# Frequenting Sequencing: How Genetics Teaches Us Cilia Biology

Genetic diseases are awesome educators. Studying rare syndromes often unearths new biologic mechanisms and therapeutic directions. In pulmonary medicine, cystic fibrosis has been the seasoned professor, instructing us on protein folding, drug development, and how to meld cutting-edge care with research. Primary ciliary dyskinesia (PCD) may be similarly informative. This disease of faulty cilia is credibly the newly minted instructor. PCD tells us much about the challenges of disease diagnosis, and by studying its genetics, we may be able to uncover new cellular mechanisms. In this issue of the *Journal*, Zietkiewicz and colleagues (pp. 440–449) describe patients bearing mutations in *CFAP300*, which was discovered in a large population of patients with PCD in Poland (1). Together with recent data from two other groups (2, 3), this study may facilitate our understanding of how motor proteins are sorted and delivered along the length of the cilia.

Motile cilia generate force to move fluid for airway clearance, reproduction, cerebral spinal fluid circulation, and the establishment of left–right asymmetry during development. Consequently, patients with PCD typically suffer from chronic rhinosinusitis, otitis media, and lung infections, leading to bronchiectasis. Infertility also occurs and cardiac defects are not infrequent.

Not surprisingly, it is difficult to establish a diagnosis of PCD, so the American Thoracic Society and European Respiratory Society provide guidelines (4, 5). The American Thoracic Society recommends the use of four criteria: *1*) unexplained respiratory distress in full-term newborns, *2*) year-round wet cough, *3*) year-round nasal congestion beginning at ⩽6 months of age, and *4*) an organ laterality defect. Meeting two of these criteria provides a specificity of 72%, and meeting all four provides a specificity of 99%. As a next step, sampling nasal nitric oxide levels for a low level can bolster the diagnosis (6). Beyond this, one can examine ciliary ultrastructure and waveform by transmission EM (TEM) (7) and videomicroscopy, respectively; however, the results are challenging to interpret and false negatives are possible (5).

Enter diagnostic genetics. PCD is an autosomal-recessive and genetically heterogeneous disease that is now attributed to mutations in over 40 different genes (8, 9). This number is bound to grow, considering that beating cilia require many moving parts, all at risk of mutation. Initially, attention was directed toward mutations in the ciliary motors, megadalton-sized dynein proteins precisely spaced at 96-nm intervals along the cilia microtubules. The motors are part of multimeric complexes called "dynein arms" that are positioned on the inner and outer edges of the ciliary microtubules. These inner and outer dynein arms (IDA and ODA, respectively) generate the characteristic waveform. Mutation of a protein within the IDA or ODA can cause the loss of that structure on transmission EM (7). However, some patients are mutant in a single gene yet are missing *both* dynein arms. It was in such patients that Zietkiewicz and colleagues sought to establish a diagnosis of PCD.

So far, mutations in about a dozen genes have been shown to result in loss of both IDA and ODA in individuals with PCD. These genes, which are known as dynein axonemal assembly factors, code for proteins that are present only in the cytoplasm and never in the cilia (10). They include *DNAAF1*, *DNAAF2*, *DNAAF3*, *DYX1C1 (DNAAF4)*, *HEATR2 (DNAAF5)*, *SPAG1*, *ZMYND10*, *LRRC6*, *C21ORF59*, and *PIH1D3*. Emerging proteomics data suggest that these factors chaperone IDA and ODA components to engage the professional folding protein, HSP90, to assemble the "arms." The mechanisms for interaction have not been elucidated, but clues come from their puncta-like presence in the cytoplasm (10).

To explore this issue, Huizar and colleagues expressed fluorescently labeled assembly proteins in *Xenopus* embryos (11). The cytoplasm of the multiciliated cells of the larvae lit up with multiple puncta, each of which included the assembly proteins, HSP90, and dynein proteins. The particles were distinct from the Golgi and endosomes, and demonstrated characteristics of a phase-separated structure. Time-lapse imaging of fluorescent recovery after photobleaching showed the rapid exchange of assembly factors in and out of the liquid-phase particle, while the dynein proteins stayed put. How the assembled arms then engage the ciliary intraflagellar transport (IFT) system for delivery to specific locations along the cilium remains unresolved.

