De novo mutations in FOXJ1 result in a motile ciliopathy with hydrocephalus and randomization of left / right body asymmetry

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Abstract:

Hydrocephalus remains the most prevalent forms of developmental central nervous system (CNS) malformation treated by neurosurgeons. The cerebrospinal fluid (CSF) flow depends on both heartbeat and body movement. Furthermore it has been shown that CSF flow within and across brain ventricles depends on cilia motility of the ependymal cells lining the brain ventricles, which play a crucial role to maintain patency of the narrow sites of CSF passage during brain formation in mice. Using whole exome and whole genome sequencing we...
identified the first autosomal dominant cause of a distinct motile ciliopathy related to defective ciliogenesis of the ependymal cilia. Heterozygous de novo mutations in FOXJ1, which encodes a well-known member of the forkhead transcription factors important for ciliogenesis of motile cilia, cause a motile ciliopathy that is characterized by hydrocephalus internus, chronic destructive airway disease and randomization of left / right body asymmetry. Mutant respiratory epithelial cells are unable to generate a fluid flow and exhibit a reduced number of cilia per cell, as documented by high-speed video microscopy, transmission electron microscopy (TEM) and immunofluorescence analysis (IF). TEM and IF demonstrate mislocalized basal bodies. In line with this finding, the expression of the focal adhesion protein PTK2 is reduced and aberrant in the cytoplasm of the mutant respiratory epithelial cells.

Main Text:

Hydrocephalus remains the most prevalent form of developmental central nervous system (CNS) malformation treated by pediatric neurosurgeons. While trauma, intraventricular hemorrhages, and CNS infections account for the majority of cases, heritable genetic mutations in human hydrocephalus are relatively rare. Here, we identify a novel genetic cause related to dysfunction of the CNS ependymal cilia.

Multiple motile cilia in the respiratory tract, the ependyma or the female fallopian tubes as well as motile monocilia in the embryonic left / right organizer generate the mechanical force to drive extracellular fluid flow in a continuous and coordinated fashion. While formation of a single cilium is a complex process depending on several hundreds of different factors, ciliogenesis in multiciliated cells additionally requires development of a network of oriented cilia within a short period of time. Defects in ciliary generation or motility lead to a mucociliary clearance disorder associated with laterality defects, male infertility and very rarely hydrocephalus, which is referred to as primary ciliary dyskinesia (PCD). So far recessive mutations in 44 genes have been identified to cause PCD [refs].

Here, to our knowledge, we present the first autosomal dominant cause of a distinct motile ciliopathy. Heterozygous de novo mutations in FOXJ1, which encodes a well-known member...
of the forkhead/winged-helix transcription factor DNA binding proteins, cause a motile ciliopathy that is characterized by hydrocephalus internus, chronic destructive airway disease and randomization of left/right body asymmetry.

 Whole exome sequencing performed as previously described, in a cohort of heterozygous loss-of-function mutations in FOXJ1 in two non-related individuals (OP-1743 II1 c.901G>T, p.Glu301*; OP-1933 II1 c.826C>T, p.Gln276*). In an additional cohort of individuals with mucociliary clearance disorder, a proportion of whom had hydrocephalus, whole exome sequencing revealed four further affected individuals with heterozygous variants in FOXJ1 (OP-2950 II1 c.868_871dup, p.Thr271Lysfs*12; US-1 II1 c.826C>T, p.Gln276*; US-2 II1 c.939delC, p.Ile314Serfs*19; RBH II1 c.967delG, p.Glu323Serfs*10) (Figure 1, Supplemental Figure S1). These FOXJ1 variants were not identified in any of the parents or the non-affected siblings in the families and were therefore considered to have arisen de novo. For affected individuals, parental DNA was not available. No mutations in other motile ciliopathy-related genes were identified (Figure 1, Supplemental Figure 1). We systematically screened of a total 354 individuals with mucociliary clearance disorders but without co-occurrence of hydrocephalus and did not identify any FOXJ1 mutations.

