Genetic Diagnosis and Respiratory Management of Primary Ciliary Dyskinesia

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ENGLISH SUMMARY

Primary ciliary dyskinesia (PCD) is a rare inherited disease characterized by a defect in motile cilia that line the airways. Cilia sweep the airways by transporting mucus containing inhaled noxious substances, viruses and bacteria. By doing so they are an important part of our innate immune system. PCD patients suffer from mucus that builds up in the airways. As a result they have chronic rhinitis and are vulnerable for infections of the middle ear, sinuses and lungs. Eventually, this leads to permanent lung damage (bronchiectasis) in almost all patients. PCD is difficult to diagnose. In addition, male patients are usually sub –or infertile and in up to 50% of cases, the internal organs are in mirror image. This happens because respiratory cilia, nodal cilia and sperm flagella are remarkably similar in their core structure. Nodal cilia are responsible for the left-right asymmetry of the internal organs.

PCD is difficult to diagnose. The diagnosis is currently made by evaluating ciliary ultrastructure and movement in cells that are obtained by nasal curette biopsy. These tests require expertise, are costly, invasive and the results can take up to several months. A genetic test would be a major improvement in the diagnosis of PCD. However, this is not possible yet as only 60-70% genes that are linked to PCD are known. As motile cilia consist of >200 proteins, many genes can be involved in PCD. After we give an overview of PCD symptoms, diagnostics and treatment options in **chapter 1**, we describe the identification of two novel PCD-related genes in **chapter 2** and **3**. In **chapter 2** we show that a homozygous mutation in the *CCDC114* gene is the cause of PCD in all patients originating from the Dutch town of Volendam. In contrast to an estimated prevalence of 1 in 15,000-30,000 in the rest of the world, 1 in 400 inhabitants of Volendam suffer from PCD. This is related to the settlement of Volendam in 1462. Twenty families founded the village and lived relatively isolated ever since because of geographical, religious and cultural reasons. As a result, several inherited diseases occur more frequently in this population. The identification of the gene defect in *CCDC114* led to the availability of a simple diagnostic genetic test for inhabitants of Volendam. In this chapter we also show that fertility is preserved in PCD patients originating from Volendam, in contrast to fertility of other male PCD patients. The other novel PCD-related gene that we describe in **chapter 3**, *PIH1D3*, lies on the X-chromosome. As a consequence, PCD is inherited in an X-linked recessive manner in patients with this gene defect. This is the first proof of X-linked inheritance in PCD patients without syndromal co-segregation. This finding is important because it influences the analysis of next-generation sequencing data and the counselling of patients on the risks of passing on the disease to their offspring. In **chapter 4** we investigate the diagnostic yield of screening DNA of a Dutch cohort of 74 PCD patients. A preselected part of their DNA was screened by using targeted next-generation sequencing. We tested a panel of 26 genes that were already linked to PCD at that time and 284 PCD candidate genes. We show that in almost 68% of Dutch PCD patients a gene defect can be identified. PCD patients originating from Volendam were not included in this study. PCD candidate genes were selected by investigating differentiating gene expression during *in*

vitro cell culture of nasal curette biopsies from healthy volunteers. Defects in genes with high expression during ciliogenesis could theoretically be related to PCD. The list of approximately 5,500 genes with upregulation during ciliogenesis can now be used in the search for novel PCD-related genes. It may also lead to a reduction of exome sequencing data analysis (in which all coding parts of the DNA is read) to approximately 21% of total

In **chapter 5**, in the second part of this thesis, we summarize the available data on exhaled breath (VOCs; volatile organic compounds) in lung disease. We describe the progress and challenges in capturing and analyzing exhaled breath analysis in the diagnosis and monitoring of pulmonary diseases. In **chapter 6** we show that exhaled breath from children with PCD and CF (cystic fibrosis) can be distinguished from the breath of healthy volunteers and from one another, by use of a so-called "electronic nose". This is an important observation as these diseases can mimic each other in clinic, but have a distinct pathophysiological mechanism. The difference in exhaled breath pattern may reflect this. Moreover, we observed that the breath of patients with a pulmonary exacerbation could be differentiated from the breath of patients in a stable phase. Future research has to evaluate whether individual changes in exhaled breath indicating an exacerbation can be detected. In this way treatment could start earlier. No curative treatment exists for PCD. Due to a lack of scientific evidence, treatments in PCD are extrapolated from CF care. It is important to conduct intervention studies specifically designed for PCD patients. As CF and PCD have a distinct pathophysiology, patients may respond differently to a certain treatment. In **chapter 7** we describe the results of the first randomized controlled trial in adult PCD patients. Inhalations with hypertonic saline, highly concentrated saline water may hydrate airway mucus and thereby lower viscosity. As a result this may improve cough transportability in PCD patients, keeping their airways "cleaner". In 22 PCD patients that received hypertonic and isotonic inhalations (saline water with low concentration) for 12 weeks in a random order, we did not observe a difference in quality of life, based on the "St. George's Respiratory Questionnaire", a questionnaire primarily developed to measure quality of life in COPD patients. The "Quality of Life Bronchiectasis questionnaire", which is more disease-specific but was not yet validated at the time of the study, did show an improvement in general health perception after hypertonic saline treatment. Because we observed large inter-subject variations it is important to use sensitive disease outcome measures and a larger sample size in future PCD studies. In **chapter 8** we reflect on the main results and describe methodological considerations and directions for future research.

The main conclusions derived from the studies of this thesis are:

- One defect in the novel PCD-related gene CCDC114 is the cause of PCD in all PCD patients originating from Volendam. A genetic test has become available for these patients.
- A defect in the novel PCD-related gene PIH1D3 causes recessive X-linked PCD, without syndromal cosegregation. This is in contrast to the (normal) autosomal recessive inheritance mode.
- By using a targeted genepanel including 26 PCD-related genes and 284 candidate genes, we were able to identify the gene defect in almost 68% of a Dutch PCD cohort of 74 patients (who did not originate from Volendam)
- The exhaled breath pattern of VOCs, measured by an electronic nose, was significantly different in children with CF and PCD, compared to healthy children and compared to one another. In addition, exhaled breath patterns of children with and without a pulmonary exacerbation were significantly different in both CF and PCD patients.
- Twelve weeks of hypertonic saline (highly concentrated saline water) nebulizations do not improve quality of life in 22 adult PCD patients, measured by the "St. George's Respiratory Questionnaire". When measured by the Quality of Life Bronchiectasis questionnaire, a more disease-specific questionnaire, we did observe a small positive effect on general health perception after hypertonic saline.