

Primary Ciliary Dyskinesia (PCD) via RSPH4A Gene Sequencing (Test #747)

Brief Description of Clinical Features: Primary Ciliary Dyskinesia (PCD; OMIM 244400) is a genetically heterogeneous disorder affecting the function of motile cilia (reviewed by Leigh et al. *Genetics in Medicine* 11:473-487, 2009). Motile cilia line the upper and lower respiratory airways, the ventricular system of the brain and spinal cord, and the female fallopian tubes. They are also components of the male sperm flagellum and required for sperm motility. Ciliary movement sweeps mucus, dirt and bacteria out of the lungs, nasal passageways, and ear canals, thus protecting them from recurrent infections. In the developing embryo, nodal cilia generate a rotational motion that determines the position of the internal organs. Without functional nodal cilia, thoracoabdominal orientation is random. The hallmark features of PCD are neonatal respiratory distress, chronic coughing, and recurrent sinus and/or ear infections; 80-100% of all PCD patients have one or more of these symptoms. In about 50% of individuals with PCD, the major visceral organs are reversed from their normal positions (also called *situs inversus* or Kartagener’s syndrome). Fetal cerebral ventriculomegaly and hydrocephalus can also occur due to impaired circulation of the cerebrospinal fluid. In adults with PCD, male infertility and female sub-fertility are also common features. Prompt diagnosis of PCD is critical for the prevention of secondary respiratory complications, such as bronchiectasis, pneumonia and/or progressive loss of lung function.

Genetics: Motile cilia are complex structures composed of roughly 250 proteins (reviewed in Ferkol & Leigh *Sem Perinatol* 30:335-340, 2006). Planar motion cilia (i.e. from the respiratory tract, brain, and reproductive tract) consist of nine microtubule doublets that surround a central core of two microtubules (9+2 configuration). Rotary motion cilia (i.e. those in the embryonal node) lack the central core microtubules (9+0 configuration). All motile cilia have inner and outer dynein arms attached at regular intervals to the nine peripheral microtubule doublets, which serve as molecular motors that drive microtubule sliding. For 9+2 cilia, radial spokes form a signal-transduction scaffold between the peripheral dynein arms and the central-core microtubule pair, giving these cilia their characteristic planar (i.e. forward and backward) motion. Recessive mutations in two genes encoding Radial Spoke Proteins, RSPH4A and RSPH9, have recently been found to cause PCD (Castleman et al. *Am J Hum Genet* 84:197-209, 2009). The respiratory cilia in these patients either lack the central-core microtubules, having a 9+0 configuration, or have a single peripheral microtubule pair transposed into the center, resulting in an abnormal 8+1 configuration. Remarkably, the cilia in these patients have normal beat frequencies but display a rotational beat pattern, rather than the normal forward and backward planar motion. *RSPH4A* is comprised of 6 exons and, to date, just three nonsense mutations (two in exon 1 and one in exon 3) have been described in 5 unrelated families (Castleman et al. *Am J Hum Genet* 84:197-209, 2009). All patients with *RSPH4A* mutations had cilia with transposed microtubules (i.e. 8+1 configuration).

Description of This Particular Test This test involves bidirectional DNA sequencing of coding exons 1-6 of the *RSPH4A* gene, plus ~50 bp of flanking non-coding DNA on either side of each exon. As indicated, we will also sequence one (Test #100; \$190) or two exons (Test #200; \$340) in family members of patients with a known mutation, or to confirm research results.

Reference Sequences: Genomic: NC_000006.11 mRNA: NM_001010892.2 Protein: NP_001010892.1 CCDS 34521.1

Indications for Test: Candidates for this test are patients with Primary Ciliary Dyskinesia, particularly those with central-core microtubule defects and normal *situs* laterality.

Sensitivity of Test: This test is predicted to detect at least one causative mutation in ~1-2% of all patients diagnosed with PCD, and ~75% of PCD patients with central-core microtubule defects (Castleman et al. *Am J Hum Genet* 84:197-209, 2009).

Turnaround Time: Maximum of 40 calendar days, although many tests are completed in 2-3 weeks.

Specimen Requirements: See page 4 of the Requisition Form.

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| Price: | Sequencing of the RSPH4A Gene: | \$ 620 |
| CPT Codes: | | |
| Sample Ascertainment x1 | 83890 \$ 30 | DNA Isolation x1 83891 \$ 40 |
| Amplification x9 | 83898 \$ 170 | Sequencing x9 83904 \$ 250 |
| Separation x1 | 83894 \$ 40 | Interpretation/Report x1 83912 \$ 90 |

Accreditation: CLIA ID #: 52D1027685 (expires 1/18/13) (CAP#: 7185561, AU ID: 1407125 expires 12/20/12)

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