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**Analysis of new genes involved in Primary Ciliary Dyskinesia (PCD)**

**Analiza nowych genów zaangażowanych w patogenezę pierwotnej dyskinezy rzęsek**

## **ABSTRACT**

Primary ciliary dyskinesia (PCD) belongs to the group of ciliopathies, symptoms of which are caused by kinetic dysfunction of motile cilia of the respiratory epithelium, flagella in spermatozooids, and primary cilia in the embryonic node. This is the genetically heterogeneous recessive disease with the prevalence of 1/16,000.

Since more than one third of PCD cases cannot be explained by genetic defect in any of already known PCD-related genes, it remains crucial to identify new genes and their mutations causative for PCD. The main goal of the PhD project was the identification of new genetic defects causing PCD and functional analysis of novel PCD genes and characterization of identified mutations.

The research was carried out using biological material from PCD individuals collected in the Clinic for Child and Adolescent Medicine, General Pediatrics at the University Hospital of Münster (Germany) as well as in the Department of Molecular and Clinical Genetics of the Institute of Human Genetics PAS in Poznań (Poland).

The methods and techniques used in this project were: bioinformatic analyses including linkage analysis and homozygosity mapping, transmission electron microscopy (TEM) data analysis, DNA extraction from patient blood samples, PCR amplification of DNA, sequencing via Sanger method, immunolocalization assays, immunofluorescence staining of respiratory tissue samples with the use of specific antibodies and high resolution melt (HRM) analysis.

As a result of this PhD project, ten distinct mutations in three novel PCD causative genes (*LRRC6*, *ZMYND10* and *CCDC151*) were identified. This broadens the current knowledge on the genetic

basis of PCD and its heterogeneity. It can be used to expand the diagnostic tests, leading to an earlier identification and better treatment of children with PCD.

All three novel genes are considered to be involved in the dynein arm assembly, causing ODA/ODA+IDA defects, when mutated. This was shown by the high-resolution immunofluorescence staining and confirmed by the transmission electron microscopy analysis. ODA/ODA+IDA defects are the most frequently observed structural lesions of cilia in PCD, together accounting for over 80% of all PCD cases (Papon et al, 2010; Escudier et al, 2009). Former studies were focused on genes encoding axonemal proteins, like *DNAH5*, *DNAI1*, *DNAI2*, *DNAL1* or *NME8* (Zariwala et al, 2007; Loges et al, 2008; Mazor et al, 2011; Duriez et al, 2007). However, more recent research on PCD have revealed mutations in several genes encoding cytoplasmic proteins, which are assumed to act in dynein arms assembly, transport or docking to the microtubule A, like *DNAAF1* (LRRC50), *DNAAF2* (KTU), *DNAAF3*, *CCDC103* or *CCDC114* (Loges et al, 2009; Omran et al, 2008; Mitchison et al, 2012; Panizzi et al, 2013; Onoufriadis et al, 2013).

All these recent findings indicate the existence of different sequential stages of dynein arms assembly. The three genes identified in this PhD project also encode cytoplasmic proteins not found in the axoneme. This can help to explain the processes during the dynein arm assembly pathway. Moreover, as cytoplasmic proteins, all three of them might be more sensitive/responsive to potential drugs/new treatment modalities due to their better physical accessibility than the structural axonemal proteins.