EDITORIAL

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Do variations in the *ITPA* gene determine the risk of hepatitis C virus relapse?

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***ITPA* polymorphisms & ribavirin-induced anemia**

A recent genome-wide association study demonstrated that two genetic variants, a missense variant in exon 2 (rs1127354, P32T) and a splice-altering single-nucleotide polymorphism in intron 2 (rs7270101, IVS2) of the *ITPA* gene, entailing reduced ITPase activity, protecting against ribavirin-induced hemolytic anemia during interferon and ribavirin therapy for hepatitis C virus (HCV) genotype 1 [1]. This protection against anemia has been subsequently confirmed in numerous studies [2–8], but divergent results regarding the association with outcome have been reported.

***ITPA* polymorphisms & sustained virological response**

Several, but far from all studies have reported associations between homo- or hetero-zygosity at A_{rs1127354} or C_{rs7270101}, which are present in 37% of HCV patients [2], and a higher likelihood of achieving sustained virological response (sustained virological response [SVR]; i.e., undetectable HCV RNA 24 weeks after termination of therapy), mediated by reduced

relapse rates [2–5]. This reduction in relapse resembles that reported when ribavirin was added to interferon, thus markedly improving outcomes as compared with prior interferon monotherapy [9]. In contrast to the aforementioned studies, others have not been able to demonstrate any significant association between *ITPA* variants and outcome [1,6–8]. These discordant results may potentially be explained by differences in adherence monitoring and ribavirin dosing.

When evaluating the impact of potential response predictors, monitoring of adherence to medication is essential in order to minimize the dilution effect caused by the inclusion in the analysis of patients not achieving SVR due to premature treatment termination secondary to neuropsychiatric side effects of interferon. Furthermore, the well-established relationship between reduced ITPase activity and protection against anemia may act as a confounding factor, as the latter subsequently improves ribavirin adherence [8], which consequently impacts on outcome.

When evaluating a cohort of northern European treatment-naïve patients with

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chronic HCV genotype 2/3 infection receiving peg-IFN- α 2a 180 μ g once weekly and the standard, lower ribavirin dose of 800 mg daily (NORDynamIC study), a significant association between *ITPA* polymorphisms and treatment efficacy was noted, mediated by reduced relapse [2]. This observation could not be explained by improved adherence, protection against anemia or more efficient early elimination of the virus [10]. In the NORDynamIC study, adherence was closely monitored both by case report forms and patient diaries. The strong association between reduced ITPase activity and therapeutic outcome remained significant when patients were grouped by HCV genotype, *IL28B* or liver fibrosis. It was also noted in this study that reduced ITPase activity was significantly associated with lower plasma ribavirin levels [2], which are otherwise correlated with increased risk of treatment failure. Similarly, Clark *et al.* reported that among 70 genotype 2 or 3-infected real-life clinic patients receiving 800 mg ribavirin daily and 123 HCV genotype 1 or 4-infected treated with 1000–1200 mg of ribavirin in combination with peg-IFN- α , reduced ITPase activity was significantly associated with an increased likelihood of achieving SVR by means of reduced relapse [4]. However, in this study, the authors could not exclude improved adherence secondary to protection against anemia, even though reduced ITPase activity remained significantly associated with SVR despite the inclusion of ribavirin dose reductions and anemia in the multivariate analyses.

The aforementioned studies contrast with those by Thompson *et al.* [6] and Eskesen *et al.* [7], who also assessed HCV genotype 2/3-infected patients, finding that *ITPA* variants protected against anemia, but did not impact on SVR. In both of these studies, higher ribavirin dosing was used, (i.e., 1000/1200 mg/day [6] and 800–1400 mg/day weight-based dosing [7]) instead of the conventional, lower 800-mg daily dose.

Similar to the observations noted among HCV genotype 2/3-infected patients, in a subset of 81 genotype 1b-infected Japanese patients with the favorable *IL28B* rs8099917 TT genotype treated with 600–1000-mg daily weight-based dosing of ribavirin and peg-IFN- α for 48-weeks, carriage of the minor A_{rs1127354} *ITPA* allele was associated with an improved likelihood of achieving SVR, as well as reduced relapse [3], possibly secondarily to improved ribavirin

adherence. In another Japanese study among HCV genotype 1-infected patients treated with ribavirin 400–1000 mg/day and peg-IFN- α for 72 weeks because of late viral response, A_{rs1127354} *ITPA* carriage was associated with a significantly higher likelihood of achieving SVR [5], again mediated by reduced relapse. In this latter study, both ribavirin concentration at week 44 and *ITPA* genotype were independently associated with SVR in a multivariate analysis [5].

