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PNPLA3, the History of an Orphan Gene of the Potato Tuber Protein Family That Found an Organ: The Liver

See Article on Page 2170

To fall in love is to create a religion that has a fallible god. — Jorge Luis Borges (1952)

The patatin-like phospholipase domain containing 3 gene (*PNPLA3*) was an orphan gene looking for a disease until 2008 when the first genome-wide association study (GWAS) on nonalcoholic fatty liver disease (NAFLD) showed the association between the risk of liver fat accumulation and the nonsynonymous rs738409 C/G variant.[1] Months later, the variant was significantly associated with NAFLD disease severity[2] and alanine aminotransferase (ALT) levels,[3] and the role of *PNPLA3* as a modifier of the natural history of NAFLD was unequivocally replicated in different populations around the world, from children to adults.[4] The enthusiasm about *PNPLA3* led to the search for other biologically plausible liver disease associations, for instance, alcoholic liver disease (ALD)[5-7] and an increased risk of steatosis in patients with chronic hepatitis C (CHC)[8,9] and B.[10] The variant was not only associated with cirrhosis and the occurrence of hepatocellular carcinoma (HCC),[11] but the G allele was also identified as an independent risk factor for death and poor prognosis.[12]

At last, *PNPLA3* found an organ: the liver.

In this issue, Trépo et al.[13] explore the strength of the association between the rs738409 and the prevalence of HCC in cirrhosis patients by means of a meta-analysis of 2,503 individual participant data (IPD) from Europe. The authors found that the risk allele G was significantly associated with HCC (odds ratio [OR] per allele = 1.77), after adjusting for sex, age, and obesity. Although the association was also found in patients with HCV-related cirrhosis (OR = 1.55), in which the variant showed a small but statistically significant effect on the risk of HCC, the effect was more pronounced among patients with ALD (OR = 2.20), suggesting that the variant influence is stronger, if not exclusive, on fat-derived HCC.

Altogether, these results suggest some reflections: 1) rs738409 is the most consistent genetic modifier of the natural history of common chronic liver diseases in which inflammation and fibrogenesis are induced by environmental factors (dietary fat, alcohol, or viruses). 2) rs738409 is consistently associated with disease progression in liver diseases in which liver fat accumulation matters (NAFLD, ALD, and also CHC). Hence, instead of being "the cause" of the disease in terms of traditional monogenic disorders, the variant seems to be an important link between environmental stressors and the susceptibility of the liver to develop a more aggressive phenotype. 3) From a theoretical perspective, it can be argued that rs738409 has *per se* a substantial role in hepatocarcinogenesis because the variant is involved in related histological outcomes, such as steatosis and inflammation.[4] Because the report of Trépo et al. is a cross-sectional study, we cannot rule out the possibility that patients enrolled in this meta-analysis have had fatty liver prior to developing cirrhosis; thus, we do not know whether carriers of the G allele that developed HCC were those who had increased susceptibility to steatosis (in other terms, adjustment by steatosis). Considering that IPD is a unique opportunity to have full access to patients' records, it would have been interesting to know whether the association between the variant and HCV-HCC remained significant after adjusting for well-

Abbreviations: ALD, alcoholic liver disease; ALT, alanine-aminotransferase; AUC, area under the curve; CHC, chronic hepatitis C; GWAS, genome-wide association study; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain containing 3; ROC, receiver operating characteristic; SNP, single nucleotide polymorphism

