Multigene Testing for Primary Ciliary Dyskinesia (PCD): Diagnostic Yield and Phenotypic Summary

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Primary ciliary dyskinesia (PCD) is a rare genetic condition caused by abnormal ciliary action or structural defects in embryonic and postnatal life. Symptoms can include situs inversus or situs ambiguous, respiratory disease with sinusitis and bronchiectasis, chronic otitis media, and male infertility. Historically, mutations in *DNAI1* and *DNAH5* were estimated to account for up to 30% of all cases of PCD, while mutations in other genes accounted for only a small percentage. With the availability of next generation sequencing, simultaneous assessment of genes implicated in PCD beyond *DNAI1* and *DNAH5* has become timely and cost effective. We sought to determine the contribution of 11 genes to PCD when analyzed concurrently and to identify the phenotypic spectrum of disease among those with a genetic diagnosis identified.

DNA samples from 692 individuals with a clinical suspicion of PCD were referred for clinical genetic testing between November 2011 and June 2014 and were analyzed with a PCD multigene sequencing panel that included the following genes: *DNAH5, DNAI1, DNAI2, DNAH11, TXNDC3, RSPH4A, RSPH9, DNAAF1, DNAAF2, RPGR, OFD1,* and *CFTR*. Due to the high carrier frequency of cystic fibrosis (CF) and phenotypic overlap between PCD and CF, the *CFTR* gene is included on the panel. PCD is a recessive condition, and the majority of implicated genes are autosomal. However, two of the genes are located on the X chromosome, *RPGR* and *OFD1*, and are also associated with retinitis pigmentosa (RP) and intellectual disability, respectively. Clinical information submitted by ordering healthcare providers was reviewed.

Out of the 692 individuals, a genetic diagnosis (2 pathogenic mutations in autosomal recessive genes or 1 hemizygous pathogenic mutation in X-linked genes) was provided in 42 individuals (6%). Of the 42 positive cases, 57% (n=24) had mutations identified in *DNAH5* (45%, n=19) and *DNAI1* (12%, n=5). In the remaining 19 cases (45%), individuals had mutations in *DNAH11* (23%, n=10), *RSPH4A* (9%, n=4), *RPGR* (5%, n=2), and *CFTR* (5%; n=2). Although genetic diagnoses were not identified in the remaining 6 genes, heterozygous carriers of mutations were identified across all genes except *OFD1*. In fact, 40 individuals (6% of total cohort) were found to be heterozygous carriers of mutations in *CFTR*. Some of these individuals were also heterozygous for variants of unknown significance (VUSs), which have the potential to be pathogenic. Many of the VUSs were missense mutations and were not classified as pathogenic due to the limited evidence and literature available for these genes. Of note, samples were not analyzed for gross deletions or duplications.

Clinical histories were provided in 76% (n=32) of the positive cases. Of those, 34% (n=11) were reported to have situs inversus or situs ambiguous and had mutations in *DNAH5*, *DNAH11,* or *DNAI1*. Only 9% (n=3) of cases with clinical histories provided reported abnormal electron microscopy (EM) results and also had mutations in *DNAH5*, *DNAH11*, or *DNAI1*. Other commonly reported symptoms in positive cases included recurrent or chronic sinusitis, bronchitis, otitis media, and cough. The 2 males with an *RGPR* mutation had no reported signs of RP at the time of testing. One of the 2 individuals with *CFTR* mutations reported sweat chloride levels >100 mmol/L.

These results indicate that multigene testing for PCD increased diagnostic yield by 45% compared to testing for *DNAH5* and *DNAI1* alone. Although only 3 genes were implicated in individuals reported to have situs abnormalities or abnormal EM results, these clinical findings were only reported in a small number of individuals. These results support a multigene panel approach for PCD, particularly in the absence of situs abnormalities or abnormal EM findings. These results also support the inclusion of *CFTR* on a panel for PCD due to the clinical overlap of symptoms and the diagnosis of CF in 5% of positive cases.