By contrast, Holmes *et al.* observed among 546 HCV genotype 1-infected patients receiving 1000–1200-mg weight-based ribavirin that the major *ITPA* variant, entailing normal ITPase activity, was associated with higher cumulative dose reduction, but not with ribavirin concentration at week 8 or SVR [8]. Thus, higher ribavirin dosing may possibly overshadow the impact of *ITPA* variants, as higher intracellular ribavirin levels may overcome the negative impact of normal ITPase activity.

How can reduced ITPase activity have a ribavirin-like effect on relapse rates?

ITPase is a highly conserved enzyme that recycles purines by converting ITP into IMP, thus protecting against the accumulation of rogue nucleotides (e.g., ITP and dITP), which otherwise may lead to erroneous incorporation into RNA and DNA, resulting in mutations, recombinations, altered RNA structures, irregular proteins and altered ATP-dependent signaling [11]. The consequences of ITPase deficiency remain largely unclear; however, it has been associated with increased drug toxicity when treating with purine analogs [12,13].

Thus far, the underlying mechanism of action through which lower ITPase activity protects against relapse remains unclear; however, it possibly differs from that suggested regarding protection against ribavirin-induced anemia (i.e., diminished oxidative stress in erythrocytes through the evasion of ATP reduction) [14]. By contrast, reduced ITPase activity has been hypothesized to have a ribavirin-like mechanism of action [2]. In part, the antiviral effects of ribavirin, which also has little effect on viral load during HCV treatment [15] but markedly reduces relapse after discontinuation of therapy [16], are assumed to be achieved by inhibition of IMPDH, leading to depletion of GTP and a circuitous increase of ITP via the induction of IMP [17]. This parallels findings in genetic knockout models, where ITP concentrations surge as a consequence of a lack of ITPase [18],

whereas intracellular levels of IMP (the substrate of IMPDH) diminish, which in turn results in lower concentrations of GTP. Therefore, it may be hypothesized that increased intracellular concentrations of ITP and decreased levels of GTP, whether as a result of lower ITPase activity or the extrinsic addition of ribavirin, lead to increased incorporation of this noncanonical nucleotide [11] by the nonstringent HCV RNA-dependent RNA polymerase, which lacks proofreading capacity, with ensuing random mutagenesis. This mechanism could potentially be augmented further by a similar incorporation of ribavirin triphosphate. Supportive of this notion is the reported strong synergistic effect of the inhibition of both the IMPDH and ITPA homologs in *Escherichia coli* and *Saccharomyces cerevisiae* leading to increased dITP and ITP levels in DNA and RNA [19]. In addition, inosine-containing RNA has been proposed to act as a potential danger signal, triggering the activation of both adaptive and innate immune responses [20].

Possible clinical applications in HCV & other viral infections

There are potential clinical applications of determining *ITPA* genetic variants (e.g., lower daily dosing of ribavirin might be sufficient for HCV-infected patients with polymorphisms that encode reduced ITPase activity when receiving IFN-based therapy, whereas higher dosing might be preferential for patients who are homozygous for both major alleles with normal ITPase activity). This could affect future HCV treatment guidelines, even in more affluent regions in spite of the therapeutic improvements secondary to the recent introduction of the relatively costly direct-acting antiviral agents. Indeed, the majority of patients have normal ITPase activity and so may potentially benefit from ribavirin addition to some of the

new IFN-free treatment regimens for HCV. Similarly, wild-type *ITPA* carriers may also gain more from the use of ribavirin against other viral infections (e.g., respiratory syncytial virus, hepatitis E virus, Lassa fever and polio, among others). In addition, pharmacological inhibition of ITPase activity could also potentially mitigate the need for ribavirin, and thus be an innovative target for drug development.

Conclusion

ITPA polymorphisms entailing reduced ITPase activity are associated with an improved likelihood of achieving SVR resulting from the reduced risk of relapse following IFN and ribavirin combination therapy for HCV infection, and this impact on outcome is possibly more pronounced when lower ribavirin dosing is used. The underlying mechanism of this remains to be elucidated, but the incorporation of the potentially mutagenic, immunostimulatory and structure-altering ITP into the viral genome by less stringent viral polymerases lacking proofreading capabilities offers an intriguing hypothesis. This could potentially reveal a novel feature of innate immunity in addition to being an innovative target for future drug development. Thus, in conclusion, the impact of *ITPA* polymorphisms on HCV and other viral infection warrants further study.

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