



## Editorial

# Nonalcoholic steatohepatitis pharmacotherapy and predictors of response: dual role of aminotransferases as biosensors of metabolism and biomarkers of histological improvement

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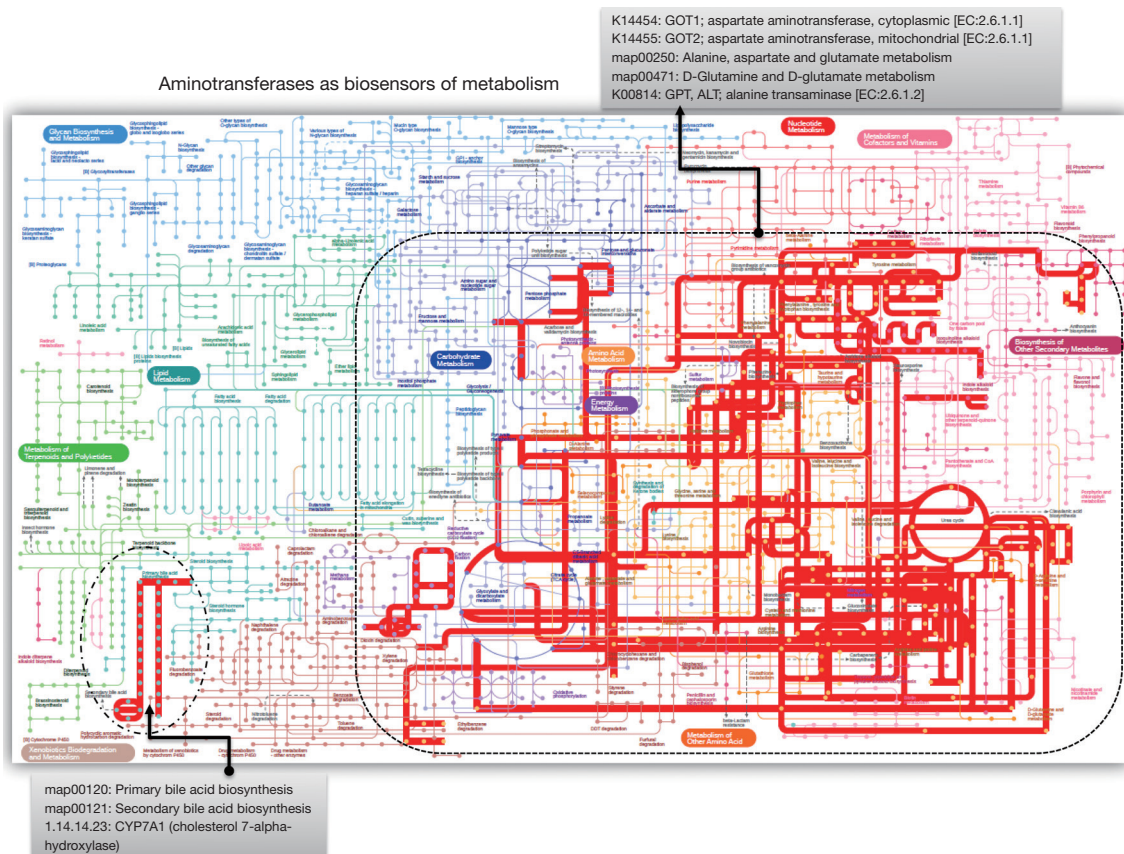
Nonalcoholic steatohepatitis (NASH)—the severe histological form of nonalcoholic fatty liver disease (NAFLD)—is regarded as a major health problem worldwide (1,2). The disease can progress to liver cirrhosis and eventually to hepatocellular carcinoma (1,3). Treatment of NASH and NASH-fibrosis is thus increasingly being given priority in the clinical field. Yet, these efforts face challenges not only related to the design and validation of novel pharmacological agents, but also to the need to optimize treatment options as well as improve access to therapies. In fact, while many drugs are currently being tested for safety and efficacy, only a few approved options for the treatment of NASH presently exist (4,5). It is thus expected that, in the near future, emphasis will be given to selecting the right drug for treating NASH patients from a wide spectrum of treatment choices. Hence, identification of early predictors of treatment response represents an unmet and relevant need.

