Molecular diagnosis of primary ciliary dyskinesia: experience from a clinical laboratory

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Background

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder that arises from defects in motile cilia. Affected individuals have a wide range of phenotypes, including chronic cough, frequent respiratory infections, otitis media, neonatal respiratory distress, bronchiectasis, congenital heart defects, and organ laterality defects. The incidence of PCD is estimated to be ~1 per 10,000-20,000 births in parts of Scandinavia and Japan (Torgersen Acta Radiol 1947; Katsuhara et al. Chest 1972), but the prevalence in the United States has been difficult to determine due to challenges in making a clinical diagnosis (Leigh et al. Proc Am Thorac Soc 2011). Recently, with significant advances in DNA sequencing technology and the discovery of many new genes associated with PCD, genetic testing has emerged as a highly reliable, low cost diagnostic option (Knowles et al. Am J Respir Crit Care Med 2013).

Herein we describe our findings from 763 individuals who underwent genetic testing for PCD during the past 1.5 years. Through November 2016, we used our panel to analyze 30 genes, with the *CFTR* gene offered as an optional add-on for differential diagnosis. The analysis included both sequence and copy number variants (CNVs). Thereafter, the primary panel was expanded to include four additional genes for a total of 34 genes +/- *CFTR*.

Methods & Acknowledgments

- **Patient Cohort:** This study included 763 individuals with personal or family history of suspected PCD. Approximately 150 of these patients with personal history of disease had previously undergone genetic screening in a research setting and were found to be negative.

- **Panel composition:** The following genes were included in our analysis. Bolded genes were added to the panel in December 2016. CFTR was included as an optional add-on in ~40% of cases.

ARMC4	*C21orf59*	*CCDC103*	*CCDC114*	*CCDC151*	*CCDC39*
CCDC40	*CCDC65*	*CCNO*	*DNAAF1*	*DNAAF2*	*DNAAF3*
DNAAF5	*DNAH11*	*DNAH5*	*DNAH8*	*DNAI1*	*DNAI2*
DNAL1	*DRC1*	*DYX1C1*	*MCIDAS*	*NME8*	*OFD1*
RPGR	*RSPH1*	*RSPH4A*	*RSPH9*	*SPAG1*	*ZMYND10*
DNAH1	***GAS8***	***LRRC6***	***RSPH3***	*[CFTR]*	

- **Invitae sequencing methodology:** Sample types for this cohort include blood, saliva, and gDNA. Extracted DNA was subjected to paired-end sequencing on an Illumina next-generation sequencing platform. Bioinformatic analysis of the sequence data included GATK-based tools as well as coverage-based copy number detection algorithms. All likely pathogenic and pathogenic variants, as well as copy number variants of uncertain significance (VUS), were confirmed with an orthogonal technology. Variants were subjected to clinical interpretation using refined ACMG criteria known as Sherloc (Nykamp et al. Genet Med *in press*)

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  - PCD research group at UNC https://www.med.unc.edu/pulmonary/specialties/areas-and-programs/pcd
  - Primary investigators and coordinators of the Genetic Disorders of Mucociliary Clearance Consortium (GDMCC) http://rarediseasesnetwork.epi.usf.edu/gdmcc/index.htm
  - Invitae collaboration, accessioning, laboratory, and billing teams

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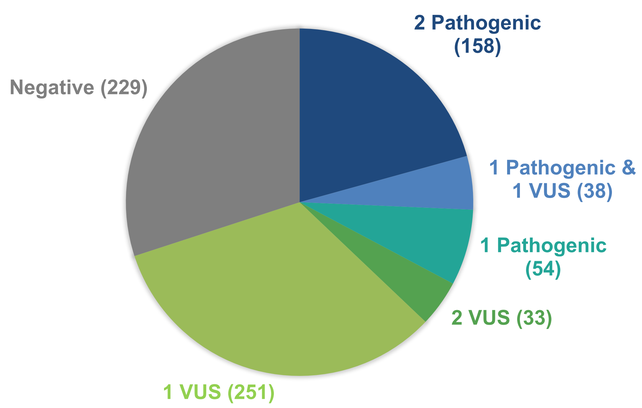
Results

Summary of individuals tested: The 763 individuals tested to date were divided into six groups based on their molecular findings. The number of individuals in each group is indicated in parentheses.

