Genetics of PFIC: Current Status and Implications
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I am writing this summary in the spring of 2018. My Ph.D. training was in human genetics, and in the 25 years since then, I have performed research at the intersection of genetics and cholestatic liver disease. My work has focused on understanding the genetic factors contributing to inherited cholestasis, including PFIC and related diagnoses such as BRIC (benign recurrent intrahepatic cholestasis) and hypercholanemia, as well as ICP (intrahepatic cholestasis of pregnancy). I have also performed genetic and physiologic research using a mouse model of PFIC1, also called FIC1, or ATP8B1, deficiency.

In this article, I will try to provide an overview of our current understanding of the genetics of PFIC, based upon our work and that of colleagues in the field. I will also indicate some of what still remains unknown.

PFIC is often divided into 2 broad categories: ‘Low-γGT PFIC,’ in which serum activity of gamma-glutamyltranspeptidase is usually low or normal, and ‘high-γGT PFIC,’ in which the activity is above normal. In reality, occasional patients have intermediate levels and can be hard to categorize according to this criterion, and every once in a while, we find a patient whose γGT doesn’t fit what was expected, based on genetic findings. Nevertheless, γGT can be a useful way to subclassify forms of PFIC.
One other point to note- while this article focuses on patients with the clinical diagnosis of PFIC, the reality is that different patients can have mutations in the same gene, but have cholestatic disease of varying severity- for example, some patients with mutation in FIC1 have PFIC, while others are diagnosed with BRIC, or appear to have a condition that is intermediate between these 2 conditions. This variation in severity of disease between patients with mutation in the same gene is at least partly explained by differing effects of mutations. Some mutations more or less completely prevent function of the protein encoded by the mutated gene, while other mutations may impair, but not completely prevent, protein function.

Most PFIC patients in whom genetic mutations have been identified appear to have autosomal recessive disease. This means that they develop PFIC because both copies of a single gene important for liver function- one inherited from mom and the other from dad- carry disease-causing mutations. All of the forms of PFIC discussed below have this type of inheritance, unless otherwise mentioned.

I will briefly describe the different ‘PFIC genes’ that have been identified to date.

(Note: in human genetics, gene names are shown in italics, while protein names are indicated in regular text. Especially since the commonly used names for particular genes are sometimes different from the typically used names for the proteins they encode, this can be confusing. I’ve tried to use the most frequently employed names for the genes and proteins discussed below.)

FIC1/ATP8B1 (PFIC1)
Some people with low-γGT PFIC carry mutations in *ATP8B1*, the gene which encodes the FIC1 protein (for Familial Intrahepatic Cholestasis 1, as it was the 1st PFIC gene discovered). FIC1 is expressed in the liver, but is also expressed in many other places in the body. FIC1 deficiency is often considered a ‘syndrome’ (a condition in which multiple organ systems are directly impacted by the loss of protein function), rather than simply a liver disease. For example, FIC1 is expressed in the intestine, pancreas, and inner ear, and FIC1 patients are more prone to diarrhea, pancreatic insufficiency, pancreatitis, and hearing loss than are most people with cholestasis.

When FIC1 deficiency was first discovered as a cause of PFIC, we didn’t know what the FIC1 protein does. Accumulating evidence now indicates that it is a ‘phospholipid flippase’ that helps maintain the proper distribution of lipids within cell membranes. The precise ways through which loss of FIC1 function leads to cholestasis aren’t certain, although there are several ideas with at least some supporting evidence. For example, incorrect distribution of lipids in liver cell membranes may make the liver cells more vulnerable to damage by bile acids, and may cause some membrane proteins important for normal bile acid transport to have impaired function. FIC1 function may also be important for movement of vesicles within cells; such movement is important for a variety of cellular processes.

**BSEP/ABCB11 (PFIC2)**

The most common genetic form of low-γGT PFIC is caused by mutations in *ABCB11*, the gene which encodes the BSEP protein (bile salt export protein). BSEP is expressed only in the liver, and transports bile acids out of the liver. When BSEP function is lost or impaired, bile acids accumulate in the liver, causing cholestasis and often, liver
inflammation and damage. Depending on the nature of the BSEP mutation(s) that a patient has, PFIC caused by BSEP deficiency may progress rapidly, or more slowly.

