The ciliary impact of non-ciliary gene mutations.

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Mutations in genes encoding centriolar or ciliary proteins cause diseases collectively known as ciliopathies. Interestingly, the Human Phenotype Ontology database lists numerous disorders that display clinical features reminiscent of ciliopathies, but do not involve defects in the centriole-cilium proteome. Instead, defects in different cellular compartments may impair cilia indirectly and cause additional, non-ciliopathy phenotypes. This phenotypic heterogeneity, perhaps combined with the field's centriole-cilium-centric view, may have hindered the recognition of ciliary contributions. Identifying these diseases and dissecting how the underlying gene mutations impair cilia will not only add to our understanding of cilium assembly and function, but may also open up new therapeutic avenues.

Ciliary defects are linked to disease

Cilia are hair-like protrusions exposed at the cell surface that can be immotile or motile (Figure 1). A single, immotile cilium is present on most vertebrate cell types and serves to detect and respond to environmental cues including mechanical, chemical, or, in the case of photoreceptors, light signals. Upon detection the cilium transduces the signal to regulate gene expression, send a nerve signal to connected neurons, or control other cellular processes [1–3]. The best characterized example is the Hedgehog signaling pathway, which in vertebrates involves the primary cilium [4]. Motile cilia are present only on certain cell types and are used to propel cells (e.g. sperm) or move fluids over the cell's surface (e.g. multi-ciliated epithelial cells of the airways) [5].

Cilia are important for various developmental processes, as well as the function and homeostasis of many cell types. Consequently, ciliary defects cause a range of diseases that affect almost all organs and tissues of the human body. Common clinical phenotypes include impaired brain development and intellectual disability, distinct facial features, obesity, skeletal abnormalities, vision and hearing loss, heart malformations, abnormal organ placement, kidney and liver cysts, and respiratory defects. Collectively these diseases are referred to as ciliopathies [5–7]. The total number of identified ciliopathies has been growing continuously over the past decade. Established ciliopathies comprise at least 38 diseases with mutations in at least 247 genes (Figure 2; Supplemental File), all of which affect the assembly, maintenance, or function of centrioles or cilia. In the majority of cases the encoded proteins also localize to these structures. Such cases were recently referred to as **'first-order' ciliopathies** [6]. In contrast, **'second-order' ciliopathies** are caused by mutations in non-centriolar, non-ciliary genes. First and second-order ciliopathies can be further grouped as motile or sensory ciliopathies, depending on the type of cilium affected. Various loss-of-function screens have implicated non-ciliary genes in ciliogenesis and ciliary function [8–11], but most mechanistic studies have focused on the centriole-cilium proteome and how it is linked to first-order ciliopathies. Here we discuss genes with indirect effects on cilia, which are linked to second-order ciliopathies and potentially to diseases that we term **'disorders with ciliary contribution' (DCCs)**. In the case of DCCs, only a subset of the clinical manifestations may result from ciliary impairments, and these may be obscured by additional, non-ciliary phenotypes. Using **Human Phenotype Ontology (HPO)** data (see: http://hpo.jax.org), we identify candidate second-order ciliopathies and candidate DCCs and discuss how the underlying gene defects may impair cilia. This information may serve as starting point for experimental validation and for further mechanistic studies in the future.

The many roads to ciliopathy

Cilia are highly complex organelles that depend on a plethora of proteins for their assembly and roles in signaling and motility. Ciliopathies can result from defects in the cilium itself or from defective centrioles, which serve as platforms for cilium assembly (Box 1). The most direct impact is expected from mutations in the ciliome, which comprises between ~300 (high confidence SYSCILIA 'gold standard' list, based on experimental evidence) [12] and ~1000 ('CiliaCarta', includes SYSCILIA and adds GO annotations and predictions) [13] proteins.

Involvement of the ciliome

Proteins of the ciliome linked to first-order ciliopathies can be classified in four major groups that work together to form functional cilia (Figure 1). All first-order ciliopathies have causative mutations that can be linked to one of these groups. Motile ciliopathies have in common that they specifically affect the motility apparatus, impairing ciliary beating or altering the beating pattern (Figure 1) [6,7]. Some proteins of the ciliome assemble together with a set of scaffold proteins into 70-100 nm cytoplasmic granules known as centriolar satellites [14,15]. Centriolar satellites traffic around centrioles in a microtubule-dependent manner and were found to be important for ciliogenesis in some cell line models [16,17], but a PCM1 knockout mouse only displayed defects in long-term cilia maintenance in some brain tissues [18]. Loss of the centriolar satellite scaffold protein PCM1 interferes with the ciliary localization of several proteins. This suggests that is not the formation of satellites per se, but the incorporation of satellite-associated proteins into cilia, which is crucial [19]. This notion is also consistent with the proposed role of centriolar satellites in protein targeting and cellular proteostasis [15,20].

Apart from defects in the ciliome, mutations that impinge on proteins in other cellular compartments can also cause ciliopathies.

Involvement of proteins not associated with centrioles or cilia

By comparison with the SYSCILIA ciliome [12] and the ciliary protein list compiled by Reiter and Leroux [6], we observed that only about half of the genes associated with 38 established, non-motile ciliopathies (Supplemental File) encode components of centrioles or cilia. The other half localizes to the nucleus and the plasma membrane, as major compartments, is secreted to the extracellular space, or is associated with the ER, the Golgi,

99 and other sites. How do mutations that affect proteins residing in these diverse
100 compartments result in ciliary defects?