 FOXJ1 (CCDS32739) is located on chromosome 17q25.1 and comprises two coding exons and one alternative first exon, encoding a 2,641 bp transcript and predicting a 421 amino acid protein (Figure 1). Consistent with a mutational hotspot in the FOXJ1 C-terminal region (Figure 1), all identified mutations localize within a small region of exon 3. Interestingly the variant (c.826C>T, p.Gln276*) occurred de novo in two non-related individuals, respectively (OP-1933 II1 and US-1 II1), emphasizing, that this gene region is especially susceptible to de novo variants.
Consistent with haploinsufficiency being the disease cause, all variants reported are predicted to result in a premature termination codon (nonsense or frameshift type allele). In addition, both the The GnomAD gene constraint scores for FOXJ1 both utilized to predict likely haploinsufficient genes (pLI=0.97, oe=0) place it also indicate a high LOF intolerance consistent with in the haploinsufficient/haploinsufficient gene category i.e. the a high intolerance to loss-of-function alleles. The significance of our genetic findings is also supported by the fact that de novo mutations are very rare events in humans. While 45 to 60 de novo single nucleotide variants occur on average per individual, only one to two de novo mutations affect the coding sequence. Interestingly, haploinsufficiency has also been reported in other genes encoding forkhead transcription factors such as FOXC1 (FKHL7) (pLI=0.95, oe=0) and FOXC2 (FKHL14), which are associated with aberrant ocular development.

We next performed 3' mRNA-sequencing in FOXJ1 mutant respiratory cells (OP-1743 II1 and OP-2950 II1) to analyze the effect of the detected de novo FOXJ1 mutations on the transcript level. Consistent with haploinsufficiency, FOXJ1 transcripts are reduced compared to healthy controls (Supplemental Figure S8). Furthermore, also direct FOXJ1 gene targets encoding for ciliary axonemal proteins are also reduced in FOXJ1 mutant cells. FOXJ1, also referred to as hepatocyte nuclear factor-3 / forkhead homologue 4 gene (HFH-4), has been studied in detail in the past. Consistent with a distinct functional role for motile cilia, FOXJ1 expression has been detected in ciliated cells of the ependyma lining the brain ventricles, airways, oviduct, and the embryonic left / right organizer. Targeted mutation or knock-down of Foxj1 in zebrafish, Xenopus laevis and mice resulted in a motile ciliopathy characterized by a reduced number of multiple motile cilia (MMC) and mislocalized basal bodies, which nucleate ciliary axonemes. These findings demonstrate the important functional role of FOXJ1 for the generation of motile cilia.

All six individuals with pathogenic FOXJ1 variants exhibited hydrocephalus internus (Figure 2, Supplemental Figure S2). In five affected individuals (OP-1743 II1, OP-1933 II1, RBH II1, Wallmeier et al.)
US-1 II1 and US-2 II2 obstructive hydrocephalus was detected within the first few weeks of life, which required immediate treatment by insertion of a ventriculo-peritoneal shunt system to relieve elevated intracranial pressure. Interestingly, hydrocephalus internus in OP-2950 II1 was detected at age 54 years, when clinical examination revealed macrocephaly, gait ataxia and optic atrophy consistent with a long-standing, increased intracranial pressure. Cranial magnetic resonance imaging (MRI) confirmed obstructive hydrocephalus internus.

The flow of cerebrospinal fluid (CSF) depends on heart-beat as well as body movement. In zebrafish, it has been proposed that the distribution of CSF within and across brain ventricles depends on ciliary motility. CSF is produced by the choroid plexus in both lateral ventricles and the choroid plexus of the third and the fourth ventricle. Before its re-absorption, the CSF traverses several narrow spaces such as the aqueduct of Sylvius (to enter the fourth ventricle), three openings including the foramen of Magendii, and the lateral apertures (to enter the subarachnoid spaces). We have previously reported that motile cilia of the ependymal cells lining the brain ventricles play a crucial role to maintain patency of the narrow sites of CSF passage during brain formation. Consistent with the role of FOXJ1 for the generation of motile ependymal cilia, cranial MRI studies demonstrated stenosis of the cerebral aqueduct and/or foramina Magendii and Luschka responsible for the obstructive hydrocephalus internus in all six patients (Figure 2, Supplemental Figure S2). In agreement with this observation, Foxj1-deficient mice develop hydrocephalus. Therefore, we assume that hydrocephalus is a characteristic clinical finding in FOXJ1-mutant individuals.

