# Molecular Testing by Next Generation Sequencing for mutations associated with Primary Ciliary Dyskinesia / Kartagener Syndrome

The UNC Hospitals Molecular Genetics Laboratory performs DNA sequencing of 36 genes to detect mutations that are associated with primary ciliary dyskinesia/Kartagener Syndrome by massively parallel (Next Generation Sequencing) on the Illumina MiSeq and an additional two genes associated with conditions that present similarly to PCD. An additional set of candidate genes are sequenced for research purposes with patient consent.

**Biology of the Disease:**
Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disease associated with abnormalities in the structure and function of cilia of the respiratory tract and flagella of the sperm. It is usually inherited as an autosomal recessive trait, although occasionally other modes of inheritance have been observed. It is a rare genetic disorder, with an incidence of approximately 1 in 16,000, which corresponds to a carrier rate of approximately 1 in 63. It is estimated that there are 12-17,000 patients in the USA affected with PCD. Clinically, PCD is associated with recurrent sinusitis and bronchitis, and in severe cases patients may develop end-stage bronchiectasis and require lung transplantation. In addition, the disease affects other organs and patients may exhibit otitis media and infertility. Approximately 50% of patients with PCD present with situs inversus totalis, termed Kartagener syndrome (KS), and at least 12% present with heterotaxy (abnormal placement of organs due to failure to establish the normal left-right patterning during embryonic development.). Diagnosis is made on the basis of clinical criteria and electron microscopic analysis for ultrastructural defects of the cilia. PCD presents with extensive genetic heterogeneity and multiple chromosomal loci have been identified. Mutations in the 36 genes targeted in the sequencing panel are estimated to account for approximately 67% of patients with PCD.

**Clinical Indications for Molecular Genetic Testing:**
PCD molecular genetic testing is performed for the purpose of diagnosis of PCD, to determine carrier status, or as confirmatory diagnostic testing. The chance of identifying a mutation in these genes increases if the patient population is selected based on the presence of defined dynein arm defects by Electron Microscopy (EM).

Indications include: 1) patients with clinical disease compatible with PCD, but without a defined etiology such as cystic fibrosis (CF), 2) neonatal respiratory distress in term neonates, 3) suppurative airways disease of unknown etiology, even with normal situs, 4) persistent/chronic cough and sinusitis, 5) non-CF bronchiectasis, 6) severe middle ear disease, 7) situs inversus totalis or situs ambiguus/heterotaxy, 8) congenital heart disease with situs inversus totalis or situs ambiguus 9) non-CF male infertility in conjunction with other features of PCD, for example airway disease or situs hydrocephalus, and 11) a family history of PCD/KS.

**Laboratory Testing for PCD-associated mutations:**
Requests for testing must be accompanied by a clinical criteria form (available on our website:
https://www.uncmedicalcenter.org/mclendon-clinical-laboratories/directory/molecular-pathology-and-genetics/)
The preferred sample is ACD anticoagulated blood (pale yellow top) which may be refrigerated up to 48 hours before analysis. Obtaining informed consent for testing is the responsibility of the ordering

physician. Genetic counseling is recommended; for help with genetic counseling please call 919-966-4380.

The test is performed by sequencing the complete coding region and flanking intronic regions of 36 genes that have been associated with PCD using a massively parallel sequencing assay developed in our laboratory. This assay utilizes a custom target capture Nextera Rapid Capture hybridization reagent (Illumina) to generate libraries for custom massively parallel sequencing on an Illumina MiSeq instrument. The panel targets the full exonic sequence of 99 genes (Table 1) that includes 36 genes with evidence of association with PCD in the medical literature, as well as the sites of known pathogenic intronic variants. Also included are two genes associated with disorders that present with symptoms similar to PCD and which are often included in the differential diagnoses (*CFTR* and *RAG1*). Additionally, 62 candidate genes linked to ciliary function in preclinical studies, which may be used for research or future clinical analysis as knowledge of PCD genetics advances, are included. Details of the genes included in the panel are shown in Table 2.

