum, ampulla and isthmus using antibodies against DNAH5 and acetylated α-tubulin showed that all segments were populated with MMC, indicating that ciliary beating takes place along the entire length of the FTs (Fig. 2). The density of multiciliated cells was highest in the fimbria and decreased towards the isthmus. As in respiratory cilia, both antibodies co-localized throughout the entire length of ciliary axonemes in all FT segments (Fig. 3).

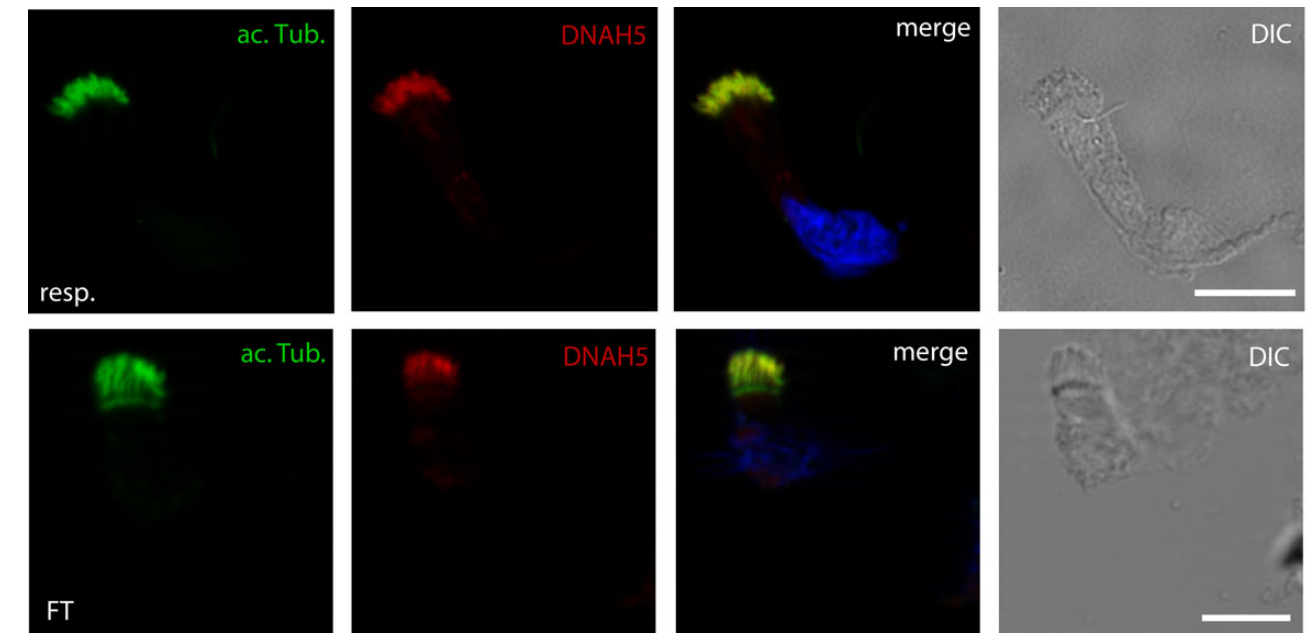

Figure 3 Respiratory epithelial (resp) and fallopian tube (FT) cells from healthy control persons after brush biopsy were double labelled with antibodies directed against acetylated α-tubulin (green) and DNAH5 (red). Both proteins co-localize (yellow) along the entire length of the ciliary axoneme in both type of cells. Nuclei were stained with Hoechst33342 (blue). DIC represents differential interference contrast. Scale bar equals 10 μm.

Motor protein composition of human FT cilia

To further analyse the composition of FT ciliary motor proteins, cryosections as well as epithelial cell strips from the infundibulum segment were used for additional IF stainings. The subcellular localization of several ODA components, the axonemal dynein heavy chains DNAH5 (Fig. 3) and DNAH9 (Fig. 4A) as well as the axonemal dynein intermediate chains DNAI1 and DNAI2 (Fig. 4B and C), showed an identical distribution in human FT cilia as in respiratory cilia (Supplementary data, Fig. S1). Similarly, components of the IDA, DNALI1 (Fig. 4D), and N-DRC, LRRC48 (Fig. 4E), and the N-DRC-related protein, CCDC39 (Fig. 4F), showed the same axonemal staining pattern in human FT as in respiratory cilia (Supplementary data, Fig. S1).