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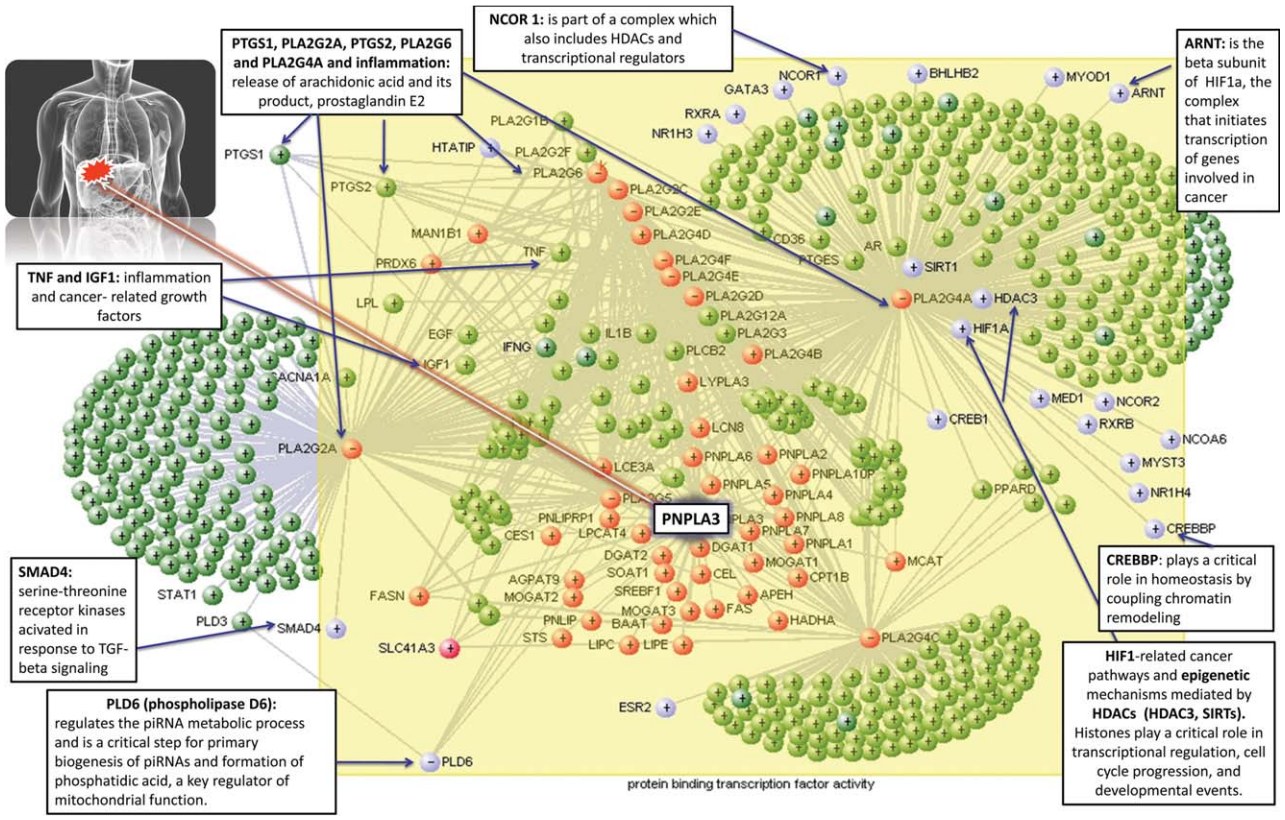

Fig. 1. Proposed scheme of *PNPLA3*-mediated hepatocarcinogenesis. Analysis of interaction pathways of *PNPLA3* performed by the VisANT resource (http://www.openhelix.com/cgi/tutorialInfo.cgi?id=102). The metagraph structure allows the visualization of bio-networks constructed by integrative data-mining features that permit a large number of functional associations for 103 different species. Few nodes are labeled for the sake of simplicity; in this figure, we highlighted the networks putatively associated with inflammation and cancer development. Direct *PNPLA3*-related nodes are shown in red. The nodes marked in blue depict results of putative protein binding transcription activity. Specifically, we focused on potential epigenetic factors that have never been explored before in association with *PNPLA3,* such as remodeling of the chromatin structure and function by histone deacetylases (HDACs), which are involved in tumor development, cell proliferation, cell-cycle regulation, and apoptosis. A novel hypothesis about the relation of PNPLA3 with PLD6 (the mammalian homolog of *Drosophila*'s Zucchini protein) and its role beyond the phospholipase activity and phosphatidic acid generation as an important participant in PiwiRNA biogenesis is proposed. Gene labels are official names. *PLA2G6*: phospholipase A2, group VI (cytosolic, calcium-independent); *PTGS1*: prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase); *PLA2G4A*: phospholipase A2, group IVA (cytosolic, calcium-dependent); *PLA2G2A*: phospholipase A2, group IIA; *IGF1*: insulin-like growth factor 1; *TNF*: tumor necrosis factor; *NCOR1*: nuclear receptor corepressor 1; *ARNT*: aryl hydrocarbon receptor nuclear translocator; SMAD4: SMAD family member 4; *PLD6*: phospholipase D family, member 6 (mitochondrial phospholipase); *HIF1A*: hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor); *HDAC3*: histone deacetylase 3; CREBBP: CREB binding protein.