101 Nuclear proteins may control the expression of centriolar and ciliary genes. Examples
102 are the transcription factors CRX, NRL, and NR2E3 that cooperate in the regulation of
103 photoreceptor differentiation and homeostasis and that have been linked to retinitis
104 pigmentosa and other retinal diseases [21–25]. Another example is GLIS2, a suppressor of
105 transcription downstream of the Hedgehog signaling pathway that is implicated in
106 nephronophthisis [26,27]. Other nuclear proteins are splicing factors (DHX38, RP9) and core
107 spliceosome proteins (PRPF3, PRPF4, PRPF6, PRPF8, PRPF31, SNRNP200). Some were
108 identified in screens for factors required for centriole biogenesis [28] and ciliogenesis [9,11],
109 and all are linked to retinal degeneration [29–31]. A high demand for splicing activity may
110 explain why the retina is particularly sensitive to systemic splicing defects [32,33].

111 Plasma membrane-associated proteins include surface receptors and associated
112 signal transduction machinery, ion channels, as well as cell adhesion proteins. Many of
113 these may also be present and have important roles in the ciliary membrane. This includes
114 the receptor FGFR1, which was shown to localize to the kinocilium of mechanosensory hair
115 cells in the inner ear [34]. Mechanotransduction involves adjacent rows of actin-based
116 stereocilia, which are inter-connected and linked to the kinocilium by the membrane-
117 associated adhesion molecules CDH23 and PCDH15 [35]. Recruitment of PCDH15 to the
118 kinocilium involves FGFR1-dependent PCDH15 phosphorylation and loading on IFT
119 transport complexes [34]. Mutations in FGFR1, CDH23, and PCDH15 have all been linked
120 to ciliopathies that involve hearing loss.

121 Photoreceptors are cells that receive light signals via opsins, G-protein-coupled
122 receptors (GPCRs) in the outer segment, a highly specialized cilium. Transduction of the
123 signal involves hydrolysis of the second messenger cyclic GMP (cGMP) [36].
124 Phosphodiesterase subunits PDE6A, PDE6B, and PDE6G, the guanylate cyclase GUCY2D,
125 and the calcium-sensitive guanylate cyclase activator GUCA1B are involved in the dynamic
126 cycle of cGMP hydrolysis and re-synthesis. Mutations in these genes have been linked to
127 vision loss in a number of ciliopathies [37–41].

128 Factors secreted to the extracellular space such as extracellular matrix proteins,
129 growth factors, and other intercellular signaling molecules are also linked to established
130 ciliopathies. The most prominent example is SHH, the ligand of the receptor PTCH1 that
131 initiates the Hedgehog signaling cascade [4].

132 The remaining proteins are associated with various other organelles. ER enzymes
133 (DHDDS, ALG9, GANAB, PRKCSH) participate in protein glycosylation. During N-linked
134 glycosylation precursor oligosaccharides linked to the lipid dolichol are synthesized and
135 transferred by ER-resident glycosyltransferases to nascent proteins. Mutations in the
136 glucosidase II complex subunits GANAB and PRKCSH, interfere with the biogenesis and
137 targeting of the glycoproteins PKD1 and PKD2 to the ciliary membrane and cell surface,
138 causing polycystic kidney and liver disease [42–44]. O-linked glycosylation is initiated in the
139 ER and completed mainly by Golgi-resident enzymes such as the glycosyltransferase
140 POMGNT1. POMGNT1 mutations result in the loss of a complex carbohydrate modification
141 on the cell surface protein α-dystroglycan (DAG1), which is required for photoreceptor
142 function and survival [45,46].
143 In summary, apart from the ciliome, major groups of genes implicated in established
144 ciliopathies comprise regulators of gene expression, as well as surface receptors and
145 secreted factors that mediate signaling through the ciliary membrane. An additional
146 contribution is made by factors controlling protein glycosylation and trafficking of ciliary
147 building blocks. Of note, some of the ciliopathy-linked gene products that are not generally
148 considered part of the ciliome, may in fact localize to cilia in certain cell types or transiently
149 in response to specific signals. Moreover, ciliary proteins may also have additional functions
150 outside of the cilium [47].
151
152 **Identifying novel ciliary disease genes**
153 The prevalence of non-ciliome gene mutations in established ciliopathies suggests that cilia,
154 owing to the complexity of their biogenesis and function, are affected by a wide range of
155 cellular processes. Thus, disorders with mutations in genes that are seemingly unrelated to
156 centrioles or cilia may include ciliary defects as part of their phenotypic spectrum. To
157 address this, we probed the HPO database for any diseases that display at least a subset
158 of features observed in established ciliopathies. Since motile ciliopathy presents with a
159 relatively narrow range of specific phenotypes, we excluded it from this analysis. For each
160 disease we scored the different organs and body parts that were associated with ciliopathy-
161 like phenotypes, scoring those that are more frequently affected higher than those in which
162 phenotypes are less frequent (Figure 2; Supplemental File 1). This resulted in a ranked list
163 of candidate diseases that are not generally classified as ciliopathies but include ciliopathy-
164 like phenotypes (Supplemental File 2). Most of the associated genes encode proteins that
165 do not localize to centrioles or cilia, but there are a few exceptions (Figure 3). Meier-Gorlin

