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Mini-symposium: Primary Ciliary Dyskinesia

Genetics and biology of primary ciliary dyskinesia

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EDUCATIONAL AIMS

The reader will come to:

1) Appreciate the heterogeneity and complexity of primary ciliary dyskinesia genetics.
2) Understand how mutations in affected genes impact cilia structure and regulation, leading to primary ciliary dyskinesia.
3) Recognize issues related to the use of genetic testing as a diagnostic tool for primary ciliary dyskinesia.

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SUMMARY

Ciliopathies are a growing class of disorders caused by abnormal ciliary axonemal structure and function. Our understanding of the complex genetic and functional phenotypes of these conditions has rapidly progressed. Primary ciliary dyskinesia (PCD) remains the sole genetic disorder of motile cilia dysfunction. However, unlike many Mendelian genetic disorders, PCD is not caused by mutations in a single gene or locus, but rather, autosomal recessive mutation in one of many genes that lead to a similar phenotype. The first reported PCD mutations, more than a decade ago, identified genes encoding known structural components of the ciliary axoneme. In recent years, mutations in genes encoding novel cytoplasmic and regulatory proteins have been discovered. These findings have provided new insights into the functions of the motile cilia, and a better understanding of motile cilia disease. Advances in genetic tools will soon allow more precise genetic testing, mandating that clinicians must understand the genetic basis of PCD. Here, we review genetic mutations, their biological impact on cilia structure and function, and the implication of emerging genetic diagnostic tools.

Primary ciliary dyskinesia (PCD) is a rare disease of childhood, the prototype for motile ciliary dysfunction. PCD results from abnormal ciliary function, leading to neonatal respiratory distress, chronic sinopulmonary disease causing sinusitis, bronchiectasis, recurrent ear infections, and infertility. The cilia are also important for establishing left-right asymmetry during embryogenesis, thus cilia dysfunction can lead to *situs inversus* and a spectrum of *situs ambiguous* with associated congenital heart defects. The first report of bronchiectasis and situs abnormalities was in 1904 by Zivert [1]. This same association was later coined Kartagener's syndrome, based on the description of a series of patients, presenting with recurrent sinusitis, lung infections, and dextrocardia [2,3]. Inclusion of siblings and other family members suggested a genetic cause. Almost 40 years passed before Afzelius provided biological credence to the pathogenesis of PCD in a description of ultrastructural changes in the ciliary axoneme of individuals with immotile cilia and Kartagener's syndrome [4–6]. During the past decade, advances in DNA sequencing, genomics, and proteomics have driven the identification of mutations in almost 30 genes that are causative of motile cilia defects in PCD. At the same time, the discovery of each mutant gene has expanded our understanding of cilia biology and ciliogenesis.

The goal of this review is to provide an update regarding the genetics of PCD so that the clinician can be familiar with the importance of biologic defects in cilia functions, recognize the potential genotype-phenotype relationships, as well as understand the biologic and genetic bases of PCD. This is particularly important when evaluating the patient with recurrent respiratory tract infections. It must be recognized that the genetic causes of PCD are

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in contrast to cystic fibrosis, another well-known genetic cause of bronchiectasis. While cystic fibrosis is a result of mutations in a single gene causing dysfunction of a single protein (CFTR), PCD is the result of recessive mutations in one of many genes, owing to the complexity of the cilia structure and the process of ciliogenesis. Moreover, most mutations are private, unique to families and reflect the personalized nature of the genetics of PCD.

Secondly, genetic testing for PCD continues to evolve and is currently available on a limited basis for analysis of mutations in a few genes. Academic and commercial development of platforms is anticipated to make testing available for many more mutations in the coming years. Thus, it is essential that clinicians are familiar with the genes that when mutated can cause PCD. Moreover, the genetic basis of an estimated 30 to 40% of patients with PCD and overlapping syndromes are unknown. An appreciation of the structure of the cilia and fundamental stages in ciliogenesis will aid the clinician in understanding the role of new genes as they are discovered. In addition, as mutations in novel genes are identified using genome sequencing, it is important to understand how the protein product is validated as cilia-related. Finally, though still conceptual, the reader will gain insight into the challenge of developing therapies for PCD.

CILIA TYPES AND CILIOPATHIES

Cilia are segregated into two classes, motile and primary. Syndromes associated with defects in cilia of either class are termed ciliopathies. Primary cilia have sensory and signaling roles, and are present on most non-dividing cells, with prominent functions in osteoblasts, neurons and renal tubule cells during development and for homeostasis [7,8]. Primary cilia have evolved unique functions in the cells of the retina and inner ear, as well as in the epithelia of the renal tubule, where cilia sense flow [9–11]. Like PCD, primary cilia syndromes are the result of mutations in one of many genes with specific functions in primary cilia. Unlike PCD, features of these syndromes consist of combinations of sensory (blindness, deafness), skeletal, neural tube, developmental, and cognitive defects, as well as renal cysts. Genes mutated in primary cilia syndromes are also expressed in cells with motile cilia, but rarely is ciliary motility affected, with the exception of some overlap syndromes [12].