While the prevalence of infant hydrocephalus occurs with a frequency of approximately one per 1,000 births, most cases are of post-haemorrhagic nature due to prematurity (malabsorption of CSF). About 10% are related to primary causes but so far only a small number of associated genes have been identified. In PCD individuals with severely impaired cilia beating due to altered axonemal motor protein composition of multiciliated cells (MCC), the prevalence of hydrocephalus is only slightly increased (approximately 110). Individuals carrying recessive mutations in MCIDAS and CCNO, which cause severe multiciliogenesis
defects of MCCs, develop hydrocephalus much more often (10%). Nevertheless, hydrocephalus is not an obligatory finding in cases arising from these multiciliogenesis defects and hydrocephalus has been shown by Behan et al. to not be indicative for overall PCD, because hydrocephalus is very rarely present in PCD individuals. Therefore, aberrant beating or reduced numbers of ependymal cilia alone is not sufficient to explain the occurrence of human hydrocephalus.

Besides its function in motile ciliogenesis, FOXJ1 is known to be essential for ependymal cell maturation, which might contribute to the development of hydrocephalus in FOXJ1-mutant individuals. During early postnatal periods, radial glial cells in various ventricular zones of the brain differentiate into ependymal cells and astrocytes. In mice, it has been shown that Foxj1 expression in the lateral ventricle is required for the differentiation of radial glial cells into ependymal cells and a small subset of Foxj1(+) astrocytes into a postnatal neural stem cell niche. A chemical screen for modulators of ependymal cell differentiation found that the mature multiciliated ependymal needs constant FOXJ1 expression to prevent cellular dedifferentiation back to a glial-like morphology. Interestingly, Abdi et al. reported that ependymal FOXJ1 has a short half-life, requiring non-canonical IkB kinase activity to prevent rapid degradation via the ubiquitin proteasome system. Thus, constant Foxj1 expression is crucial to maintain ependymal cell differentiation and prevent hydrocephalus. Interestingly, autosomal dominant mutations in FOXG1, another member of the forkhead gene family, cause a neurodevelopmental disorder associated with brain malformations including corpus callosal dysgenesis, indicating that forkhead transcription factors play a crucial role in neurodevelopment.

Consistent with a mucociliary clearance disorder also affecting the generation of MMC of the airways, all FOXJ1-mutant individuals suffered from recurrent infections of upper and lower airways, chronic productive cough, bronchiectasis as well as chronic rhinitis and sinusitis (Figure 2, Supplemental Figure S2). Postnatal respiratory distress syndrome was present in four individuals. This respiratory phenotype resembles that of PCD. Interestingly, the nasal
nitric oxide (NO) production rate is usually markedly reduced in PCD individuals. However, nasal NO production rates of the four tested FOXJ1-mutant individuals OP-1933 II1 (141 nl/min), OP-2950 II1 (122 nl/min), RBH II1 (215 nl/min) and US-2 II1 (328 nl/min) were within normal ranges. Thus, nasal NO-measurement cannot be used to screen for individuals with FOXJ1 mutations, and affected individuals will not be identified by the nasal NO testing implemented early within the current diagnostic workup for PCD.

Affected OP-2950 II1, US-1 II1 and US-2 II1 exhibited situs inversus, consistent with previous observations in mice, *Xenopus laevis* and zebrafish, indicating that FOXJ1 has an important functional role in left / right body asymmetry determination during early embryogenesis. Interestingly, OP-1933 II1 presented with a ventricular septal cardiac defect, which might reflect the increased incidence of congenital heart defects in motile ciliopathies associated with randomization of left / right body asymmetry compared to the healthy population.

The female patient, RBH II1, was diagnosed on imaging with hydrosalpinx following presentation with abdominal pain at 15 years old. OP-2950 II1 was unable to become pregnant even after in-vitro fertilization. MMC also line the fallopian tube, and there are several reports of individuals with PCD where fallopian tube cilia defects mirror those in the respiratory tract, possibly causing subfertility and ectopic pregnancy.

Consistent with a defect also affecting sperm flagella, the male affected individual US-2 II1 was not able to father a child. Fertility evaluation revealed an adequate sperm count but with severe reduction in the motility and number of spermatozoa as well as a change in sperm morphology (severe oligoasthenoteratospermia). Further studies will reveal whether male infertility is indeed a constant finding in FOXJ1 mutant males.