166 syndrome-associated ORC1, a subunit of the origin recognition complex in the nucleus,
167 additionally localizes to the centrosome and its depletion impairs ciliogenesis in cell lines
168 and in a zebrafish model [48,49]. Centrosome localization has also been observed for
169 Fanconi anemia-associated BRCA1, BRCA2, XRCC2, and RAD51, which function in the
170 nucleus in the DNA damage response [50–52]. While there is evidence for a relationship
171 between DNA damage response and ciliogenesis, its mechanistic basis is still unclear
172 [53,54]. The AAA ATPase family member PEX6, which mediates protein import into
173 peroxisomes and is mutated in Zellweger syndrome, was recently suggested to have
174 peroxisome-independent roles in the ciliary structures of photoreceptors [55].
175 Below we will highlight a few of the remaining genes and associated diseases, which
176 are candidates for second-order ciliopathies or DCCs.
177
178 *Nuclear factors*
179 Most of these genes may affect cilia via the regulation of gene expression. The splicing
180 factor SON controls specifically the expression of various centriolar and ciliary genes
181 [9,56,57] and was shown to affect centriole biogenesis [28]. The chromatin regulators
182 KMT2D and ANKRD11, linked to Kabuki and KBG syndromes, respectively, were among
183 the hits in a screen for factors involved in ciliary Hedgehog signaling [8]. Both were also
184 identified by exome sequencing in patients with congenital heart disease [58,59], a disease
185 in which cilia have a central role [60]. Additional genes identified in the exome sequencing
186 studies are PTPN11, ADNP, NSD1, KMT2A, and DYRK1A, which are associated with
187 various human disorders. SMARCA4, associated with Coffin-Siris syndrome, has not yet
188 been shown to affect cilia, but mutation of the mouse ortholog is associated with congenital
189 heart disease [60].
190
191 *Plasma membrane and secreted proteins*
192 As discussed above, this group may include components of ciliary signaling pathways.
193 Notch signaling, for example, may function upstream of ciliogenesis, but may also be
194 mediated by cilium-localized NOTCH receptors such as NOTCH3 [61]. Mutation of the
195 NOTCH2 receptor is associated with cystic kidneys among other phenotypes in Alagille
196 syndrome and Hajdu-Cheney syndrome, and impairs primary cilia during renal tube
197 morphogenesis [62]. Recent work in zebrafish showed that primary cilia control
198 hematopoiesis through Notch signaling [63]. Apart from various signaling pathways that are
199 initiated in the cilium [64], others function upstream of the cilium. An example are

200 noncanonical Wnt signaling ligand WNT5A and planar cell polarity DVL proteins, which have
201 been implicated in primary cilium assembly/disassembly [65–67].
202
203 *Secretory pathway*
204 Components of the secretory pathway may affect ciliary membrane composition. Coatomer
205 proteins (ARCN1, SEC24C) are involved in vesicle trafficking between ER and Golgi and
206 TBC domain-containing proteins (TBC1D20, TBC1D25, TBCK) function as Rab-GTPases
207 activating proteins to mediate specific trafficking steps. For instance, TBC1D20 is required
208 for the ER-Golgi-plasma membrane trafficking of GPCRs [68]. Several candidate genes of
209 the ER and Golgi, linked with congenital disorders of glycosylation (CDGs), affect protein
210 glycosylation and glycoprotein trafficking (MOGS, SSR4, ALG12, COG1, COG5, COG6).
211 Two genes are involved in related GPI-anchor disorders (PIGT, PIGQ). Others are involved
212 in phospholipid synthesis and distribution (PIK3C2A, VAC14, FIG4), as well as cholesterol
213 metabolism (EBP, SC5D, DHCR7). The phosphatidylinositol and phosphatidylinositol-4-
214 phosphate kinase PIK3C2A is associated with oculoskeletodental syndrome and has been
215 implicated in primary cilium formation and function [69,70]. Similarly, DHCR7, mutated in
216 Smith-Lemli-Opitz syndrome and involved in cholesterol biosynthesis, was shown to localize
217 at the ciliary base and promote Hedgehog signaling [71,72]. RAB18, RAB3GAP1,
218 RAB3GAP2, mutated in Warburg Micro syndrome, have been implicated in the mobilization
219 of cholesterol by promoting lipid transfer between distinct membrane compartments [73].
220 Also implicated in lipid transport are VPS13 family members such as Cohen Syndrome-
221 associated VPS13B, a Golgi protein [74].
222
223 *Peroxisome proteins*
224 A number of PEX genes, associated with Zellweger syndrome, and other peroxisomal
225 components (HSD17B4, ACOX1) are also found in our candidate list. Interestingly, acute
226 depletion of peroxisome biogenesis factors PEX1 and PEX3 by knockdown was shown to
227 partially inhibit ciliogenesis [75]. Thus, peroxisomes and not only peroxisomal proteins that
228 localize to cilia (as discussed above for PEX6) may be important for cilia, possibly owing to
229 their role in cholesterol trafficking. Cells from Zellweger patients were shown to have
230 reduced ciliary cholesterol and microtubule-dependent transport of peroxisomes was
231 proposed to deliver cholesterol to the ciliary pocket [76].
232
233 *Mitochondria*

234 A functionally diverse set of mitochondrial factors, linked to various disorders, may also
235 affect cilia, possibly by regulating cellular ATP levels. Indeed, knockdown of a set of genes
236 with roles in mitochondria and mutated in patients with heterotaxy (misplacement of visceral
237 organs) was shown to reduce cellular ATP levels and increase the length of cilia in cultured
238 cells and caused ciliopathy-like phenotypes in zebrafish [77]. Depletion of ACOX1, an
239 enzyme of the peroxisomal β-oxidation pathway, has similar effects, potentially involving
240 mitochondrial dysfunction that is secondary to disrupted peroxisomal β-oxidation. Reduction
241 in cellular ATP was proposed to inhibit the ATP-dependent kinesin KIF19A, which limits
242 ciliary length by its microtubule depolymerase activity [77].
243
244 *Protein degradation machinery*
245 The protein degradation machinery has been identified as an important ciliary regulator in
246 several screens [8,9,11]. Interestingly, our candidate list also contains several components
247 of the ubiquitin-conjugation machinery (SPOP, FANCL, HUWE1, RFWD3, RNF113A,
248 UBE3B, UBE2T, UBA1).
249
250
251 **Concluding remarks**
252 The association of ciliopathy-like phenotypes with a large number of human genetic
253 disorders suggests a much broader involvement of ciliary dysfunction in human disease than
254 generally appreciated. It also suggests that the underlying gene mutations frequently do not
255 directly interfere with ciliary function, but impair other organelles, with secondary effects on
256 cilia. The discussion provided here may serve as starting point for future studies aimed at
257 uncovering such links. Integrating seemingly unrelated cellular compartments and
258 processes with the assembly and function of cilia will allow a better grasp of the true
259 complexity of these organelles. Moreover, being able to add rare disorders with poorly
260 understood etiology to the ciliopathy disease spectrum will raise urgently needed awareness
261 among clinicians and researchers and may allow identification of new therapeutic strategies.
262
263
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Declaration of Interests

The authors declare no competing interests.