Loomba and coworkers have recently published results yielded by a post-hoc analysis of clinical predictors of histologic response (6), whereby the data was sourced from the FLINT trial—a double-blind, placebo-controlled, randomized clinical trial conducted at multiple medical centers across the U.S. involving non-cirrhotic NASH patients—to assess the treatment efficacy of obeticholic acid (OCA, a farnesoid X receptor agonist) given orally (25 mg daily) for 72 weeks (7). Specifically, the authors aimed at identifying baseline and early on-treatment

factors that might predict histologic response. By utilizing a multivariable-adjusted model, the authors found that baseline NAS (NASH activity score) >5, baseline triglyceride levels ≤154 mg/dL, baseline international normalized ratio ≤1, baseline aspartate aminotransferase (AST) level ≤49 IU/L, and a decrease in alanine aminotransferase (ALT) levels at week 24 by at least 17 IU/L were significantly associated with histological improvement (6). These interesting and promising findings deserve some reflections.

## What is the biological meaning of these findings?

Notably, all predictors in the model with the exception of the NAS score are indeed biochemical measurements that include liver enzymes, specifically aminotransferases, and circulating levels of triglycerides. One might argue that liver enzymes are expected predictors of treatment response because ALT and AST have been widely used in prior studies not only as surrogate biomarkers of liver disease severity (8,9) but also to monitor response to any liver disease, particularly the treatment of chronic viral hepatitis. Nevertheless, more recent data indicate that serum concentrations aminotransferases are linked to cardiovascular disease and the metabolic syndrome (9-11). Therefore, it has been postulated that elevated aminotransferase values approaching the upper-normal threshold reflect high levels of hepatic transamination in response to increased NAFLD-associated metabolic



**Figure 1** Aminotransferases as biosensors of metabolism. Metabolic map showing the complexity of the pathways and enzyme-related modules associated with aminotransferases (GPT1, GPT2, GOT1 and GOT2). Figure also shows primary and secondary bile acid biosynthesis in the right section of the map; bile acid biosynthesis has been highlighted because OCA is a regulator of bile in the liver. OCA is conjugated with glycine or taurine in the liver and secreted into bile; these conjugates of OCA are absorbed in the small intestine leading to enterohepatic recirculation. Metabolic map was done by iPath3.0 in the web interface available at: <https://pathways.embl.de/>. Input data for map construction: map00120, map00250, K14455, K14454, map00471, K00814, map00121, 1.14.14.23; pathways and enzyme modules are highlighted in red. Detailed information on pathways can be found in the KEGG database (<https://www.genome.jp/kegg/kegg2.html>), including KEGG pathways maps (KEGG Pathways), reactions and reaction classes (KEGG reactions) and KEGG modules. Glutamic-Pyruvate Transaminase (alanine aminotransferase); Glutamic-Oxaloacetic Transaminase (aspartate aminotransferase). OCA, obeticholic acid.

demands (9,11). In fact, we have evaluated the expression levels of aminotransferases in the liver of patients with NAFLD and have explored their relationship with global metabolic changes in the circulation (11). Profiling of circulating metabolites indicated that serum aminotransferase concentrations are a signature of liver metabolic perturbations, particularly at the amino acid metabolism and Krebs cycle level (11). Robust confirmation of that finding can be found in the representation of GPT and GOT—including their isoenzymes—in the global metabolic map (Figure 1). The pathways in which

aminotransferases are involved cover not only an extensive portion of the carbohydrate and amino acid metabolism, but energy metabolism as well (Figure 1). The role of GOT1 [aspartate aminotransferase, cytoplasmic (EC:2.6.1.1)] is particularly interesting, as it presents 8 direct hits and 256 indirect hits in the global metabolic map (Figure 1). Consequently, it is plausible to suggest that baseline levels of AST—as reported by Loomba and coworkers (6)—are not necessarily prognostic indicators of histological improvement but also dynamic biosensors of the patients' metabolic status.