To study the effects of FOXJ1 haploinsufficiency at the cellular level, we analyzed respiratory epithelial cells from FOXJ1-mutant individuals and healthy controls by high-speed video microscopy analysis (HVMA). HVMA in OP-1933 II1, OP-2950 II1 and RBH II1 showed a...
reduced number of motile cilia per MCC. The number of cilia per MCC varied between zero to
almost normal numbers, whereas however most cells were lined with very few cilia per cell
(Supplemental Figure S3). To corroborate these findings, we also investigated the amount of
cells with i) normal (>100 cilia per MCC), ii) slightly reduced (4-100 cilia per MCC) and -iii)
severely reduced (0-4 cilia per MCC) numbers of cilia in FOXJ1-mutant individuals (OP-1743
II1, OP-1933 II1 and OP-2950 II1) and healthy controls. In all FOXJ1 mutant individuals the
number of cells with normal amounts of cilia was reduced compared to healthy controls
Supplemental Figure S3). The residual cilia of their MCCs exhibited a stiff beating pattern with
reduced beating amplitude (Supplemental Video files S1-S4). To distinguish between
reduced generation and secondary loss of MMC, we cultured primary respiratory epithelial
cells from OP-1743 II1, OP-1933 II1 and OP-2950 II1 and performed in-vitro ciliogenesis
experiments in spheroid and air-liquid interface (ALI-) cultures. HVMA after in-vitro ciliogenesis
of spheroid cultures confirmed reduced numbers of cilia per MCC, as well as the abnormal
beating pattern, in samples from OP-1933 II1 and OP-2950 II1. This is consistent with a
primary defect of ciliogenesis, as previously reported in various FOXJ1-deficient model
organisms10,12,30 (Supplemental Video files S5-S7). Next, we tested whether the residual
motile cilia of FOXJ1-mutant cells are still able to generate a directed fluid flow. To mimic the
process of particulate lung clearance in-vitro, we added fluorescent particles to the apical
compartment of the ALI-Transwell® inserts from OP-1743 II1 and OP-2950 II1 as well as
healthy controls and performed particle-tracking experiments. Consistent with aberrant
mucociliary clearance, FOXJ1-mutant cilia were not able to propel mucous along the surface
of the differentiated epithelium (Figure 3, Supplemental Video files S8-S13).

To further characterize the ciliogenesis defect at the cellular level, we performed
transmission electron microscopy (TEM) as previously described22 on native respiratory
epithelial cells after nasal brushing (OP-1743 II1, OP-1933 II1, OP-2950 II1, RBH II1, US-1,
US-2) as well as after spheroid cultures (OP-1933 II1; OP-2950 II1). Consistent with a defect
in ciliogenesis, the number of cilia per MCC was markedly reduced in most cells across all the
different samples (Figure 4). The apical cell regions showed a severe decrease of basal
Wallmeier et al.
Whereas the overall number of basal bodies per MCC did not seem to be altered, basal bodies were mislocalized within the cytoplasm, indicating a defect in apical docking that is consistent with previous reports on MCCs in Foxj1-deficient mice\textsuperscript{10,36} (Figure 4).

To corroborate these findings, we performed high-resolution immunofluorescence microscopy analyses (IFs) with antibodies targeting acetylated α-tubulin (marker of the ciliary axonemes) and the centrosomal protein 164 / CEP164 (marker of basal bodies), as previously described\textsuperscript{22}. As expected, the number of cilia per MCC in samples of FOXJ1-mutant individuals varied, but was severely reduced in most cells, consistent with our findings obtained by HVMA and TEM. The number of CEP164-positive basal bodies per MCC was not altered but basal bodies were mislocalized within the cytoplasm, consistent with a basal body docking defect also documented by TEM (Figure 4).

Because focal adhesion components have been shown to be responsible for anchoring basal bodies to the actin network of multiciliated cells\textsuperscript{37}, we analyzed PTK2 / protein tyrosine kinase 2 (also known as focal adhesion kinase / FAK) localization in respiratory epithelial cells of healthy controls utilizing anti-PTK2 antibodies. Consistent with previous findings in Xenopus\textsuperscript{37}, PTK2 localizes in the basal body area in respiratory epithelial cells of healthy controls (Figure 5). In line with a basal body docking defect, PTK2 localization was markedly reduced in FOXJ1-mutant respiratory epithelial cells (Figure 5).