Glossary

First-order ciliopathy: disease caused by ciliary defects due to mutations in genes encoding ciliary or centriolar components.

Second-order ciliopathy: disease caused by ciliary defects due to mutations in genes encoding proteins that are not present at centrioles or cilia.

Disorders with ciliary contribution (DCCs): disorders caused by mutations in proteins that do not localize to the centriole-cilium compartment but affect cilia indirectly and that may cause additional, non-ciliary phenotypes.

Intraflagellar transport (IFT): motor-dependent bidirectional traffic of molecules along axonemal microtubules.

BBSome: heterooctameric BBS protein complex that acts as a ciliary cargo adapter in retrograde transport, linking membrane-bound ciliary proteins to the intraflagellar transport machinery. Mutations in the BBSome genes cause the ciliopathy Bardet-Biedl syndrome (BBS).

Human Phenotype Ontology (HPO): standardized vocabulary of phenotypic abnormalities encountered in human disease. It provides more than 150,000 phenotype and gene annotations to human hereditary diseases.

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519 **Box 1: Step-wise cilium assembly**

520 Before serving as platform for cilium assembly, the mother centriole acquires distal and
521 subdistal appendages. Only distal appendages are essential for ciliogenesis. Subdistal
522 appendages are not required for cilium formation, but for its positioning, including its
523 submerged configuration in the ciliary pocket by tethering the mother centriole to the Golgi
524 [78,79]. Loss of subdistal appendages results in farther protrusion of cilia on the cell surface,
525 increasing their susceptibility to chemical and mechanical stimuli [78].

526 Ciliogenesis is coupled to the cell cycle. Cilia assemble during G0/G1 phase and, as
527 cells re-enter or progress in the cell cycle, disassemble again at some point prior to mitosis.
528 The first step of cilium formation is the docking of small, pre-ciliary vesicles, presumably
529 originated from the Golgi, at the distal appendages of the mother centriole, a process that
530 requires the distal appendage protein CEP164 [80,81]. The kinase TTBK2 promotes the
531 removal of the inhibitory factors CP110 and CEP97 from the distal centriole end [82,83].
532 Ciliary membrane growth and protein composition are established by vesicle trafficking
533 mediated by members of the Rab and Arf GTPase families, such as Rab8A and Rab11, in
534 cooperation with the actin network. Fusion of pre-ciliary vesicles gives rise to a larger ciliary
535 vesicle that spans the entire distal centriole end [84]. Axoneme formation by elongation of
536 the centriolar doublet microtubules is accompanied by growth of the ciliary vesicle, forming
537 a sheath around the nascent axoneme. An important event is assembly of the transition
538 zone complex, which controls protein transition into and out of the cilium [85,86]. These
539 processes involve the **intraflagellar transport (IFT)** and vesicular trafficking machineries
540 that are recruited to the ciliary base and into the growing cilium. Finally, the membrane-
541 bounded axoneme reaches the cell surface where the ciliary sheath fuses with the plasma
542 membrane, allowing the docking of the distal appendages to the plasma membrane and
543 extension of the membrane-enclosed axoneme into the exterior of the cell [84,87].

544 Alternatively, some cells (e.g. polarized epithelial cells) employ a plasma membrane-
545 associated cilium assembly pathway. Here, the mother centriole first migrates and docks to
546 the apical cell membrane, where the entire ciliary assembly process takes place. Other cells
547 (e.g. multi-ciliated cells) use a combination of both pathways [87].

548

549 Figure I. **Key steps of ciliogenesis**

550 The key steps of cilium assembly are shown from left to right. The first step is the tethering
551 of small vesicles at the distal appendages of the mother centriole. Multiple small vesicles
552 fuse to form a larger ciliary vesicle. The axoneme assembles and the ciliary vesicle forms a

553 sheath around it. Fusion of the ciliary sheath with the plasma membrane and membrane
554 docking of the mother centriole exposes the growing cilium at the cell surface.
555
556

Outstanding questions

Are genes linked to second-order ciliopathies truly non-ciliary?
Several examples suggest that proteins that are not considered components of centrioles or cilia may localize to these structures transiently or only in certain cell types. Thus, it will be important to investigate their localization in a disease-relevant tissue and context.

How are cilia affected by defects in other cellular compartments?
Many disorders with mutations in non-ciliary genes share phenotypes with known ciliopathies, suggesting that cilia are sensitive to a range of different cellular impairments. Identifying such cases and deciphering the underlying mechanisms will provide new insight into disease pathophysiology and increase our understanding of cilia biology.

Is the crosstalk between cilia and other cellular compartments or processes enhanced in particular cell types?
As for centriolar and ciliary genes, mutations in non-ciliary genes may not necessarily have general effects on cilia. Thus, it will be crucial to study their impact in a context that is relevant to the disease.

Do ciliary proteins have functions beyond the cilium?
There are examples of centriolar or ciliary genes having additional roles outside the cilium. Mutations in these genes may produce phenotypes not consistent with ciliopathy, resulting in misclassification.

