Genetic findings from a multi-gene panel for primary ciliary dyskinesia

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Introduction

- Primary ciliary dyskinesia (PCD) is a genetically heterogenous disease in which pathogenic variants lead to abnormalities in the structure and function of cilia, typically resulting in chronic respiratory and sinus infections, male infertility, and possible laterality defects.
- Recent American Thoracic Society guidelines suggest for patients with a strong clinical phenotype for PCD the use of extended (>12 genes) genetic testing panels instead of traditional transmission electron microscopy (TEM) as a diagnostic test when nasal nitric oxide (nNO) is not available.
- To further investigate the use of expanded genetic testing in this patient population, we retrospectively analyzed the genetic findings identified in a cohort of patients with PCD undergoing testing with a next-generation sequencing (NGS)-based multigene PCD panel.

Methods

- A retrospective review of deidentified patients who underwent testing with a PCD panel at Blueprint Genetics was performed.
- Testing included analysis of both sequence and copy number variants (CNVs) in up to 47 genes by NGS data from a validated assay.
- The patient sex, age at testing, and clinical history of PCD were collected from test requisition forms.
- Variant classification was performed using a modified ACMG/AMP variant classification scheme.
- A positive result was defined as the identification of a pathogenic (P) or likely pathogenic (LP) variant(s) consistent with the patient's reported phenotype.

Demographic	Number of individuals (n=404)	% of the cohort
Female sex	204	50%
Male sex	200	50%
Prenatal cases	3	0.7%
Pediatric (0-17yrs)	324	80%
Adult (≥18yrs)	77	19%

Table 1. Patient demographics

Gene(s)	MOI	% of total diagnoses
CCDC40	AR	17% (17/101)
DNAH5	AR	13% (13/101)
DNAH11	AR	11% (11/101)
HYDIN	AR	7% (7/101)
LRRC6	AR	6% (6/101)
CCDC39, *RSPH9*, *ZMYND10*	AR	each 5% (5/101)
DNAAF3	AR	3% (3/101)
CCDC114, *CFAP300*, *DNAAF1*, *DNAI2*, *DNAL1*, *GAS8*, *ODAD2 (ARMC4)*	AR	each 2% (2/101)
RPGR	XL	2% (2/101)
CCDC103, *CCNO*, *DNAI1*, *DRC1*, *DYC1C1*, *SPAG1*	AR	each 1% (1/101)
DNAAF6, *PIH1D3*	XL	each 1% (1/101)
CFTR	AR	5% (5/101)

Table 2. Genes with diagnostic findings in 101/404 cases and the associated mode of inheritance mode (MOI). Genes highlighted in pink are complicated by >90% or >98% sequencing homology.

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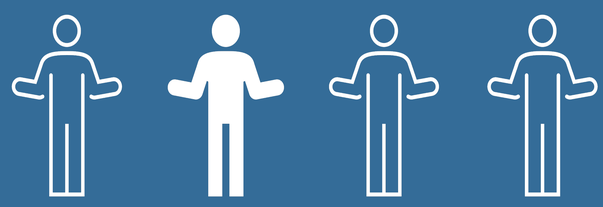
Conflict of interest statement: All authors are employed by Blueprint Genetics.

Results

- 404 patients (50% female) were included in the analysis, with a median age at testing of 7 years (ranging from fetal to 70 years) (Table 1).
- A molecular diagnosis was identified in 25% (101/404) of patients with 124 (93 unique) P or LP variants reported in 26 unique genes (Table 2).
- CNVs were identified in 10/101 diagnoses, 60% of which were single-exon deletions (Figure 1).
- P/LP variants in the *HYDIN* gene were identified in 7 patients, including both sequencing variants and CNVs. Of the 11 total variants reported in *HYDIN*, 10 occurred in exons 7-84, which are complicated by >98% sequence homology to the pseudogene *HYDIN2*.
- P/LP *CFTR* variants represented 5% (5/101) of positive results. Patients with this finding presented with respiratory failure, bronchiectasis, recurrent infections, or atypical cystic fibrosis.



One in 4 patients undergoing multi-gene panel testing for primary ciliary dyskinesia received a molecular diagnosis

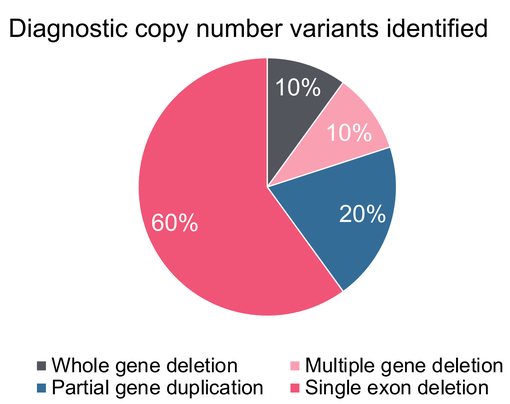


Figure 1. Types of copy number variants identified in 10/101 diagnostic reports.

Gene	Event	Exon(s)	Size
DNAL1, n=2	Deletion	5	240bp
DNAH5	Deletion	56	363bp
DNAH5	Deletion	76	334bp
DNAAF6	Deletion	3	274bp
DYX1C1	Deletion	10	482bp
PIH1D3	Deletion	Whole gene	~30.5kb
GAS8 and *URAHP*	Deletion	2-9 (*GAS8*)	~13kb
DNAH5	Duplication	1-50	~35.1kb
HYDIN	Duplication	19-23	~13.9kb

Table 3. Specific copy number variants identified in 10/101 diagnostic reports.

Conclusions

- 25% of patients received a molecular diagnosis, with P/LP variants identified in 26 genes.
- Of patients with positive results, 38% (n=38) had variants in genes not listed in the standard 12-gene panel referenced in guidelines[a], including 7 variants in the difficult to sequence *HYDIN* gene.
- CNVs made up 10% of all diagnoses, most commonly single exon deletions.
- The results of this study support the use of expanded multi-gene panels in patients with suspected PCD, and demonstrate the added value of high-resolution CNV analysis, and coverage of exons complicated by >98% sequence homology in *HYDIN*.

Reference:

a. Kim RH, A Hall D, Cutz E, Knowles MR, Nelligan KA, Nykamp K, Zariwala MA, Dell SD. The role of molecular genetic analysis in the diagnosis of primary ciliary dyskinesia. Ann Am Thorac Soc. 2014 Mar;11(3):351-9.