Ciliary beating pattern and frequency in human fallopian tubes

HVMA analysis of 25 videos of FT cell strips at 25°C revealed a mean ciliary beat frequency of 3.48 Hz, which is slightly below the normal range (4.25–11.63 Hz) in respiratory cilia (Raidt *et al.*, 2014). HVMA of ciliated cell strips showed a similar ciliary beating pattern to that in respiratory cells with distinct forward and recovery strokes (Supplementary data, Videos S1 and S2). Top view analysis showed a regular planar beating along the longitudinal axis without major deviation to the side. Ciliary beating of neighbouring cilia was coordinated (Supplementary data, Video S3). We did not observe any significant differences in beat frequency or pattern in cilia of the different anatomical FT segments.

Cilia-driven transport in human fallopian tubes

We next assessed by HVMA whether FT cilia could generate a directed fluid flow. Indeed, the fast and synchronized movement of FT cilia produced a laminar fluid flow on the surface of the FT cells. This laminar flow resulted in a prompt transport of fluorescent beads from the lateral margin of the FT towards the uterus at a mean velocity of 32.43 μm/s (Fig. 5; Supplementary data, Video S4).

Child bearing in female PCD individuals

Based on our analysis that FT and respiratory motile cilia share an identical motor protein composition and a similar ciliary beat pattern and frequency, we searched our PCD database for information on gestation and childbirth in PCD-affected females with severe defects of ciliary motor proteins. We identified nine PCD-affected females who had given birth to children without any reproductive assistance. Each of these individuals displayed classical PCD features including chronic rhinosinusitis, chronic wet cough, recurrent pneumonia and bronchiectasis. In three females (OP-118, OP-527 III, OP-515), genetic analysis revealed biallelic mutations predicting the complete absence of the motor protein DNAH5 in the ciliary axoneme. In OP-1795 II3, a homozygous mutation in the gene *RSPH4A* leads to the complete absence of radial spoke head proteins RSPH1, RSPH4A and RSPH9. In seven out of nine patients, respiratory MMC were available for IF, which showed the absence of essential motor proteins in all of these individuals. A summary of diagnostic details is given in Table I.

Discussion

We here report a detailed analysis of motor protein composition and function of human FT MMC. We identified MMC along the entire oviduct (Fig. 2). The density of multiciliated cells was highest in the fimbria and decreased towards the uterus, consistent with the findings described by TEM (Crow *et al.*, 1994). A detailed analysis of ciliary motor protein composition by IF identified an identical localization of proteins known to be essential in driving ciliary motion (ODA components, DNAH5, DNAI1, DNAI2 and DNAH9) and regulating ciliary beating pattern (IDA component, DNALI1, and N-DRC-related components, LRRC48 and CCDC39) compared with multiciliated respiratory cells (Fig. 4). This includes the presence of two different ODA types localizing to distinct proximal (type-1 containing DNAH5) and distal compartments (type-2 containing DNAH5 and DNAH9) of the ciliary axonemes (Fliegauf *et al.*, 2005).

Direct analysis of ciliary transport using HVMA revealed a prompt transport of particles towards the uterus (Supplementary data, Video S4 and Fig. 5). Consistent with our IF findings showing an identical motor protein composition, we observed an identical ciliary beating pattern in FT cilia as in respiratory cilia by HVMA (Supplementary data, Videos S1–S3). We observed a slightly lower ciliary beat frequency in FT cilia than in respiratory cilia and in FT samples from non-pregnant individuals (Lyons *et al.*, 2002). This is possibly caused by high progesterone levels at the end of pregnancy. Progesterone has been shown to decrease ciliary beat frequency (Mahmood *et al.*, 1998; Bylander *et al.*, 2013). In another study, measurements performed from the isthmic parts of oviducts from women undergoing hysterectomy for benign disease conditions revealed a much higher ciliary beat frequency of >10 Hz (Liao *et al.*, 2012). A positive correlation between ciliary beat frequency and temperature is well known (Sisson *et al.*, 2003). Thus, this discrepancy with our findings can be explained at least partly by the higher temperature of 37°C used during measurements in that study. Similar to previous findings (Lyons *et al.*, 2002), we did not observe variations in ciliary beat frequency or pattern in the different anatomical sites of the oviduct.