known risk factors of HCC development, such as viral load and genotype; undoubtedly, this finding should be confirmed in a better than 0.6-powered sample. Moreover, adjustment by insulin resistance would have been desirable in both cohorts. Furthermore, to be generalized, these results should be replicated in non-European patients. Hence, to fall in love with the idea that *PNPLA3* is *per se* involved in hepatocarcinogenesis is to create a hypothesis that has *a priori* a fallible biological plausibility.

We can envision some questions raised by the readers. For example, clinicians may ask: If I have a patient with cirrhosis who carries the GG genotype, should I make a quick decision to reach an early diagnosis and treatment of HCC? In other words, are the results from a *PNPLA3* test clinically applicable? According to Trépo et al., the probability with which HCC will occur in carriers of the G variant is ~64% higher per allele (OR 1.77 converted to absolute risk). In terms of personalized medicine, rs738409 is still not a feasible predictive test that can accurately discriminate between individuals who will develop HCC and those who will not, because very large ORs (probably over 10-200 to increase the area under the curve [AUC] in a receiver operating characteristic [ROC] curve) are typically needed to improve predictive accuracy above that provided by clinical information.

People from the genomic world may ask: Why did previous GWAS on HCC not uncover the *PNPLA3* association? Although a comprehensive search of

markers encompassing the entire genome was previously performed on the risk of CHC-induced HCC,[14,15] single nucleotide polymorphisms (SNPs) at *PNPLA3* were not identified. There are a number of explanations for this lack of association: i) small effects for which GWAS are underpowered to detect owing to the large number of tested SNPs and the need of multiple testing correction; ii) spurious associations; and iii) the "missing heritability" explained by epistasis and gene-environmental interactions, and so forth. The simplest explanation is that hypothesis-driven candidate gene studies still have a place for understanding the genetic architecture of complex traits using approaches such as the one described by Trépo et al.

Finally, scientists may wonder whether *PNPLA3* has another function in addition to the known triacylglycerol lipase and acylglycerol O-acyltransferase activities that explain a putative role in liver injury and carcinogenesis. The identification of the molecular mechanisms by which rs738409 is linked to hepatocarcinogenesis is certainly challenging because, disappointingly, the mechanisms by which the variant modulates cancer-linked phenotypes, such as inflammation and fibrogenesis, still remain unknown. *In silico* analysis of interaction pathways of *PNPLA3* suggests that some of the genes on the pathway are involved in the release of arachidonic acid and prostaglandin E2 (Fig. 1), a finding previously associated with the transition from steatosis to nonalcoholic steatohepatitis (NASH).[16]

Nevertheless, there are many things we do not know; for example, the impact of the variant on chromatin structure, and how these changes might impact the transcriptional machinery that orchestrates the *PNPLA3*-related network. Putative mechanisms associated with *PNPLA3*, epigenetic changes, and hepatocarcinogenesis are depicted in Fig. 1. Finally, we do not know almost anything about the role of *PNPLA3* on liver function beyond its participation in lipid remodeling. A recent report showed that *pnpla3* knockdown in zebrafish decreased the expression of hepatic progenitor cells and significantly impacted liver growth and global homeostasis.[17] This finding allows us to speculate that *PNPLA3* might exert a phospholipase D6 (PLD6)-like activity, which may modulate the biogenesis of Piwi-interacting RNAs (piRNAs, small RNAs associated with proteins of the Argonaute family) or even regulate the release of phosphatidic acid on the mitochondrial surface (Fig. 1).

In summary, we learned that *PNPLA3* is associated with increased liver fat accumulation and, consequently, with inflammation and even fibrosis. We need to refocus our research on both the physiologic role of the *PNPLA3* on the human liver and the pathogenic role of the rs738409 on the progression of liver diseases, including liver cancer.

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