Understanding the Role of PNPLA3 Genetic Variants in Patients with Chronic Hepatitis C Infection

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In 2008, in an attempt to identify genetic determinants of liver steatosis, Romeo et al. ran an independent genome-wide association study on 2,121 patients enrolled in the Dallas Heart Study that had hepatic fat content assessed by proton magnetic resonance spectroscopy [1]. By concentrating just on nonsynonymous sequence variations the authors were able to discover a variation (rs738409 C>G) at position 148 in the patatin-like phospholipase-3 (PNPLA3) gene as the strongest determinant of steatosis in patients. The genetic polymorphism encodes an isoleucine-to-methionine substitution. The PNPLA3 gene encodes a 481 amino acid protein of unknown function that belongs to the patatin-like phospholipase family; the progenitor of this family, patatin, has nonspecific lipid acyl hydrolase activity. Following this breakthrough discovery, several candidate gene studies have demonstrated that the G allele of PNPLA3 single nucleotide polymorphism (SNP) influences liver fat accumulation, is associated with disease severity in adult and pediatric populations with NAFLD, and ultimately increases the risk of NASH [2–5]. These results did not go unnoticed among colleagues working in the hepatitis C virus (HCV) field, as several studies were designed to assess the clinical role of the PNPLA3 polymorphisms in patients with chronic hepatitis C. The reasons for this interest are many fold. First, steatosis is known to negatively impact the natural history of HCV infection as it accelerates progression to cirrhosis [6, 7]. Second, liver steatosis has been shown to be a negative

moderator of treatment outcome to interferon (IFN)-based therapies, with patients showing a fatty liver achieving lower sustained virological response rates across all HCV genotypes [8]. Third, with respect to the plethora of clinical trials currently investigating future potential anti-HCV drugs, the identification of a further genetic polymorphism able to influence treatment outcome would have an enormous impact in the design of future studies [9]. Among the first to demonstrate the impact of the PNPLA polymorphism in patients with HCV infection were the studies by Valenti et al. and Trèpo et al. who, by analyzing large cohorts of HCV patients in Europe, almost simultaneously reported the G allele of rs738409 SNP to be associated not only with the presence of histologically determined liver steatosis, but also with the presence of cirrhosis and accelerated fibrosis progression [10, 11]. Ginanni-Corradini et al. further expanded these findings by reporting an increased rate of hepatocellular carcinoma (HCC) development in patients with the GG genotype of rs738409 in a cohort of 222 HCV patients [12]. Indeed by multivariate analysis the GG genotype was an independent factor associated with HCC development carrying a 2.23 odds ratio. Taken together these data rather unequivocally correlate the G allele of the PNPLA3 SNP with a worse prognosis of chronic hepatitis C.

On the other hand the first studies were not able to solve if the G allele of the PNPLA3 SNP had an impact on IFN-based treatment efficacy. Indeed Valenti et al. were able to show a modest effect only in HCV-1 patients, with GG patients achieving a 10 % SVR rate compared to the 36 % obtained by the CG/CC counterpart, while Trèpo et al. could not replicate these findings in their cohort. These discrepant results are not surprising given the extreme heterogeneity of the cohorts in terms of known moderators of treatment outcome such as disease severity, HCV

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genotype, age and gender. Moreover, these studies were not specifically designed to assess treatment efficacy and as a consequence often included patients who received IFN monotherapy or who had already failed a previous course of therapy. To override these biases, Clark et al. analyzed the IDEAL study cohort with the specific aim of assessing the role of the PNPLA3 SNP on hepatic steatosis and treatment outcome [13]. The IDEAL cohort has the benefit of being composed of previously untreated HCV genotype 1 patients, in all cases characterized by a liver biopsy and by Interleukin 28B (IL28B) genotype [14]. Although patients were treated either with PegIFNalpha2a or alpha2b, that have been shown to achieve slightly different SVR rates in some clinical trials, still given the similar SVR rates obtained by the two regimens in the IDEAL study this is unlikely to have played a confounding role in the present analysis [15–17]. In their analysis of 972 HCV-1 Caucasian patients, Clark et al. replicate the association between the PNPLA3 risk allele and presence of any steatosis, clinically significant steatosis (>5 % of hepatocytes) and severe steatosis (>32 % of hepatocytes). Although the authors were able to reproduce previous data showing the T allele of the IL28B rs12979860 SNP to be associated with steatosis [18], they could not replicate the finding that the CC genotype of IL28B protects from steatosis only in patients with the risk allele of the PNPLA3 SNP that has been recently reported by others [19, 20].

Interestingly, although steatosis was an independent predictor of treatment outcome, no association between PNPLA3 genotype and SVR was observed. This suggests metabolic steatosis rather than genetic steatosis to be the reason for treatment failure in this cohort of patients. The lack of association between PNPLA3 genotype and SVR has been recently confirmed by a collaborative study conducted in Italy and Austria where 602 naïve patients of any HCV genotype were treated with PegIFNalpha plus Ribavirin [21]. Overall, in that study SVR rates were 51 % in rs738409 GG and 59 % in CG/CC patients. When breaking down the data the SVR rates were influenced by the GG genotype only in HCV-1 and -4 patients with bridging fibrosis/cirrhosis (GG: 17 % vs CG/CC 46 %, $p = 0.03$). This finding could not be replicated by Clark et al., as a likely consequence of the small number of patients with advanced fibrosis (12 %) enrolled in the IDEAL study. Overall, based on more than 1,500 patients analyzed in the Clark et al. study and the Valenti et al. study, it is safe to conclude that PNPLA3 polymorphisms have little to no impact on SVR rates to PegIFN and Ribavirin. This information coupled with the increased antiviral effect of triple therapy regimens containing Telaprevir or Boceprevir in HCV-1 patients [22] de facto suggests against routine assessment of PNPLA3 genotype before starting anti-HCV therapy.

So does this effectively end the enthusiasm for PNPLA3 in HCV patients once and for all? Well, thanks to the data by Clark et al., it is safe to say that PNPLA3 testing has no place as a decision-making step to start or defer treatment at the individual patient level, nor should it be used to stratify patients enrolled in studies on investigational agents. Still, as physicians we should keep in mind that the ultimate goal of anti-HCV treatment is not to eliminate the virus but actually to cure the patient by preventing the occurrence of liver disease complications [23]. And in this respect PNPLA3 might have a prominent role. Indeed patients with NAFLD or NASH do progress to cirrhosis, suggesting that an SVR patient with the risk allele for PNPLA3 should probably be kept monitored despite the eradication of the virus. Moreover, PNPLA3 might be the missing piece in the puzzle that is HCC development in cirrhotic patients with an SVR. Indeed HCC is known to occur at a 0.3 % yearly rate in cirrhotic HCV patients following an SVR, with most studies showing an increased incidence in those with metabolic co-factors [24]. In a recent study by Asahina investigating 2,166 HCV patients receiving IFN based therapies, steatosis >10 % was an independent risk factor for the development of HCC in patients with an SVR, carrying a 3.5 risk ratio [25]. Although this does not prove genetic steatosis to be the culprit, it suggests that we still have a lot to investigate before shutting the door on future studies on PNPLA3 genetic variants in patients with HCV infection.

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Conflict of interest None.

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