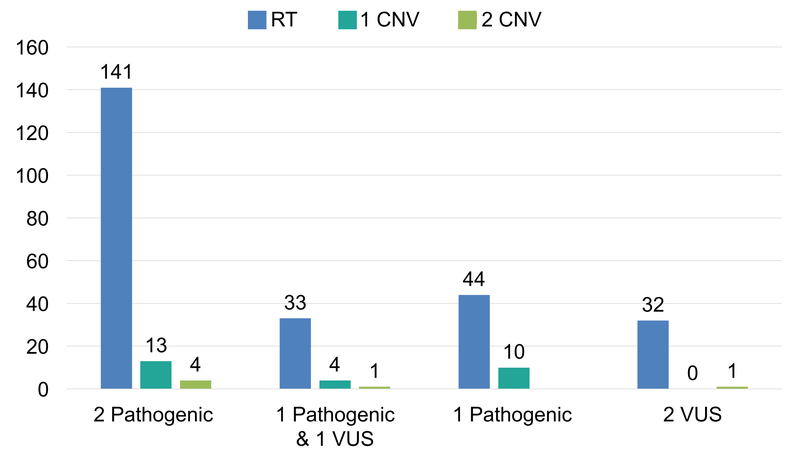
Abbreviation	Molecular finding
2 Pathogenic	Two **likely pathogenic or pathogenic** variants in the same gene
1 Pathogenic & 1 VUS	One **likely pathogenic or pathogenic** variant and one **variant of uncertain significance** in the same gene
1 Pathogenic	One **likely pathogenic or pathogenic** variant in a gene
2 VUS	Two **variants of uncertain significance** in the same gene
1 VUS	One **variant of uncertain significance** in a gene
Negative	**No** pathogenic, likely pathogenic, or variants of uncertain significance detected in any genes



Individuals with VUS require additional functional studies, case reports, or family studies to clarify their molecular diagnoses. Note that 54 individuals were found to only carry a single likely pathogenic or pathogenic variant – it is currently unclear whether these individuals have either a second variant that is not detectable using our assay or an alternative, currently undiscovered explanation for disease.

***CFTR* analysis can be useful as a differential diagnosis:** The phenotypic spectrum of cystic fibrosis (CF) overlaps with that of PCD, and can make definitive diagnosis challenging. Of the 763 individuals tested for PCD, 321 were also screened for variants in *CFTR*, and five individuals had two pathogenic variants in that gene. This result is consistent with a molecular diagnosis of CF.

Compared with read-through (RT) analysis alone, CNV analysis increases yield across molecular groups:



Including CNV analysis as part of the diagnostic test allowed for the identification of an additional 17 cases with two likely pathogenic or pathogenic variants, increasing the positive case yield by >10%.

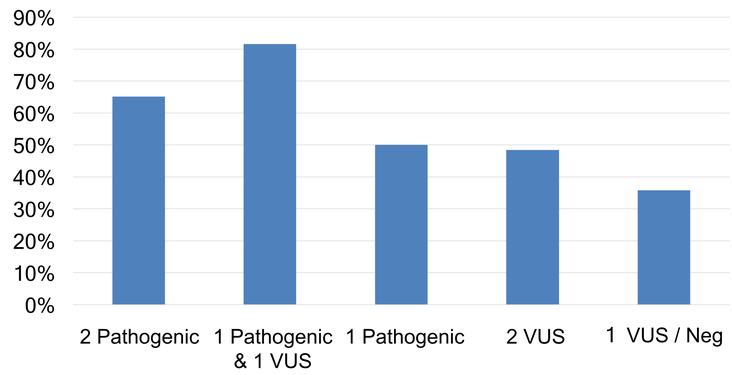
Results (cont.)

Individuals present with variable phenotypes:

Defining strict clinical criteria for PCD (individuals must have at least two of the following and/or low nasal nitric oxide levels)
Unexplained neonatal respiratory distress
Early onset, year-round wet cough and/or bronchiectasis
Early onset, year-round nasal congestion and/or sinusitis
Laterality defect

Leigh et al. Ann Am Thor Soc 2016; GeneReviews

Percentage of cases that meet strict clinical criteria



Review of the phenotypic information provided by the ordering clinician revealed that 103 of 158 (65%) individuals with a molecular diagnosis of PCD met strict clinical criteria for the disease. This outcome may be an under-representation of the actual number because detailed clinical information is often not provided by the clinician when ordering genetic testing. However, it also suggests that individuals with PCD do not always present with clearly defined clinical symptoms, as 35% of individuals with a molecular diagnosis of PCD did not apparently meet strict clinical criteria. Conversely, 36% of individuals with a single VUS or no reportable variants met strict clinical criteria for PCD, suggesting that there are likely additional contributory genes for this condition.

Conclusions

- A striking number of individuals with clinical features of PCD carry a single likely pathogenic or pathogenic variant as determined by standard exon-sequencing methods.
  - Further research is needed to determine the cause of disease in these individuals.
- Including CNV analysis in clinical testing increases the detection of positive cases by more than 10%.
- Phenotypic variability can introduce challenges when making a clinical diagnosis of PCD – not all individuals with a molecular diagnosis of PCD present with well-defined symptoms.
- Individuals who meet strict clinical criteria for PCD do not always have two pathogenic variants in currently recognized genes.
- *CFTR* analysis may be a helpful differential diagnostic tool for individuals with suspected PCD.