CILIOPATHIES: ONE OF THE FAULTS IN THE HUMAN SYSTEM

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ABSTRACT:

This comment reviews the article by Li et al, published in 2004, whom compiled a dataset of proteins that presumably have a function in the cilia, flagella and/or basal bodies or its assembly. The purpose of this comment is to summarise their findings and put them into context. It is interesting to see how research was done about a decade ago and how science has grown since then. New techniques have been developed since 2004, which can facilitate further research. This comment is meant to inform the scientific community and inspire them to do more research on this subject.

KEYWORDS: Ciliopathies, Genetics, Proteins

The cilium is a very small organelle, mostly present on the cell membrane of quiescent or differentiated cells [1]. The basal body, derived from modified centrioles, forms the site where the cilium attaches to the cell [2, 3]. The cilia have a sensory function and transduce signals from the environment to the cell, like an antenna. Some cilia are important in tasks where motion is required. An organelle that slightly differs from the cilia is the flagella, which is also important for movement of the cell. The flagella also originates from the basal body. These functions of the cilia and flagella also play an important role in embryogenesis [1, 4]. A disease in which the cilia, flagella and/or basal bodies dysfunction is called a ciliopathy. Ciliopathies can lead to a vast range of phenotypes in multiple organs, because cilia are common across the human body. We will refer to the cilia, flagella and/or basal bodies as CFB.

An example of a ciliopathy is the Bardet-Biedl syndrome (BBS), a serious, but not well-known, rare, autosomal recessive disorder, from which symptoms occur before the age of 10 [5]. The disease is characterized by obesity, polydactyly, retinopathy, hypogonadism and cognitive impairment. In adulthood, patients with BBS will inevitably become blind, experience loss of hearing and often present themselves with Diabetes Mellitus type II [6, 7, 8]. The prevalence of BBS ranges from 1 in 100,000 to 1 in 175,000. [6, 7]. Another example is situs inversus, in which the major organs are reversed or mirrored.

Li et al. wanted to find the proteins that are involved in the assembly and function of CFB found in many eukaryotic organisms. In the paper, these proteins are referred to as the flagellar apparatus-basal body (FABB) proteome. To identify these proteins, and genes coding for these proteins, Li et al. used a comparative genomics approach, which means they compared the genomes and proteins of multiple organisms, by using in silico, in vitro and in vivo studies. Using WU-BLASTP, an algorithm used in bioinformatics to compare biological sequence information, like amino-acid sequences or DNA sequences, a dataset was compiled of the FABB proteins present in both Chlamydomonas reinhardtii and Homo sapiens, two organisms which are known to have cilia and basal bodies. Next, the proteins that were also present in Arabidopsis, an organism without cilia and basal bodies, were dismissed from the dataset that now assumably only contains proteins associated with cilia and basal bodies, because organisms that have lost CFB will also have lost the genes coding for them and their regulators [9, 2].

To determine whether the dataset of the FABB proteome indeed contained proteins involved in the CFB, Li et al. set the following five criteria that should be met: (1) the FABB proteome should contain genes coding for proteins that are known to be associated with CFB; (2) the characteristics of the domains in the FABB proteome should be like known domains of CFB; (3) after deflagellation a certain amount of genes should be upregulated; (4) interfering with the transcript of these genes should cause certain phenotypes; and finally, (5) there should be novel genes present involved in disorders of the human CFB in the FABB proteome.

Li et al. have met all five criteria. To begin with, 52 genes that encode for proteins that are associated with CFB were also found in the FABB proteome. Secondly, the paper found domains in proteins, with the algorithm Pfam, in the FABB proteome. The Pfam algoritm detects functional regions named domains in DNA sequences of proteins. Identifying a domain can lead to insight into the function of a protein. Those domains were virtually exclusively present in genes known to be associated with CFB. Furthermore, upregulation of several genes in the FABB protein followed deflagellation. This makes it likely these encoded proteins have a part in function and/or assembly of the flagella. Additionally, the genes that were not upregulated after deflagellation were impaired using RNA interference to investigate whether these genes coding for proteins in the FABB proteome were false positives or not. The authors found that the interference lead to a range of phenotypes: the flagella became malformed, dysfunctional or disorganized, dependent on the amount of interference. The gene expression of the impaired genes was measured using RT-PCR. Finally, Li et al. found a novel gene, BBS5, in which mutations in humans lead to the Bardet-Biedl syndrome. Further exploration of the BBS5 gene found that the gene and protein is truly part of the function/assembly of CFB. This means that there are possibly more genes in the FABB-proteome that, when mutated, lead to disorders in the human CFB.

There are many people who would benefit from research like Li et al.'s, e.g. individuals who suffer from diseases caused by dysfunctional CFB, their doctors and their family. Research on CFB may find clues on how to diagnose patients better and sooner, but also give them a more realistic prognosis. Besides, successfully understanding an organelle can give information about the cells or interactions with the environment. The paper also provided a way of investigating other organelles like the endoplasmic reticulum, which is suspected to have a part in Alzheimer's disease [10]. Also, this study could be relevant to cancer research, since tumors have often lost the ability to form cilia [11].

A few techniques that are used in the paper are WU-BLASTP, the Pfam algorithm, RNA interference and RT-PCR. Since this paper's release in 2004, 12 years ago, some of these techniques have become obsolete and others have been improved. WU-BLASTP, for example, was taken over by AB-BLAST and other BLAST databases exist as well. Today, databases contain much more information, which means more knowledge

can be used and compared. For example, in 2004 scientists assumed the human genome contained 30,000 genes, but now it is clear there are only 19,000, which means that proteins could be doubly represented [12]. Also, in the times this paper was written there was not much insight in the 3D structure of proteins, which diminishes the understanding of the complete assembly process, protein-protein interaction and cellular functions in the CFB.

New techniques have been developed as well. In the paper all experiments were done in lab conditions. Nowadays we can simulate an environment more similar to in vivo conditions, like with a kidney on a chip. [13]. This leads to a better translation of the outcomes to the clinic. Besides that, mass spectrometry can be particularly useful in the identification of proteins, among other things in protein complexes, resulting to a more thorough insight in the FABB proteome.

Since the release of Li et al.'s paper in 2004, the possibilities to do research have grown vastly. This means it has become easier to explore the causes of ciliopathies and develop new techniques to treat patients, like genetic modification, a technique focused on repairing faulty genes. Compared with 12 years ago, the amount of scientific knowledge has advanced greatly. Nevertheless, further research on new techniques and ciliopathies is still required to enable doctors to help their patients in a way they desire the most.

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