PCD is a disorder of motile respiratory cilia displaying a remarkable genetic heterogeneity. Currently, mutations in >30 different genes have been linked to PCD (Werner *et al.*, 2015). Phenotypically, ciliary beating in PCD can be divided into variants with static cilia, variants with normal or increased beat frequency but abnormal ciliary beating pattern, and variants with failure in generating MMC (Boon *et al.*, 2014; Raidt *et al.*, 2014; Wallmeier *et al.*, 2014). Of note, ciliary beating is always highly dyskinetic in PCD even in variants with normal or increased frequency, and therefore, individuals with these subtypes suffer from severe respiratory disease due to massively impaired mucociliary clearance (Schwabe *et al.*, 2008; Olbrich *et al.*, 2012; Wirschell *et al.*, 2013). Beyond respiratory cilia, PCD affects motile cilia in other organs as well. Dysfunction of cilia of the embryonic node results in randomization of the left–right organ distribution leading to *situs inversus totalis* in 40–50% of individuals (Lucas *et al.*, 2014; Werner *et al.*, 2015) and heterotaxia in a smaller subset of patients (Kennedy *et al.*, 2007; Nakhleh *et al.*, 2012). In PCD-affected males, dyskinesia of the sperm flagella commonly leads to infertility (Fliegauf *et al.*, 2005, 2007). Although it is well known that female oviducts are lined with MMC, the role of FT ciliary function in female reproduction is poorly understood. Based on conclusion by analogy, females with PCD are suspected of impaired fertility