Because FOXJ1 is not only known to be involved in ciliogenesis in multiciliated cells but also for expression of axonemal proteins related to ciliary motility\textsuperscript{12,13}, we thoroughly examined the axonemal structure and composition of FOXJ1-mutant respiratory cilia by TEM and IF. Interestingly, ciliary cross sections often exhibited various ultrastructural abnormalities. Whereas some cross sections did not show any abnormalities of the 9+2 axonemal architecture, all affected individuals exhibited defects of tubular organization, or missing central tubules (Supplemental Figure S6). Thus, we found heterogeneous ultrastructural defects in FOXJ1-mutant cilia indicating that TEM is not a sufficient method in FOXJ1 mutant individuals.
In PCD, ciliary defects are typically restricted to specific structures depending on the underlying genetic defect (e.g., ODA defects in DNAH5 or DNAI2 mutations). However, cilia in FOXJ1-mutant cells showed various abnormalities. To further elaborate these findings, we next studied respiratory cells from FOXJ1-affected individuals using antibodies targeting distinct axonemal structural components such as i) the ODA intermediate chain DNAI2 and heavy chain DNAH5 (absent in PCD subjects with ODA defects), ii) the nexin-dynein regulatory complex protein GAS8 (absent in PCD subjects with defects of microtubular organization e.g. due to pathogenic CCDC40 or CCDC39 variant), iii) the radial spoke head protein RSPH4A (absent in PCD subjects with pathogenic RSPH4A variants). However, subtle reductions of protein content cannot be detected by IF. Thus, normal IF analyses of the residual cilia did not show any gross abnormality but this does not rule out subtle axonemal defects as detected by TEM (Supplemental Figure S7). We previously reported mutations in CCNO or MCIDAS causing a mucociliary clearance disorder referred to as reduced generation of multiple motile cilia (RGMC), due to a defect of mother centriole generation and migration at the late stage of MMC generation. FOXJ1 probably acts downstream of MCIDAS and in parallel to CCNO in the NOTCH1-dependent pathway of multiciliogenesis (Figure 6). While FOXJ1 deficiency can be classified as RGMC, the cellular disease phenotype is distinct from CCNO or MCIDAS defects. FOXJ1 haploinsufficient MCC in the respiratory tract exhibit a normal number of basal bodies, which are mislocalized within the cytoplasm due to a basal body docking defect. OP-2950 II1 exhibits situs inversus, which is consistent with previous reports in mice, zebrafish and Xenopus laevis that FOXJ1 deficiency causes randomization of the left / right body asymmetry. This implies that determination of left / right asymmetry is independent of the NOTCH1-dependent multiciliogenesis pathway. NOTO is a homeodomain transcription factor specifically expressed at the left / right organizer of mouse and other vertebrate embryos; NOTO transcriptionally activates FOXJ1 and thus also regulates ciliogenesis. This NOTO-dependent activation of FOXJ1 at the left / right organizer is crucial for proper determination of the left / right asymmetry, probably explaining situs anomalies in some FOXJ1-mutant individuals (Figure 6).
This study emphasizes the pathophysiological link between the development of hydrocephalus and a severe mucociliary clearance disorder, which should be considered in clinical care of affected individuals with hydrocephalus and respiratory symptoms. Early clinical and genetic diagnosis will aid implementation of appropriate neurological as well as respiratory care in FOXJ1-mutant individuals.
Supplemental Data:

Supplemental Figure S1: Pedigrees of the families OP-1743, OP-1933, OP-2950, RBH, US-1 and US-2.

Supplemental Figure S2: FOXJ1-mutant individuals showing bronchiectasis, randomization of the left / right body asymmetry obstructive hydrocephalus and hydrosalpinx.

Supplemental Figure S3: FOXJ1-mutant respiratory epithelial cells show variable number of cilia per cell.

Supplemental Figure S4: PTK2 localizing in the basal body region is mislocalized in FOXJ1 mutant cells.

Supplemental Figure S5: Western blot analysis with the monoclonal antibody directed against PTK2.