557 **Figure Legends**

558

559 **Figure 1: The structure and composition of cilia.**

560 The base of a cilium is formed by the mother centriole. An extension of its distal wall
561 constitutes the axoneme, a scaffold structure at the core of the cilium that is surrounded by
562 the ciliary membrane [88]. Only the mother centriole carries subdistal appendages, which
563 anchor cytoplasmic microtubules, and distal appendages, which mediate docking to the
564 ciliary and plasma membranes. Cargo trafficking along the axoneme, mediated by
565 intraflagellar transport (IFT) particles composed of IFT-A complex and dynein (retrograde
566 transport) or IFT-B complex and kinesin-2 (anterograde transport) is required for cilium
567 biogenesis and function [1,3]. Cargo entry into the cilium can be passive (small proteins),
568 chaperone-assisted (e.g. lipidated cargo), or TUB/TULP3-mediated (e.g. GPCRs) (not
569 shown). The **BBSome** cargo adapter is involved in retrograde transport and export [1]. As
570 depicted in the cross-section views, the axonemes of most motile cilia contain a central pair
571 of microtubules and additional features that are required for ciliary motility. The transition
572 zone is a higher-order protein assembly that functions as a diffusion barrier, controlling entry
573 and exit of lipids and proteins [85,89]. The ciliary membrane is enriched in
574 phosphatidylinositol-4-phosphate (PI4P) and also contains microdomains composed of
575 sterols and sphingolipids. The specific lipid composition together with protein glycosylation
576 and lipidation are crucial for the targeting and function of various ciliary membrane proteins
577 such as receptors, ion channels, and associated signal transduction proteins [90,91].
578 Colored boxes indicate major groups of proteins that constitute the distinct centriolar and
579 ciliary sub-compartments and participate in their function. First-order ciliopathies are caused
580 by mutations in genes that encode proteins of these groups [6].

581

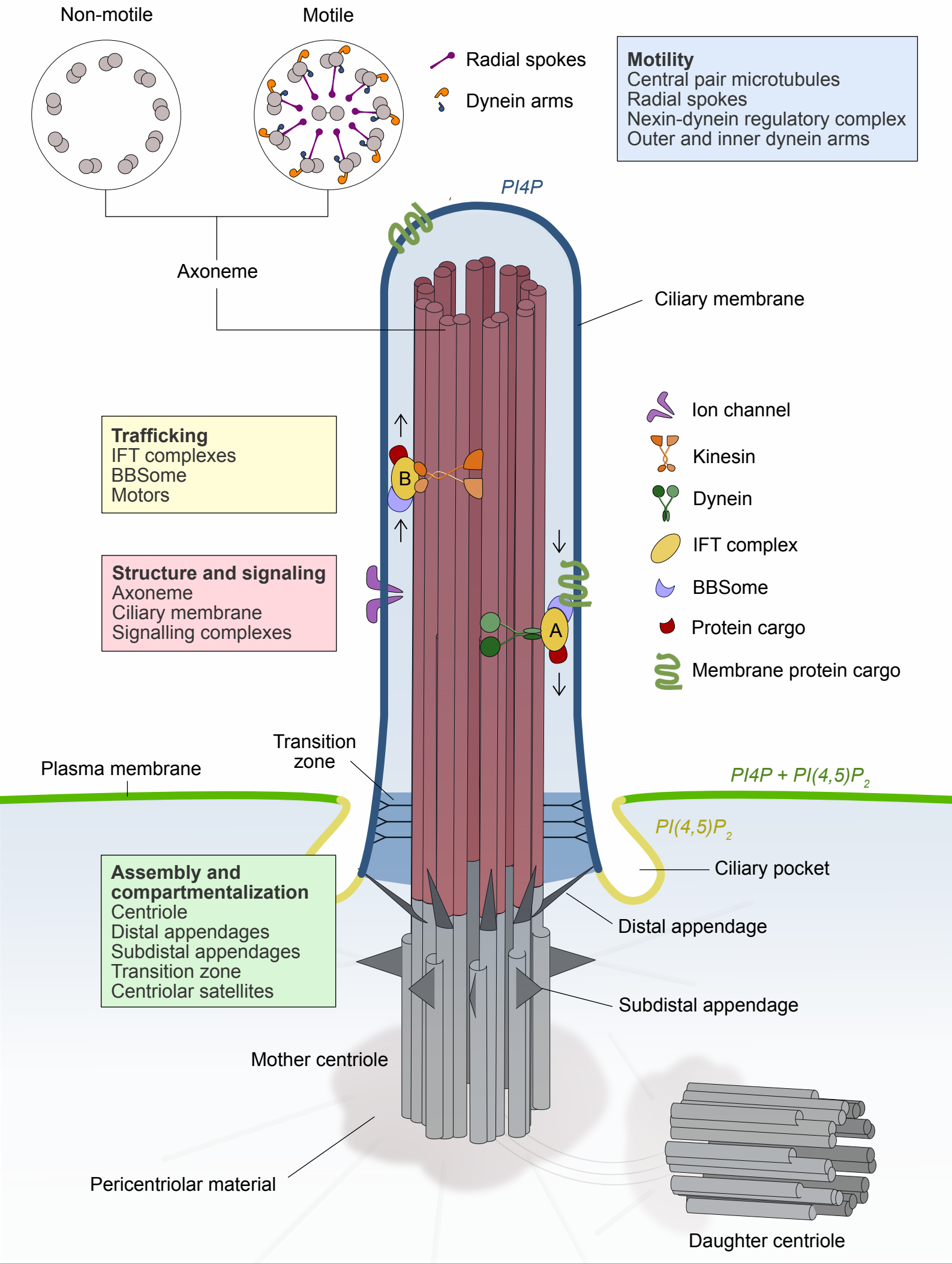
582 **Figure 2: Probing the HPO database using phenotypes of established ciliopathies**