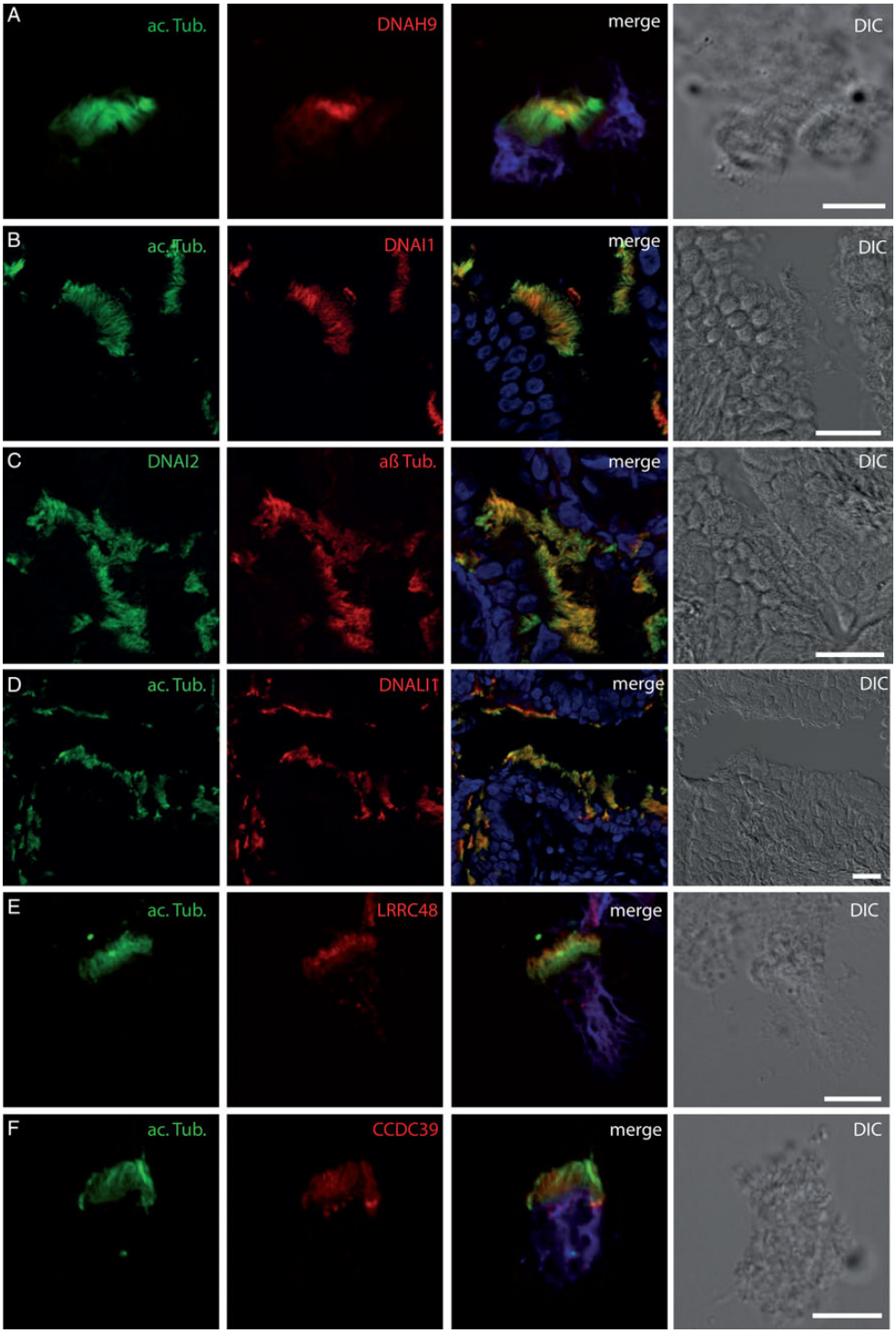

Figure 4 Subcellular localization of ciliary motor proteins in human FT multiciliated cells from healthy control persons. Antibodies directed against acetylated tubulin (**A** and **B**, **D**–**F**; green) or α/ß-tubulin (**C**; red) were used as markers of the ciliary axoneme. Antibodies against ciliary motor proteins DNAH9 (A; red; dynein arm heavy-chain component known to localize to the distal part of the ciliary axoneme in respiratory cilia), DNAI1 (B; red; outer dynein arm intermediate chain), DNAI2 (C; green; outer dynein arm intermediate chain; green), DNALI1 (D; red; inner dynein arm component), LRRC48 (E; red; nexin–dynein regulatory complex subunit) and CCDC39 (F; red; nexin–dynein regulatory complex subunit) were used. Nuclei were stained with Hoechst33342 (blue). DIC represents differential interference contrast. Scale bar equals 10 μm (A, E and F) and 50 μm (B–D), respectively. All proteins except DNAH9 localize along the entire length of the ciliary axonemes. Yellow staining (merge) indicates co-localization of both proteins within the ciliary axoneme. Like in respiratory cells, DNAH9 localizes to the distal part of the ciliary axoneme resulting in a yellow staining of the distal part of the ciliary axoneme, whereas the proximal part remains green (A; merge).