MDR3/ABCB4 (PFIC3)

Mutations in ABCB4, which encodes the MDR3 protein, can cause a high-γGT form of PFIC. MDR3 protein helps move PC (phosphatidylcholine) from within liver cells into the part of the liver where bile is formed. PC helps form micelles with bile acids in bile. In these micelles, bile acids are surrounded by lipids, and so are not able to damage the nearby liver cells. When there is not enough PC in bile, the bile acids in bile are able to damage liver cells. Patients with PFIC caused by MDR3 deficiency usually have mutations on both copies of the gene. However, unlike in FIC1 and BSEP deficiencies, people with only one mutated copy of MDR3 are known to sometimes develop liver disease due to partial MDR3 deficiency; in such cases, the liver disease may progress slowly over the life of the individual, and not be noticed until it is advanced.

TJP2/ZO2 (PFIC4)

The TJP2 protein (Tight Junction Protein 2, sometimes called ZO2) plays a role in structures called ‘tight junctions.’ Tight junctions occur where cells meet, and help to control what molecules are able to pass between cells, as well as separating different parts of the cell membrane from each other. Such junctions are important throughout the body, and TJP2 is expressed in many different tissues. PFIC due to TJP2 deficiency does not always fit perfectly into the ‘low-γGT’
versus ‘high-γGT’ categorization. The γGT tends to be close to normal- i.e. most similar to that in low-γGT PFIC, but sometimes a bit higher.

Given that TJP2 is present throughout the body, we might expect that TJP2 deficiency, like FIC1 deficiency, could be a syndrome, affecting more than just the liver. However, only a small number of patients with PFIC caused by TJP2 mutation have been studied so far, so we don’t yet have a firm understanding of what manifestations, other than liver disease and its consequences, TJP2 deficiency patients may have. So far, information indicates that TJP2 deficiency patients may have an increased risk of some respiratory and neurological conditions.

FXR/NR1H4 (PFIC5)

A seemingly especially rare genetic form of low-γGT PFIC was recently identified. This form of PFIC is caused by mutation in NR1H4, which encodes the FXR (the Farnesoid X Receptor) protein. The FXR protein is known as a nuclear receptor and transcription factor. This means it plays an important role in controlling the expression of genes. FXR is important in regulation of bile acid metabolism in the liver and intestine, as well as in other aspects of metabolism. Patients with PFIC due to FXR deficiency seem to develop rapidly progressing liver disease very early in infancy. Only 4 patients have been reported so far, but more will likely be identified over time. Then, we will be able to better understand the full spectrum of manifestations of this form of PFIC.

MYO5B/MYO5B (No PFIC number in use)
Genetic mutations in $MYO5B$ (Myosin 5B) were found some years ago to be responsible for some cases of a predominantly intestinal disorder called microvillus inclusion disease. It was noticed, that some patients with mutations in this gene had cholestasis as well as intestinal disease. Just in 2017, however, a number of patients with mutations in $MYO5B$ were reported who have low-$\gamma$GT cholestasis, without significant intestinal disease. We aren’t yet sure why some patients with mutations in this gene have intestinal disease without liver disease, others have liver disease only, and some have both.

$MYO5B$ is expressed in multiple parts of the body, and is involved in maintaining proper functioning of cell membranes and helping to move proteins, such as BSEP, to where they are needed in the cell membrane. Some patients with cholestasis due to $MYO5B$ deficiency have progressive liver disease, while others have it only intermittently. Even in the patients with progressive liver disease, progression usually seems slower than in patients with other genetic forms of PFIC.

OTHER GENETIC FORMS OF PFIC

Although many PFIC patients have mutation in one of the genes mentioned above, there are still PFIC patients in whom we do not find mutations in any of these genes. Genetic studies are underway, in my lab and other places as well, to try to identify genetic factors contributing to PFIC in such patients. There are several reasons why the identification of the genetic cause of PFIC may be more difficult in some patients. For example, it may be the case that there are a number of very rare genetic forms of PFIC, due to mutations in other genes, that haven’t yet been figured out; usually, the rarer a genetic condition is, the harder it is to identify the relevant disease gene. It may also be the case that some PFIC patients carry mutation in more
than one gene, which makes determining the genetic cause of their condition more difficult. Also, some patients may have mutations that are very hard to detect with usual genetic testing methods, and for some genetic changes that are found, it is difficult to tell if they are likely to cause disease or are just rare genetic variation without an important role in causing disease. Also, occasionally, a patient diagnosed with PFIC may turn out to have mutation in another gene implicated in a different form of liver disease; the genetic cause of disease in cases like this may be identified with genetic testing that looks at large panels of genes important for the liver, or by approaches such as whole-exome sequencing (in which the aim is to sequence all of the protein-coding sequence in our DNA) or whole-genome sequencing.