

Commentary: Loss of FOCAD, Operating via the SKI Messenger RNA Surveillance Pathway, Causes a Pediatric Syndrome with Liver Cirrhosis [1]

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Liver disease is becoming a major health concern and is estimated to be the fifth most common cause of death worldwide [2]. A systematic review from the Global Burden of Disease Study identified 1.32 million deaths due to liver cirrhosis in 2017, accounting for more than two percent of the total global deaths [3]. Liver cirrhosis is usually diagnosed late in life; it is irreversible, staying chronic for the rest of the patient's life; and it can be life-threatening, as it may evolve into hepatocellular carcinoma or lead to liver failure. Despite extensive research, to date none of the tested compounds for treating this disorder have proven to be safe and effective in human patients. Current available therapies are generally palliative and focused on slowing down the disease, with liver transplant remaining as the gold standard strategy to treat patients with decompensated cirrhosis. Therefore, there is a patent need for the identification and validation of new targets to treat, reverse and, ultimately, cure this hepatic disorder.

Liver cirrhosis is traditionally believed to be caused by environmental insults such as poor diet, viral hepatitis, or alcohol abuse. Nevertheless, little has been done to explore the existence of possible underlying genetic causes. Mendel's experiments of crossing peas to determine that certain traits are inherited as single genes are so fundamental that they are taught in high school. The enduring power of these principles lies in the fact that they allow us to tie mutations in one specific gene to an important human disease. Using the power of Mendelian genetics, we were able to pinpoint a cause for paediatric liver cirrhosis to mutations in a rarely studied gene called *FOCAD*. The finding of a monogenic disorder that leads to cirrhosis in childhood establishes a strong genetic component for liver disease.

In collaboration with hospitals and institutes across continents, we identified a total of 14 children from 7 countries (India,

USA, Saudi Arabia, Pakistan, Portugal, Brazil, and France) presenting with a multisystemic syndrome not documented before and characterized by severe neonatal liver disease. By genome/exome sequencing, all cases were found to segregate germline recessive mutations in *FOCAD* (MIM614606), a gene with no previously reported links to liver biology. Our functional studies on patient's primary cells and *in silico* prediction analysis confirmed that these *FOCAD* variants were loss-of-function.

Using the state-of-the-art CRISPR/Cas9 technology, we generated *in vitro* and *in vivo* *FOCAD*-knockout biological models that helped to delineate the cellular and molecular basis of this congenital liver syndrome. Zebrafish lacking *foCAD* phenocopied the human disease, revealing a signature of altered mRNA degradation processes in the liver. Using patient-derived primary cultures and *FOCAD* inactivation in human hepatic cell lines, we found that *FOCAD* deficiency compromises the SKI mRNA surveillance pathway by reducing the levels of the RNA helicase SKIV2L and its cofactor TTC37. The SKI pathway is a molecular quality control mechanism that assists the translation of mRNAs into proteins by destroying transcripts that are faulty [4]. The significant clinical overlap between the *FOCAD*-deficiency and the Tricho-Hepato-Enteric Syndrome (THES) caused by recessive mutations in *SKIV2L* and *TTC37* [5], further supports the functional role of *FOCAD* vis-à-vis the SKI complex. This is the first time that this translation-dependent quality control machinery has been implicated in liver health. *FOCAD*-deficient hepatic stellate cells did not appear to be overtly compromised. However, hepatocytes, the main parenchymal cells of the liver, were found to rely heavily on this mechanism compared to other cell types, showing lowered albumin expression and signs of persistent injury when *FOCAD* is absent.

Commentary

Although childhood liver cirrhosis due to mutations in *FOCAD* is infrequent, we take a rare begets common paradigm: rare mutations that cause an otherwise common disease can teach us about its origins and possible remedies. We found out that *FOCAD* deficient hepatocytes overproduce the cytokine *CCL2*, which attracts immune cells, promotes hepatic inflammation [6], and could play a key driver role in the progression of the disease. Drugs that target this or similar candidates are potential therapeutic intervention points not only for this orphan syndrome, but also for more common forms of cirrhosis and other related disorders, such as non-alcoholic steatohepatitis (NASH) and liver cancer.

In conclusion, we reported for the first time the clinical impact of recessive loss-of-function variants in the *FOCAD* gene, and provided evidence for the indispensable role of the SKI mRNA surveillance pathway for liver homeostasis. Our clinical data will help clinicians to identify new patients with this syndrome, better understand the cellular and molecular mechanisms of the disease, and hence, provide a more accurate diagnosis, prognosis, and treatment. The research also brings forth the first animal model of the human disease, as well as *in vitro* biological systems that are now being used as platforms to identify and validate new anti-fibrotic therapeutic targets.

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