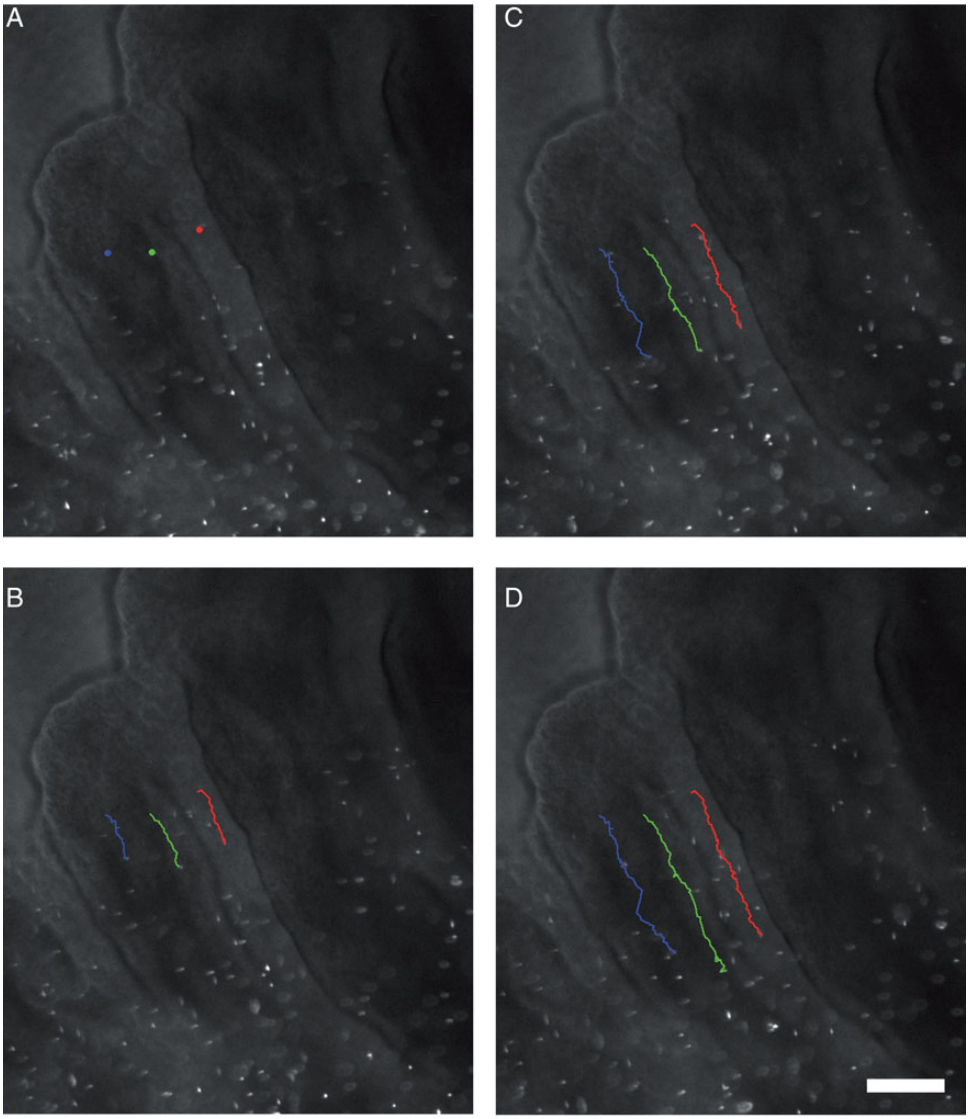

Figure 5 Colour traces of fluorescent beads generated by synchronized movement of fallopian tube cilia. Fluorescent beads were transported from the lateral margin of the fallopian tube towards the uterus at a mean velocity of 32.43 μm/s. Images were taken at times 0 ms (**A**), 100 ms (**B**), 200 ms (**C**) and 300 ms (**D**).

(Afzelius and Eliasson, 1983; McComb *et al.*, 1986; Lurie *et al.*, 1989; Halbert *et al.*, 1997; Lyons *et al.*, 2006). By contrast, we report here a series of nine female PCD individuals giving birth to children after spontaneous conception. This agrees with previous case reports (Jean *et al.*, 1979; Eliyahu and Shalev, 1996). In six out of these nine females, respiratory cilia were devoid of the ODA component, DNAH5. Biallelic mutations in *DNAH5* predicting the complete absence of this motor protein were present in three individuals (OP-118, OP-515 and OP-527 II1). In patient OP-1795 II3, a homozygous mutation in the gene, *RSPH4A*, leads to the absence of the radial spoke head proteins, RSPH1, RSPH4A and RSPH9. Only in patient OP-2049, a female with situs inversus totalis, chronic rhinosinusitis and bronchiectatic lung disease, we were not able to demonstrate the ultrastructural or genetic defect due to lack of material. Taken together, we here show that spontaneous conception can occur in females with the absence of essential motor proteins in the axoneme of motile cilia and severely impaired respiratory mucociliary clearance. This indicates that the transport of the ovum towards the uterus does not seem to be completely dependent on cilia-generated transport.

Our study exhibits limitations that warrant further discussion. As FT tissue samples from females affected by PCD were not available for HVMA or IF analysis, we were not able to demonstrate that FT cilia of PCD females indeed display the same defect as respiratory cilia. Thus, direct proof that FT cilia harbour the same pathogenic defect as respiratory cilia is lacking, even if our findings are highly suggestive. In addition, we did not investigate the role of non-cilia-dependent transport mechanisms such as muscular contractions or mucus composition (Lyons *et al.*, 2006). Ciliary beating is driven by the coordinated activity of motor proteins. This process again is regulated by multiple factors such as age (Ho *et al.*, 2001), hormones (Mahmood *et al.*, 1998; Jain *et al.*, 2012; Bylander

Table I Diagnostic findings in PCD-affected females who gave birth after spontaneous conception.