Detection of mutations has diagnostic and reproductive implications, while a negative test result does not exclude a diagnosis of PCD. Results are reported as negative for mutation(s) or mutation(s) detected. Any variant that is identified by sequence analysis is interpreted as a deleterious mutation, a variant of unknown significance, or a benign polymorphism per ACMG guidelines. The detection of two known deleterious mutations in one gene is diagnostic of PCD. The detection of one known deleterious mutation is consistent with being at least a carrier of PCD. Individuals in whom one or no mutations have been found can be referred for full gene sequence analysis and/or future genetic analysis on a research basis (with informed consent).

**Resources:**

1. Rare diseases network for PCD research: https://www.rarediseasesnetwork.org/cms/gdmcc
2. PCD Foundation: http://www.pcdfoundation.org

**References:**


1. Noone PG et al. Am. J. Respir. Crit. Care Med., 2004; 15: 459-467 PMID: 14656747
2. Zariwala MA et al. Am. J. Respir. Crit. Care Med., 2006; 174: 858-866 PMID: 16858015
3. Hornef N et al. Am. J. Respir. Crit. Care Med., 2006; 174: 120-126 PMID: 16627867
4. Berg JS et al. Genet Med. 2011 Mar;13(3):218-29 PMID: 21270641
5. Zariwala MA, Knowles MR, Leigh MW. Primary Ciliary Dyskinesia. GeneReviews 2007 Jan 24 [Updated 2019 Dec5]. http://www.genereviews.org/


**Questions?**


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**Table. List of 99 Genes included in the PCD target capture sequencing panel. The 38 genes included in the clinical testing panel are highlighted.**

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|---|---|---|---|
| *ARMC4* | *C21orf59* | *CCDC103* | *CCDC114* |
| *CCDC151* | *CCDC164(DRC1)* | *CCDC39* | *CCDC40* |
| *CCDC65(DRC2)* | *CCNO* | *CFTR* | *DNAAF1(LRRC50)* |
| *DNAAF2(KTU)* | *DNAAF3* | *DNAH1* | *DNAH11* |
| *DNAH5* | *DNAH8* | *DNAH9* | *DNAI2* |
| *DNAJB13* | *DNAL1* | *DNAI1* | *GAS8(DRC4)* |
| *HEATR2(DNAAF5)* | *LRRC6* | *DYX1C1* | *NME8(TXNDC3)* |
| *OFD1* | *RAG1* | *MCIDAS* | *RSPH1* |
| *RSPH3* | *RSPH4A* | *RPGR* | *SPAG1* |
| *ZMYND10* | *AK1* | *RSPH9* | *AK7* |
| *DCTN1* | *B9D2* | *AK5* | *DAW1* |
| *DCTN5* | *DCTN2* | *CCDC63* | *DCTN4* |
| *DNAH14* | *DCTN6* | *DCTN3* | *DNAH12* |
| *DNAH6* | *DNAH17* | *DNAH10* | *DNAH3* |
| *DNAL4* | *DNAH7* | *DNAH2* | *DNAJB1* |
| *DYNC1I1* | *DNALI1* | *DPCD* | *DYNC1H1* |
| *DYNC2H1* | *DYNC1I2* | *DYNC1LI1* | *DYNC1LI2* |
| *DYNLRB1* | *DYNC2LI1* | *DYNLL1* | *DYNLL2* |
| *GAS2L2* | *DYNLRB2* | *DYNLT1* | *DYNLT3* |
| *NAT14* | *HYDIN* | *IFT172* | *KATNB1* |
| *PPIL6* | *NME5* | *NME9* | *PCDP1* |
| *SPAG16* | *ROPN1L* | *RSPH10B* | *RSPH6A* |
| *TCTE3* | *SPAG17* | *SPAG6* | *SPEF2* |
| *TEKT2* | *TCTEX1D1* | *TCTEX1D2* | *TCTEX1D4* |
| | *WDR63* | *WDR78* | *ZMYND12* |
| | | *WDR78* | |