Again, diagnostic genetics may provide insight. Zietkiewicz and colleagues used whole-exome sequencing to study a cohort of 120 patients with PCD who were missing the IDA and ODA. One specific mutation, a deletion that introduces an early truncation of CFAP300, was present in 17 patients from 15 families (1). This is an extraordinarily large number of affected subjects with a shared mutation (without apparent consanguinity). By contrast, large European PCD research groups studying the *CFAP300* mutation found this Slavic allele in only two of eight subjects (from Germany and Italy) (2, 3). This Slavic founder effect indicates the impact of a regional population on the presence of a particular mutation. In the United States, high numbers of patients with PCD due to a single mutation are found in Amish-Mennonite communities (12) and regions with many Ashkenazi Jews (13). The Slavic founder gene will likely turn up in PCD cases within U.S. Slavic immigrant communities.

What is the function of CFAP300? Absent ODA/IDA and evidence that CFAP300 binds the assembly protein DNAAF2 suggest an assembly role. Antibodies to CFAP300 are not available. However, immunofluorescence staining for IDA and ODA proteins in *CFAP300* mutant cells suggests a transport role. All three reports (1–3) show that in mutant cells, ODA motors concentrate in the cell's apical domain, possibly as a result of failed



Supported by National Heart, Lung, and Blood Institute grant R01 HL128370 (S.L.B.).

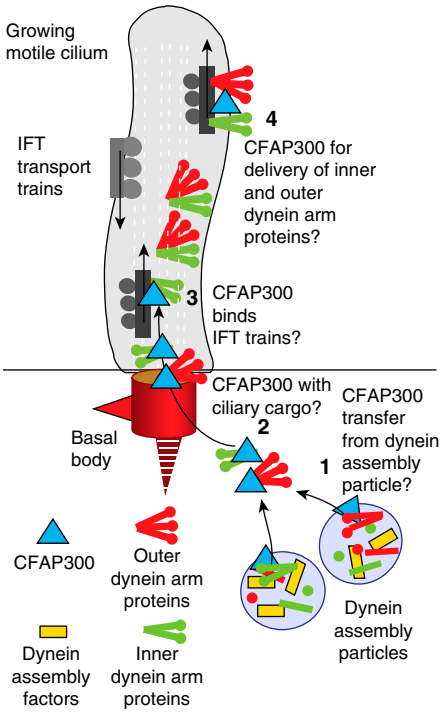


**Figure 1.** Possible sites of CFAP300 activity during cilia assembly. Recent reports suggest that CFAP300 plays a role at multiple sites during motile cilia assembly, including *1*) during cytoplasmic dynein assembly, bound to assembly factor DNAAF2; *2*) transport across the basal body; *3*) transfer of cargo to the intraflagellar transport (IFT) trains by binding IFT46; and *4*) transport of motors throughout the growing cilia. CFAP300 = cilia and flagella associated protein 300.

CFAP300-dependent transport. In contrast, the IDA proteins are either reduced or absent in the mutant (possibly degraded), or, in the Slavic mutation, restricted to the proximal portion of the cilia, suggesting that different mutations have unique effects. Moreover, the separate positions of IDA and ODA in the mutant cells point to ODA/IDA sorting pathways. The strongest data supporting a transport role come from model organisms, as CFAP300 is highly conserved evolutionarily. Fassad and colleagues showed that the orthologous CFAP300 protein is present in cilia of *Chlamydomonas* and *Paramecium* in a pattern typical of IFT (2).

Collectively, these reports suggest that CFAP300 transports ciliary cargo from the cytoplasm to the cilia, possibly as an adapter that engages IFT proteins (IFT46) for delivery along the cilia (2, 3) (Figure 1). Surprisingly, only two such IFT adapters are known (14, 15). It is likely we will find specific transporters to organize the cargoes along the cilia. The continued use of genetic diagnoses will provide satisfaction to patients, advance our understanding of disease, and teach us about cilia biology. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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