Supplemental Figure S6: Ciliary cross sections of FOXJ1-mutant respiratory epithelial cells show variable structural defects by TEM.

Supplemental Figure S7: Analysis of ciliary axonemal components in multiple motile cilia of FOXJ1-mutant respiratory epithelial by IF.

Supplemental Figure S8: Air-liquid interface (ALI) cultured FOXJ1-mutant respiratory epithelial cells show reduced transcript levels for FOXJ1 and cilia axonemal components.

High-speed video microscopy of native respiratory epithelial cells:
Video S1_Control - respiratory epithelial cells-native
Video S2_OP-1933 II1 - respiratory epithelial cells-native
Video S3_OP-2950 II1 - respiratory epithelial cells-native
Video S4_RBH II1 - respiratory epithelial cells-native

High-speed video microscopy of cultured respiratory epithelial cells:
Video S5_Control - respiratory epithelial cells-culture
Video S6_OP-1933 II1 - respiratory epithelial cells-culture
Video S7_OP-2950 II1 - respiratory epithelial cells-culture
Particle tracking (exemplary for day 44)

Video S8_Control_Particle tracking_day44_DIC
Video S9_Control_Particle tracking_day44_tracking
Video S10_OP-1743 II1_Particle tracking_day44_DIC
Video S11_OP-1743 II1_Particle tracking_day44_tracking
Video S12_OP-2950 II1_Particle tracking_day44_DIC
Video S13_OP-2950 II1_Particle tracking_day44_tracking

Declaration of Interest: The authors declare no competing interests.

Acknowledgements: We thank the PCD affected individuals and their families for their participation and acknowledge the German PCD support group “Kartagener Syndrom und Primäre Ciliaäre Dyskinesie e.V” and the UK PCD Family Support Group. We would like to thank Karl Peter Schlingmann for the discussion. We thank A. Borgscheiper, A. Dorißen, D. Ernst, S. Helms, M. Herting, A. Robbers, L. Schwiddessen, F.J. Seesing, S. Sivalingam, M. Tekaat, K. Wohlgemuth, C. Westermann and S. Wilkinson for excellent technical work. This work was supported by the Deutsche Forschungsgemeinschaft WA 4283/1-1 to J.W., OM6/7, OM6/8, OM6/10 and OM6-11/ DFG KFO 326 to H.O., OL450/1 (H.Ol.), by the IZKF Muenster to H.O. (Om2/009/12 and Om/015/16), the “Innovative Medical Research” of the University of Muenster Medical School to N.T.L. (I-LO121517) and WA 1 2 14 18 (J.W.)) and “Dekanat der Medizinischen Fakultät der WWU” to J.W. The authors participate in the COST action BEAT-PCD: Better Evidence to Advance Therapeutic options for PCD network (BM11407). H.M.M. acknowledges Great Ormond Street Children’s Charity (V1299, V2217) and the NIHR Great Ormond Street Hospital Biomedical Research Centre (GOSH BRC). We thank Sayyid Hasan (RBHT) as WL GMC Validation Coordinator for results from the UK 100K Project. We acknowledge Dr. Anne Schmidt for the clinical workup of patient RBH II-1 as well as Mitali P. Patel for the IF-stainings. The findings in patient RBH II1 were made possible through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health). The 100,000 Genomes Project is funded by the National Institute for...
Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. The 100,000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support.


Websites:

https://gnomad.broadinstitute.org/

https://nmprediction.shinyapps.io/nmdescpredictor/

References:


4. The 100,000 Genomes Project Protocol.


Figure 1: De novo pathogenic variants in FOXJ1 found to be heterozygous in affected with hydrocephalus and chronic destructive airway disease. (A) Schematic overview of chromosome 17. FOXJ1 (CCDS32739) is located on chromosome 17q25.1 (red mark). (B) FOXJ1 consists of two coding exons and one alternative first exon encoding a 2641 bp transcript and 421 amino-acid protein. (C) Electrophotographs of Sanger sequencing results for family OP-1933, OP-1743, OP-2950, RBH, and US-1. Consistent with de novo mutations, none of the variants were identified in either the parents or non-affected siblings. In US-2 no parental DNA was available.