### Clinical predictors versus genetic predictors of treatment response

Another important issue to discuss is the potentiality for including other factors, such as genetic predictors, in the model. This hypothesis could be tested, for example, via a genome-wide exploration of variants (GWAS), by necessity in a large cohort. Another, more feasible, alternative could be a rational selection of gene candidates based on biological modeling. We explored the connectivity network around NR1H4 (FXR) and aminotransferase genes (GPT1, GPT2, GOT1, and GOT2) and found some potential candidates for inclusion into the model, including CYP7A1 (cytochrome P450 family 7 subfamily A member 1) (Figure 2A). This finding is not unexpected, as FXR not only regulates bile acid biosynthesis controlling *CYP7A1* transcription through the FGFR4-JNK-HNF4 $\alpha$  pathway, but also hepatic gluconeogenesis, glycolysis and lipogenesis, including triglyceride biosynthesis through the SHP (the inhibitory nuclear receptor small heterodimer partner)-SREBP-1c pathway (12). This complex molecular interaction of the drug target may explain some adverse effects of OCA on insulin resistance (7), prompting the question of how the incorporation of plasma glucose-related variables may affect the predictive model results reported by Loomba *et al.* (6). As a proof of principle, it can be shown that variants in, or in close proximity to, *CYP7A1* are associated with lipid traits in the UK Biobank (Figure 2B), which reinforces the concept that baseline levels of lipids in patients with NASH that responded or not to OCA are genetically modulated. In addition, text mining would suggest that GPT (ALT) could be a central node in connecting the sub-network of nuclear receptors with the one composed by aminotransferases and enzymes of the glutamic acid metabolism, as GPT and FXR might be co-expressed in some species (Figure 2B).

A final question that still remains unanswered pertains to the generalizability of Loomba *et al.*'s work (6). Specifically, one may wonder whether the reported findings can be extended to all populations and to all kinds of anti-NASH drugs. Finally, as FXR and *CYP7A1* are at the core of the metabolism of bile acids, it remains to be explored whether other environmental factors, such as diet or host "microbiotas", may modulate the therapeutic response of any drug but OCA in particular, and vice versa (13).