Subject	HVMA of respiratory MMC	TEM/IF of respiratory MMC	Mutation	No. of children	Body composition
OP-118	Uncoordinated CBP; minimal residual motility	ODA defect/axonemal absence of DNAH5	***DNAH5*:** c.8029C>T [p.Arg2677*] + c.10813G>A [p.Asp3605Asn]	1	Situs inversus
OP-527 III	Uncoordinated CBP; minimal residual motility	ODA defect/axonemal absence of ODA component DNAH5	***DNAH5*:** c.10815delT [p.Pro3606Hisfs22*] + c.8642C>G [p.Ala2881Gly]	1	Situs solitus
F-725 II3	Stiff, uncoordinated CBP; strongly reduced CBF < 1 Hz	n.a./absence of DNAH5 in the distal ciliary axoneme	***DNAI1*:** c.463delA [p.Lys155Argfs2*] + n.a.	3	Situs inversus
F-649 II2	Immotile cilia	ODA defect/axonemal absence of DNAH5 and IDA component DNALI1	n.a.	1	Situs inversus
OP-515	n.a.	n.a.	***DNAH5*:** c.376delG [p.Val126Tyrfs22*] + c.13194_13197delCAGA [p.Asp4398Glufs16*]	1	Situs inversus
OP-439	Immotile cilia	ODA defect/axonemal absence of DNAH5	***DNAH5*:** c.3598+2T>C + c.10.616G>A [p.R3539H]	2	Situs inversus
OP-1795 II3	Abnormal circular CBP; normal CBF	Tubular transposition defect in subset of cilia/axonemal absence of radial spoke head proteins RSPH1, RSPH4A and RSPH9	***RSPH4A*:** c.1391G>A [p.Gly464Glu] (hom)	1	Situs solitus
OP-2049	Stiff, uncoordinated CBP; normal CBF	n.a.	n.a.	2	Situs inversus
OP-1728	Immotile cilia	ODA defect/axonemal absence of DNAH5	n.a.	1	Situs solitus

CBF, ciliary beat frequency; CBP, ciliary beat pattern; HVMA, high-speed video microscopy analysis; IDA, inner dynein arm; IF, immunofluorescence microscopy analysis; MMC, multiple motile cilia; n.a., not available; ODA, outer dynein arm; PCD, primary ciliary dyskinesia; TEM, transmission electron microscopy.

et al., 2013; O et al., 2013), calcium and cyclic nucleotide pathways (Li et al., 2000; Schmid and Salathe, 2011), chemokines (Allen-Gipson et al., 2004; Papathanasiou et al., 2008) and irritants (Zhou et al., 2009; Wyatt et al., 2013). It was the purpose of our study to analyse the motor protein composition of FT cilia and the resulting ciliary beating. We found an identical motor protein composition compared with respiratory cilia and consequently a very similar ciliary beating. A detailed analysis of ciliary beat regulation by different agents was beyond the scope of the study. Furthermore, our series of nine PCD females with apparently normal fertility does not exclude the possibility that subfertility is increased in this patient group. As it was not the main intention of our PCD database to collect information on fertility, data completeness was insufficient for a more detailed statistical analysis. Ultimately, large epidemiological studies will be needed to further delineate the exact extent of female (sub-) fertility in PCD. For this purpose and other pertinent questions on presentation, course and co-morbidity of PCD, we have set up an international PCD registry that is currently recruiting patients (www.pcdregistry.eu).

In conclusion, we have demonstrated that human female oviducts are lined by MMC and show the same ciliary motor protein composition and beating pattern as respiratory MMC. MMC activity in FTs leads to a coordinated particle transport towards the uterus. However, at least a subset of individuals with severe dysfunction of motile cilia can conceive spontaneously, indicating that impaired ciliary function may be compensated by other mechanisms. Epidemiological studies are needed to elucidate the exact extent of subfertility in these women.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

H. Omran and C.W. designed the study. C.W. and J.R. drafted the manuscript and prepared the figures. R.S. provided the FT samples. C.W. and J.R. provided clinical data. J.R. and C.W. performed the HVMA experiments. J.R., N.T.L. and P.P. performed the IF experiments. G.W.D.

and N.T.L. contributed to the interpretation of data and drafting the article. J.R., P.P. and T.M. performed and analysed the ciliary transport function experiments. H. Olbrich assisted in drafting the manuscript and figures. All authors read and critically revised the manuscript and provided their final approval for publication.

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Conflict of interest

None declared.

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