Figure 2: Affected with pathogenic FOXJ1 variants display obstructive hydrocephalus, randomization of left / right body asymmetry and a chronic destructive airway disease. (A) Cranial magnetic resonance imaging of OP-1933 II1 was performed after shunt insertion (right lateral ventricle) to relieve raised intracranial pressure. The left lateral ventricle and the third ventricle are dilated. The lateral view documents stenosis of the aqueduct of Sylvius and a small fourth ventricle. OP-2950 II1 shows massively dilated brain ventricles. Lateral view indicates a patent aqueduct and a dilated fourth ventricle due to closure of the foramen of Magendii and the lateral apertures. (B) Chest X-ray of OP-2950 II1 shows situs inversus totalis. The computed tomography scan of OP-2950 II1 and RBH II1 exhibit atelectasis and bronchiectasis of the middle lobe. (C) Summary of clinical findings in the affected individuals.

Figure 3:
Air liquid interface (ALI-) cultures of FOXJ1-mutant respiratory epithelial cells are unable to generate a directed fluid flow.

(A) Schematic depicts the experimental set-up of particle tracking analyses performed on ALI-cultured respiratory epithelial cells. (B) Respiratory epithelial cells from FOXJ1-mutants (OP-1743 II1, OP-2950 II1) as well as healthy controls were cultured under ALI-conditions. After complete differentiation, (30 days, 37 days and 44 days after airlift) 0.5 µm fluorescent particles were added to the apical compartments of the cells. Tracking videos are represented as z-stack projections, while the transport direction of each particle is summarized in polar graphs. Under healthy conditions, the fluorescent particles were transported in a linear direction along the cell layer, whereas the particle transport in FOXJ1-mutant cells (OP-1743 II1, OP-2950 II1) was highly reduced (C) and non-oriented (D). For statistical evaluation 15 videos per person were analyzed. Thereby, 253 particles were tracked per video on average. Scale bars represent 20 µm.

Figure 4:

FOXJ1-mutant respiratory epithelial cells show a reduced number of cilia and mislocalized basal bodies by TEM and IF.

(A) Respiratory epithelial cells from a control and OP-1933 II1 are cultured as spheroids. Cilia are stained with antibodies targeting acetylated α-tubulin (acet. Tub.; green) after complete differentiation. Cells of OP-1933 II1 demonstrate a variable reduction of cilia in comparison to the control. (B) TEM photographs of MCC (first row) from a healthy control show basal bodies attached to the apical membrane and nucleating multiple motile cilia. Respiratory epithelial cells from mutant individuals with pathogenic FOXJ1 variants (OP-1743 II1, OP-1933 II1, OP-2950 II1, RBH II1) exhibit mislocalized basal bodies (representative examples shown by red arrows) within the cytoplasm, consistent with a basal body docking defect. Respiratory epithelial cells are stained with antibodies targeting acetylated α-tubulin (acet. Tub.; green) and antibodies targeting mother centrioles (CEP164, red). In control cells, basal bodies (red) are aligned at the apical cell region, whereas in FOXJ1-mutant cells they are mainly mislocalized within the cytoplasm, consistent with TEM findings. Right row shows higher magnification images of regions of CEP164-positive basal bodies. Nuclei were stained with Hoechst33342 (blue).

Figure 5:
PTK2, a member of the subapical protein network, shows abnormal localization in FOXJ1-mutant cells by IF.

Respiratory epithelial cells from control and FOXJ1-mutant individuals (OP-1743 II1, OP-1933 II1, OP-2950 II1) are stained with antibodies targeting PTK2 (green). PTK2, which forms complexes named ciliary adhesions that are associated with basal bodies and striated rootlets, shows reduced localization in FOXJ1-mutant cells compared to the control. Regions around the subapical cell membrane showing PTK2 at higher magnification (right row).

Figure 6:

FOXJ1 is an essential component in signaling pathways for the generation of motile cilia.

Schematics illustrating the function of FOXJ1 in the generation of motile cilia in the NOTCH1- and NOTO-dependent pathway in (A) multiciliated cells and (B) the ciliated cells of the embryonic node, respectively. Pathogenic variants in MCIDAS and CCNO (marked in green) are known to cause a ciliogenesis defect in multiple motile cilia causing a mucociliary clearance disorder referred to as reduced generation of multiple motile cilia (RGMC).