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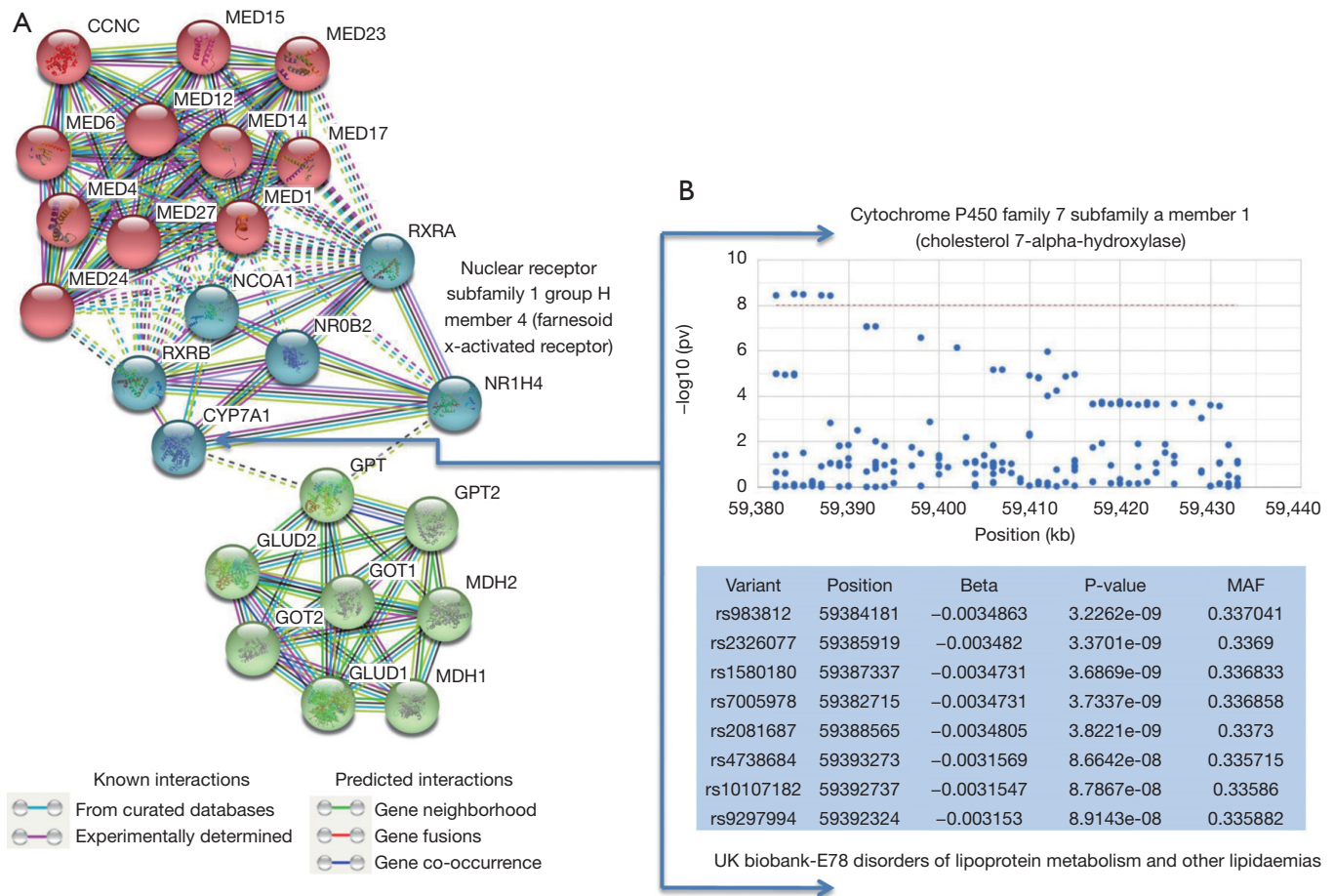
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### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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**Figure 2** Protein interaction network of FXR (NR1H4). (A) The connectivity gene/protein interaction network shows a set of genes/proteins (nodes) connected by edges representing functional relationships among them. Network shows input genes/proteins [NR1H4, also known as FXR, GPT1, GPT2, GOT1 and GOT2 and predicted genes/proteins (see below)]. The functional products of genes (proteins) interact with each other by different mechanisms; figure shows different level of evidence extracted from curated databases, experimentally determined, predicted, etc., as explained in the figure footnote. Connectivity network was generated in the STRING's website (<https://string-db.org>). Network depicts 25 nodes and 131 edges; enrichment  $P < 1.0e-16$ . Pathways overrepresented are: map04919: thyroid hormone signaling pathway ( $P = 9.11e-15$ ); map00220: arginine biosynthesis ( $P = 9.97e-12$ ); map01200: carbon metabolism ( $P = 2.63e-11$ ); map00250: alanine, aspartate and glutamate metabolism ( $P = 9.61e-11$ ); map01210: 2-oxocarboxylic acid metabolism ( $P = 9.48e-08$ ). Predicted functional partners: CYP7A1 (cholesterol 7- $\alpha$ -monooxygenase; catalyzes a rate-limiting step in cholesterol catabolism and bile acid biosynthesis by introducing a hydrophilic moiety at position 7 of cholesterol). Important for cholesterol homeostasis; cytochrome P450 family 7 (504 aa); NCOA1 (nuclear receptor coactivator 1); involved in the coactivation of different nuclear receptors, such as for steroids (PGR, GR and ER), retinoids (RXRs), thyroid hormone (TRs) and prostanoids; MDH2 (malate dehydrogenase, mitochondrial); MDH1 (malate dehydrogenase, cytoplasmic); RXRA (retinoic acid receptor RXR- $\alpha$ ); Receptor for retinoic acid and RXRB (retinoic acid receptor RXR- $\beta$ ); MED1 (mediator of RNA polymerase II transcription subunit 1); GLUD1 (glutamate dehydrogenase 1, mitochondrial); GLUD2 (glutamate dehydrogenase 2, mitochondrial); NR0B2 (nuclear receptor subfamily 0 group B member 2). Clustering was performed based on k-means clustering. NCBI taxonomy Id: 9606. (B) Manhattan plot of associations between variants in *CYP7A1* or near *CYP7A1*-locus and lipid traits (E78: disorders of lipoprotein metabolism and other lipidaemias as stated in the UK Biobank database) from individuals of the UK Biobank. The associations have been computed using 452,264 UK Biobank White British individuals (<http://genatlas.roslin.ed.ac.uk/>). Table shows effects (beta) of variants that reached genome wide-significance at a  $P < 1e-8$ . MAF, minor allele frequency.

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