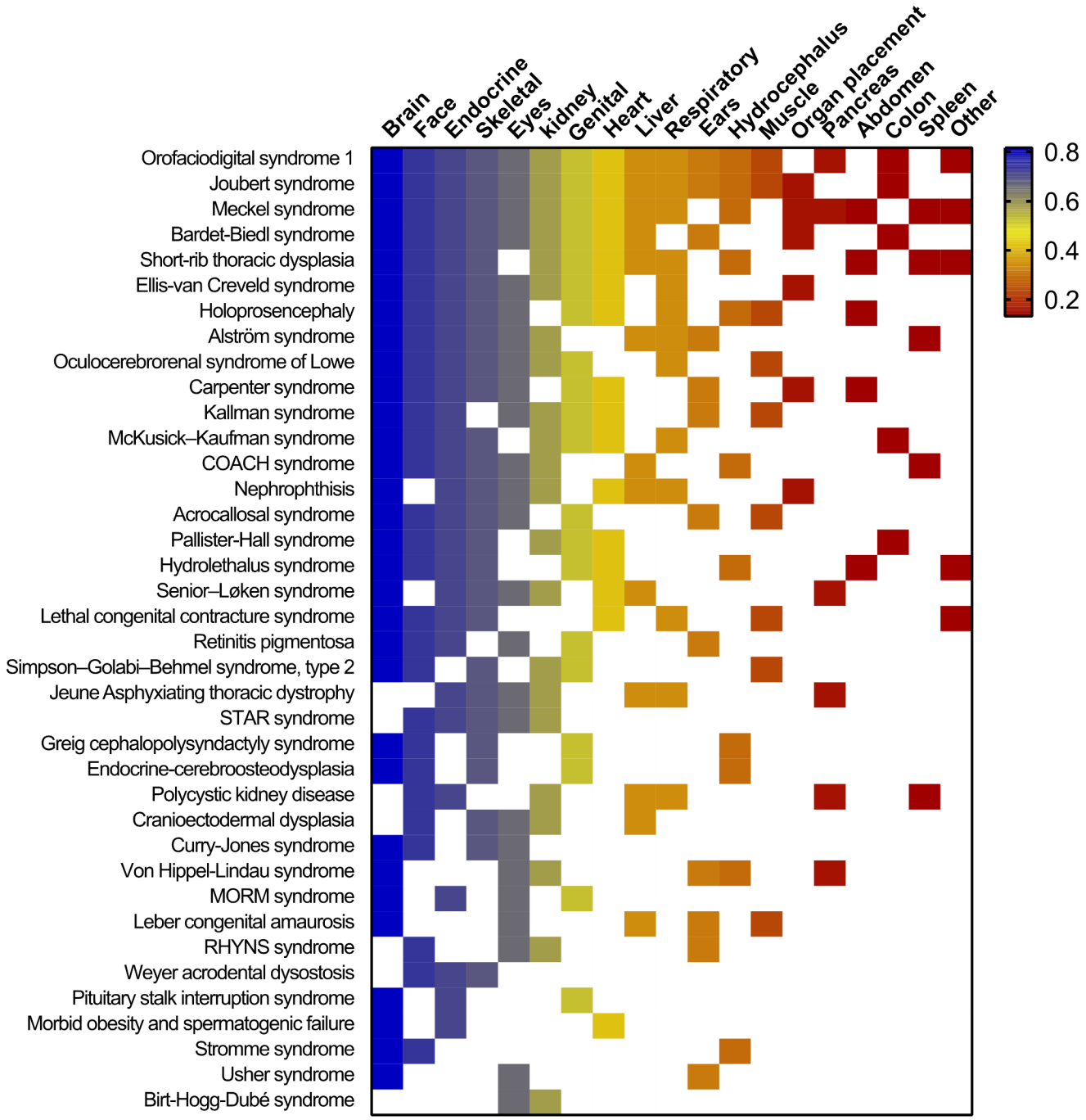
583 The matrix indicates for each of the 38 established ciliopathies (listed on the left) the organs
584 and body parts (indicated at the top) in which clinical phenotypes are observed, according
585 to the HPO database. The different categories were ordered from left to right, color-coded
586 from blue to red, and provided with a score (shown on the top right), according to how
587 prevalent they are among the 38 established ciliopathies. To identify new diseases with
588 ciliopathy-like phenotypes (flowchart at the bottom; Supplemental File S1) we compared the
589 associated phenotypes of all diseases in the HPO database with the phenotypes of
590 established ciliopathies and scored the matches. Phenotype matches in categories

frequently affected in established ciliopathies were scored higher, phenotype matches in categories less frequently affected were scored lower. The result is a ranked list of diseases with associated genes (Supplemental File S2). Top scoring diseases are candidates for previously undescribed ciliopathies or DCCs.

Figure 3: Compartments and roles of proteins associated with candidate ciliopathy-like diseases

Proteins encoded by genes mutated in 38 established ciliopathies (left column) and in candidate ciliopathy-like diseases (right column) identified as described in Figure 2 and Supplemental File S1 were ordered by compartments to which they localize. Within each compartment proteins with similar roles were grouped. For each compartment the total number of proteins is indicated. Some proteins are associated with multiple compartments. The complete lists can be found in the Supplemental File S2. For established ciliopathies only about half of the associated proteins are components of centrioles or cilia, the other half localizes to various other cellular compartments. For the novel candidate ciliopathies and DCCs the large majority of associated gene products are not part of the centriole-cilium proteome.


Non-motile
Motile
Radial spokes
Dynein arms
Motility
Central pair microtubules
Radial spokes
Nexin-dynein regulatory complex
Outer and inner dynein arms
PI4P
Axoneme
Ciliary membrane
Ion channel
Kinesin
Dynein
IFT complex
BBSome
Protein cargo
Membrane protein cargo
Trafficking
IFT complexes
BBSome
Motors
B
A
Structure and signaling
Axoneme
Ciliary membrane
Signalling complexes
Transition zone
Plasma membrane
PI4P + PI(4,5)P2
PI(4,5)P2
Ciliary pocket
Assembly and compartmentalization
Centriole
Distal appendages
Subdistal appendages
Transition zone
Centriolar satellites
Distal appendage
Subdistal appendage
Mother centriole
Pericentriolar material
Daughter centriole



Identification of candidate ciliopathies and DCCs

Probe HPO database for diseases with ciliopathy-like phenotypes

↓

Score and rank candidate diseases according to phenotypic overlap

↓

Analyze associated genes

Established ciliopathy genes (247)

