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EDITORIALS

HY-DIN' in the Cilia: Discovery of Central Pair–related Mutations in Primary Ciliary Dyskinesia

Primary ciliary dyskinesia (PCD) is a genetic motor ciliopathy that is increasingly recognized as a cause of chronic upper and lower respiratory tract infections in children but is underdiagnosed in adults. Advances in the field have made validating the diagnosis of PCD both easier and more difficult. The task has been facilitated by guidelines issued by the American Thoracic Society (ATS) (1) and European Respiratory Society (2), and simplified by the commercial availability of gene panels for genetic testing. Because there is no consensus-based gold-standard diagnostic test for PCD, the ATS guidelines suggest screening patients for four clinical features that increase the likelihood of a diagnosis, particularly in a child:

- Unexplained respiratory distress or segmental collapse in a full-term infant.
- Year-round wet cough beginning before 6 months of age.
- Year-round nasal congestion beginning before 6 months of age.
- An organ laterality defect (organs on the wrong side).

The presence of at least two of these features, along with low levels of nasal nitric oxide, provides persuasive power for establishing a PCD diagnosis in the right clinical setting. Additional support for the diagnosis may be obtained by an assessment for abnormal ultrastructure of the ciliary axoneme using transmission EM (TEM) or waveform analysis of ciliary beating. An absence of ciliary motor proteins, as detected by TEM, is supportive of a PCD diagnosis. However, many mutations exhibit no apparent change in TEM-based ultrastructure (3). TEM and waveform analysis have a high technical bar that limits their use to centers with the required expertise. In the absence of obvious abnormal TEM findings, a clinical evaluation combined with nasal nitric oxide measurements and genetic testing is increasingly favored to confirm the diagnosis (1, 4).

Whole-exome sequencing has sped genetic discovery and diagnosis, such that pathogenic variants in over 40 genes are known to be causative of PCD. Commercial panels covering ∼30 genes are now available in the clinic. Conceptually, a genetic diagnosis should be straightforward for an autosomal-recessive disease like PCD (i.e., identify pathogenic variants in the sequence of one of the known PCD genes in both alleles). In practice, of course, this is much more complex as we learn more about the growing number of genes implicated in PCD. As is the case with many genetic diseases, a genetic diagnosis of PCD is restrained by several limitations:

- Genetic variants may be pathologic or variants of uncertain significance.
- It is difficult to interpret variants that lie in introns and regulatory noncoding regions.
- Long repeat regions confound the location of variants due to failed sequence alignment.

Genetic diagnosis of PCD is hampered by another, less common problem: one of the PCD genes, called *HYDIN* (on chromosome 16), has a pseudogene, a nonfunctional duplication of the implicated gene (*HYDIN2*, on chromosome 1) (5, 6). Of the 86 exons in HYDIN, exons 6-81 are shared between HYDIN and the pseudogene and the two reference sequences are nearly identical. This makes it very difficult to identify pathogenic variants in this conserved region of *HYDIN*. The difficulty of characterizing *HYDIN* variants is compounded by three other problems: a normal TEM appearance of the ciliary ultrastructure in cilia with *HYDIN* pathogenic variants, a residual spinning-like movement of the cilia that may appear normal, and the lack of laterality defects in patients with a HYDIN variant. HYDIN is located in the central apparatus, which is the "2" in the 9+2 pattern observed in ciliary cross-sections. Defects of the central apparatus do not cause situs inversus, as these microtubules are absent in the normal cilia of the embryonic node. For all of these reasons, investigators have failed to identify *HYDIN* pathogenic variants. Consequently, *HYDIN* mutations were believed to be a rare cause of PCD (6).

In this issue of the *Journal*, Cindrić and colleagues (pp. 382–396), from the group of Heymut Omran, describe what appears to be a highly reliable way to initiate the diagnosis of *HYDIN* pathogenic variants in PCD using immunofluorescent staining of cilia for an absence of SPEF2 (Sperm Flagellar Protein 2) (7). This approach has a strong biologic and genetic basis. It has been reported that HYDIN and SPEF2 form a bridge between the two central microtubules and are interdependent in the ciliated model organism *Chlamydomonas* (8, 9). Because there is no reliable anti-HYDIN antibody, Cindrić and colleagues used an antibody to SPEF2 for immunofluorescence microscopy. They found that SPEF2 was absent from the cilia of 41 out of 189 patients with a PCD-like syndrome of unknown genetic cause. A careful segregation analysis showed that 15 of these patients had *HYDIN* pathogenic variants. Support for a HYDIN–SPEF2 interaction was provided by the identification of a patient with *SPEF2* variants in this PCD cohort. This is the first report of *SPEF2* pathogenic variants causative of PCD, which corroborates a PCD phenotype in the previously described *Spef2* knockout mouse (10). Moreover, the discovery of the *SPEF2* mutation ups the number of PCD-associated genes, which we predict will continue to rise rapidly.

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An upshot of the group's finding is that *HYDIN* pathogenic variants are more frequent than previously thought. The relatively large number of patients with PCD in this study (n = 15) eclipses prior reports of *HYDIN* mutations (6, 11) and suggests that we are failing to identify the genetic cause in a number of patients. The gnomAD database indicates that HYDIN mutations are deleterious. Only one-half of the expected number of loss-of-function alleles are found among the ∼141,000 healthy individuals who were sequenced (12). The ratio of observed to expected loss-of-function variants is similar to that reported for the well-known PCD genes *DNAH5* and *CCDC39*. Thus, it is logical that patients with *HYDIN* pathogenic variants show the typical features of PCD (e.g., chronic respiratory tract infections, infertility), with the exception of situs inversus.

Omran's team is reportedly still working through the genetics of the 25 patients who lacked SPEF2 staining but had neither *HYDIN* nor *SPEF2* pathogenic variants. It is likely that variants in additional central apparatus genes causative for PCD will be found. In addition to this SPEF2 strategy, similar approaches using antibodies to other central apparatus proteins may provide a quick screening method for patients with no obvious TEM defect. The establishment of a clinically certified pathology laboratory in the United States that can perform immunofluorescent microscopy on ciliated nasal epithelial cells from patients may help investigators routinely resolve variants of uncertain significance or find new mutations (13). ■

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Susan K. Dutcher, Ph.D.
Department of Genetics
Washington University School of Medicine
Saint Louis, Missouri

Steven L. Brody, M.D.
Department of Medicine
Washington University School of Medicine
Saint Louis, Missouri

ORCID IDs: 0000-0001-5689-5753 (S.K.D.); 0000-0002-0905-7527 (S.L.B.).

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