Motile cilia are found on the multiciliated cells that line the respiratory tract, ependymal cells of the brain ventricles, and fallopian tubes. The flagellum that propels spermatozoa has a similar ultrastructure. A unique type of solitary, motile cilia are transiently present during early development in a midline structure called the embryonic node. The current paradigm is that nodal cilia move with a directional, spinning motion, to signal the primary cilia that surround the node to govern left-right asymmetry during embryogenesis. Failure of ciliary function in their various tissues is responsible for the constellation of findings in PCD.

PROGRAMS OF CILIOGENESIS

Identifying the specific function of proteins that are mutated in PCD is a major challenge, owing to the complexity of the defined and predicted pathways for generation of ciliated cells and the assembly of motile cilia. A mutation in any one of the proteins required to build or regulate the cilium could cause PCD. The assembly of cilia is regulated by several transcription factors, each with their own transcriptome. The identification of regulatory programs has been complemented by the identification of human and experimental mutations in transcription factors that cause PCD syndromes. Notch signaling pathway is among the earliest regulators involved, with reduced activity levels driving progenitor cells to the motile ciliated phenotype, while higher levels favor secretory and mucous cell differentiation (Figure 1) [13]. Notch activation is followed by activity of MCIDAS and MYB, that regulate early steps in multilineage commitment and differentiation of airway epithelial cells [14]. Other transcription factors, RFX2, RFX3 and FOXJ1 are required for the activation of genes necessary for anchoring basal bodies at the apical surface of cells, and regulating the transcription of structural proteins during ciliogenesis [15–17]. FOXJ1 has been coined the "master" ciliogenesis gene owing to severe disruption of cilia formation in animal models lacking FOXJ1. The later is due to failure of the basal bodies to properly dock to the cell membrane. These animals also manifest the classic symptoms of motile cilia dysfunction, which includes *situs inversus*, hydrocephalus, sinusitis, and lung disease [16,17]. Roles in ciliogenesis of Regulatory factor X (Rfx) proteins were first identified in the roundworm (*Caenorhabditis elegans*), indicating the importance of less complex organisms in sorting out ciliogenesis [18]. The RFX transcription factors act at early stages in the ciliogenesis program, and their dysfunction affects both motile and sensory primary cilia [19,20]. Moreover, RFX2 and RFX3 share program with FOXJ1, and are potential candidates for mutations that causes PCD [21–24].

ANIMAL MODELS FOR DISCOVERY OF CANDIDATE PCD GENES AND VALIDATION

As noted, a challenge in the field is the identification of candidate genes that may be mutant in PCD, and to determine if a mutation is related to cilia function. The ciliary axoneme is phylogenetically conserved, which has been exploited in PCD gene discovery. *Chlamydomonas reinhardtii*, a single cell, biflagellated

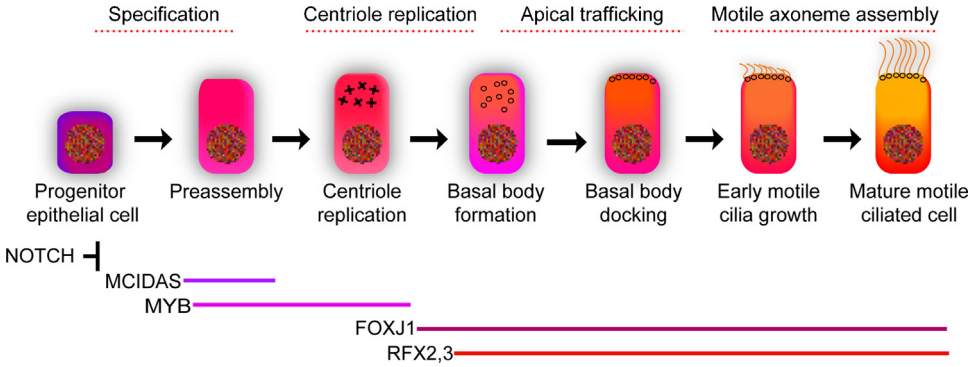

Figure 1. Multiciliated cell differentiation. An airway epithelial progenitor cell, possessing a primary cilium, is directed toward the multiciliated cell type in a low Notch signaling condition. Under the influence of multiple transcription factors, hundreds of centrioles are generated, dock as basal bodies, and nucleate motile cilia in a step-wise process.