First-order (133)

Cilium (112)

ABCA4, ADCY6, ADGRV1, AHI1, AIPL1, ANKS6, ARL13B, ARL3, ARL6, B9D1, B9D2, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, C8ORF37, CC2D2A, CCDC28B, CEP104, CEP290, CEP41, CILK1, CLRN1, CNGA1, CNGB1, CNTNAP1, CPLANE1, CRB1, DCDC2, DYNC2H1, DYNC2I1, DYNC2I2, DYNC2LI1, EVC, EVC2, EYS, FLCN, GLI1, GLI2, GLI3, GPR161, GUCA1B, IFT122, IFT140, IFT172, IFT27, IFT43, IFT52, IFT80, IFT88, INPP5E, INVS, IQCB1, KIAA1549, KIF7, KISS1R, LCA5, LZTFL1, MAK, MKKS, MKS1, NEK1, NEK2, NEK8, NPHP1, NPHP3, NPHP4, OCRL, PCARE, PDE6D, PDZD7, PKD1, PKD2, PKHD1, PTCH1, RAB23, RHO, ROM1, RP1, RP1L1, RP2, RPGR, RPGRIP1, RPGRIP1L, SDCCAG8, SMO, SPATA7, TCTN1, TCTN2, TCTN3, TMEM107, TMEM138, TMEM216, TMEM231, TMEM237, TMEM67, TRAF3IP1, TRIM32, TTC21B, TTC8, TUB, TULP1, WDPCP, WDR11, WDR19, WDR35, WHRN

Centriole (21)

ALMS1, ARL2BP, ARMC9, C2CD3, CCDC141, CEP120, CEP164, CEP19, CEP55, CEP78, CEP83, CSPP1, FAM161A, HYLS1, KATNIP, KIAA0586, KIAA0753, KIZ, OFD1, PIBF1, TOPORS

Second-order (114)

Nucleus (47)

Transcriptional regulation (20): AHR, CHD7, CRX, FEZF1, GLI1, GLI2, GLI3, GLIS2, HESX1, LHX4, NR2E3, NRL, SIX3, SOX10, TGIF1, ZBTB42, ZIC2, ZNF408, ZNF423, ZNF513

Splicing (8): DHX38, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, RP9, SNRNP200

Others (19): AGBL5, CCND1, CCNQ, CERKL, CILK1, FGFR1, GLE1, IMPDH1, KIF14, KLHL7, MEGF8, NMNAT1, NSMF, PCYT1A, PDZD7, RD3, USP45, VHL, WDR11

Plasma membrane (39)

Surface receptor signaling (17): ADGRG6, ADGRV1, CDON, ERBB3, FGFR1, FLRT3, IL17RD, KISS1R, MERTK, NSMF, PROKR2, PROM1, PRPH2, RHO, ROBO1, SEMA4A, TACR3

Cell adhesion (5): CDH23, CDHR1, DCC, FLRT3, PCDH15

cGMP hydrolysis and re-synthesis (4): GUCY2D, PDE6A, PDE6B, PDE6G

Others (14): ABCA4, ANOS1, BEST1, CA4, CLRN1, CNGB1, GPC3, GPC4, KCNJ13, KIAA1549, PIP5K1C, PRCD, SHH, SPRY4

Secretory machinery (28)

Glycosylation and glycoprotein trafficking (5): ALG9, DHDDS, GANAB, POMGNT1, PRKCSH

Lipid synthesis and transport (5): ABCA4, INPP5E, OCRL, PCYT1A, PIP5K1C

Others (18): ARSG, GUCY2D, HGSNAT, HS6ST1, IL17RD, LRAT, PROM1, RD3, RDH12, REEP6, RGR, ROBO1, RPE65, SCAPER, SLC7A14, SPRY4, VHL, WDR11

Extracellular (16)

Signaling and growth factors (9): ANOS1, FGF17, FGF8, GDF6, MEGF8, NDNF, PROK2, SEMA3A, SHH

Others (7): CRB1, ERBB3, FLRT3, IMPG2, PCDH15, RBP3, USH2A

Mitochondria (4)

Regulation of ATP production (2): IDH3A, IDH3B

Others (2): SOX10, TRNS2

Other (16)

ARHGEF18, BICC1, CIB2, DDX59, DNM2, DUSP6, ESPN, FAM149B1, FSCN2, HARS1, MYBPC1, MYO7A, RLBP1, SAG, USH1C, USH1G

Novel candidates (326)

First-order (15)

Cilium (3)

INTU, PEX6, RTTN

Centriole/centrosome (12)

ACTB, BRCA1, BRCA2, CEP57, CKAP2L, NPM1, ORC1, PPP2R3C, RAD51, SKI, TUBB3, XRCC2

Second-order or DCCs (311)

Nucleus (185)

Transcriptional regulation (45): ADNP, ARX, ASXL1, ATRX, AUTS2, BCOR, BRF1, CTBP1, CTCF, DEAF1, FLI1, FOXH1, GLIS3, GTF2E2, GTF2H5, GTF2I, GTF2IRD1, KMT2D, MAF, MED12, MED13L, MLXIPL, MYCN, NELFA, NFIX, PHF6, PRDM16, RAI1, RERE, RREB1, SALL1, SATB2, SMAD4, SMARCA4, SOX11, SOX4, SRY, TBX1, TBX4, TFAP2A, TP63, ZBTB20, ZEB2, ZMIZ1, ZNF148

Chromatin regulation (34): ANKRD11, ARID1B, ARID2, BAZ1B, BPTF, CREBBP, DKC1, DPF2, EP300, HDAC8, HIRA, JMJD1C, KANSL1, KAT6A, KAT6B, KDM1A, KDM5C, KDM6A, KMT2A, NSD1, NSD2, SETBP1, SETD2, SETD5, SIN3A, SMARCB1, SMARCC2, SMARCD1, SMARCE1, SRCAP, STAG1, STAG2, TRIP13, TRRAP

Splicing (8): NHP2, NONO, PARN, PQBP1, PUF60, RNF113A, SNRPB, SON

Others (98): ACTB, ADAT3, AHDC1, AMMECR1, ARID1A, ARVCF, BRCA1, BRCA2, BRIP1, BUB1, BUB3, CDC45, CDCA7, CHAT, COMT, CTC1, DACT1, DDX6, DHCR7, DYRK1A, EBP, ERCC1, ERCC2, ERCC3, ERCC4, ERCC6, ERCC8, ESCO2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FGFR2, FLII, FLNA, HNRNPK, HRAS, HUWE1, LAGE3, LIG4, LIMK1, MAD2L2, MAGEL2, MEG3, MPLKIP, NAA10, NCAPG2, NF1, NIPBL, NOP10, NOTCH2, NOTCH3, NPM1, NRXN1, NSUN2, NXN, ORC1, PALB2, PIK3C2A, POLR1C, POLR1D, POLR3A, PPP2R3C, PRMT7, PSMD12, PTPN11, RAC1, RAD21, RAD51, RAD51C, RBM10, RECQL4, RFC2, RFWD3, RMRP, RTEL1, SKI, SLX4, SMC1A, SMC3, SPOP, TBL1XR1, TCOF1, TERC, TERT, TINF2, UBA1, UBE2T, UFD1, USB1, WRAP53, XRCC2

Secretory machinery (58)

Glycosylation and glycoprotein trafficking (10): ALG12, B3GALT6, B3GLCT, COG1, COG5, COG6, MOGS, PIGQ, PIGT, SSR4

Lipid synthesis and transport (12): DHCR7, EBP, FIG4, PIK3C2A, PNPLA6, RAB18, RAB3GAP1, RAB3GAP2, SC5D, SLC18A3, VAC14, VPS13B

Others (36): ANK1, APC2, ARCN1, ATP6V0A2, ATP6V1A, BMP4, CCDC22, CDC42, CNTNAP2, COL13A1, COMT, CPLX1, FGFRL1, GRIP1, HRAS, IDUA, KRAS, LIMK1, MAGEL2, MBTPS2, MCTP2, MYMK, PORCN, PPP1R15B, PQBP1, RAC1, SEC24C, SNAP25, TBC1D20, TBC1D24, TBL2, TMCO1, UBA1, UFD1, VAMP1, WASHC5

Plasma membrane (52)

Surface receptor signaling (23): AMER1, CHRNG, DISP1, DLK1, DLL1, DVL1, DVL3, EFNB1, FGFR2, FGFRL1, FZD2, GABRD, GRIP1, HRAS, KRAS, MADD, NOTCH2, NOTCH3, NRXN1, PRKAR1A, RAC1, ROR2, TDGF1

Cell adhesion (8): AGRN, CD96, CNTNAP2, COL13A1, DCHS1, FAT4, MYMK, NRXN1

Others (22): ACTB, ANK1, ATP6V0A2, ATP6V1A, BRAF, CDC42, FLNA, GAS1, GP1BB, KCNAB2, NALCN, PIEZO2, PIK3C2A, SHANK3, SLC26A2, SLC5A7, SNAP25, STAC3, STRA6, SYT2, TBC1D24, VAMP1

Mitochondria (17)

Regulation of ATP production (8): ALDH18A1, COX7B, CYTB, HCCS, LETM1, NDUFB11, SLC25A24, TMEM70

Others (9): CPT2, FBXL4, KIFBP, KRAS, LONP1, PTRH2, SLC25A1, UBE3B, VAMP1

Extracellular (15)

Signaling and growth factors (6): BMP4, FGFR2, NODAL, SEMA3E, TDGF1, WNT5A

Others (9): ACTB, AGRN, COL13A1, DEAF1, ELN, FBN1, FRAS1, FREM2, SMC3

Peroxisome (15)

ACOX1, HSD17B4, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6

Other (22)

BUB1B, CLIP2, CTU2, IQSEC2, MAP2K1, MAP2K2, MYH3, MYO9A, OTUD6B, PHGDH, PPP2R1A, RPL10, RTL1, SMS, TARS1, TASP1, TBCK, TMEM94, UBR1, USP9X, WDR73, WHCR

Inventory of supplemental information

The ciliary impact of non-ciliary gene mutations.

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- **Supplemental File S1**, related to Figure 2 and Figure 3: provides a flow chart depicting the steps that were carried out in order to identify candidate ciliopathies and DCCs, as well as the localization and roles of the proteins encodes by genes associated with these diseases.

- **Supplemental File S2:** Excel file containing all the data obtained in our analysis including a list of established ciliopathies and their associated phenotypes and newly identified diseases with ciliopathy-like phenotypes. This file also contains tables showing disease-gene association, as well as encoded protein localization and function, for the genes associated with the top 300 scoring diseases from our analysis.

Supplemental File S1: Outline of the procedure used to identify candidate ciliopathies and DCCs.

The HPO database was used to extract phenotypes of 38 established ciliopathies and identify recurrent phenotypes. These were classified into different categories according to the affected body parts/tissues. For each phenotype category a prevalence was calculated based on how frequently it is among the established ciliopathies. All HPO disease entries were then probed for association with any of the ciliopathy phenotype categories and a score was assigned, calculated from the sum of prevalences of the phenotype categories the disease is associated with. This resulted in a ranked list of diseases with ciliopathy-like phenotypes. The top 300 scoring diseases were subjected to further analysis. For each disease the associated genes were identified in the HPO database. For all gene products subcellular localization and the biological process they are involved in were listed using GO terms (http://go.princeton.edu; http://pantherdb.org), information in Uniprot, and manual curation based on available literature.

Supplemental File S2: Gene lists and phenotypes of established ciliopathies, candidate ciliopathies, and candidate DCCs.

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Supplemental File S1