JOURNAL
OF HEPATOLOGY

Whole exome sequencing for personalized hepatology: Expanding applications in adults and challenges

To the Editor:

We read with interest the study by Hakim *et al.*, and the related comment by Pinto e Vairo and Lazaridis, on the application of a personalized medicine approach based on whole exome sequencing (WES) for diagnosing young patients with unexplained liver disease.[1,2]

By performing WES in 19 patients with severe cryptogenic liver disease, not related to alcohol abuse, but also including fatty liver disease not associated with obesity, Hakim *et al.* identified and characterized in 5 (25%) inherited variants in *ABCB4*, *PPARG*, *NDUFB3*, and *APOB*. These variants were selected based on the frequency (rare), predicted impact on protein activity, and localization at genomic loci previously associated with liver disease. Importantly these variants resulted in phenotypes consistent with a loss-of-function of the encoded proteins. These genetic diagnoses had implications for clinical management, treatment, and family counselling of the affected individuals.[1] Overall, their findings supported the utility of WES for diagnosing patients with unexplained liver disease, including adults with symptoms onset before the age of 40 years.[2]

We recently applied the same WES approach to a cohort of 201 patients with severe liver disease (advanced fibrosis with or without hepatocellular carcinoma), which was attributed to non-alcoholic fatty liver disease (NAFLD).[3] We used even more strict criteria for variants prioritization based on allelic frequency (<0.001), affected genes (a predefined list of 181 implicated in liver disease, lipid metabolism and hereditary cancer), and predicted impact (mutations already defined as pathogenic or with a very high likelihood of altering protein activity), to define pathogenic mutations. By consulting the Clinvar database reporting variants already defined as pathogenic, we identified a genotype consistent with a genetic diagnosis of Mendelian disease, which predisposed patients to liver disease or cancer in 22 cases (11%). In keeping with the results of Hakim *et al.*,[1] at the single gene level there was a significant enrichment in *APOB* mutations.[3] Loss-of-function variants in *APOB*, encoding for apolipoprotein B, are responsible for hypo-betalipoproteinemia, an autosomal dominant condition characterized by fatty liver related to altered very low-density lipoprotein secretion, which may also be associated with malabsorption determining low body mass and damage to the intestinal barrier.[4]

When we selected variants based on the likelihood of them being pathogenic, we confirmed a strong enrichment in multiple genes, including *APOB*, compared to healthy individuals (Fig. 1). Confirming the genetic diagnoses, carriage of such *APOB* variants resulted in a circulating lipid profile consistent with hypo-betalipoproteinemia.[3] Of the 15 patients with *APOB* mutations with advanced liver disease associated with NAFLD, 7 (47%) were obese, 5 (33%) overweight, and 3 (20%) had normal weight.

However, it should be noted at the same time that the cumulative rate of carriage of variants in genes associated with liver disease is relatively high in the general population. In addition, carriage of *APOB* mutations may even protect against cardiovascular disease in the absence of cofactors for hepatotoxicity.[5] Furthermore, as also reported in the *Journal*, the list of genes whose mutations cause development of severe fatty liver in adults is rapidly expanding.[6] Careful evaluation of the phenotype associated with single mutations and family history, coupled with *in silico* and functional studies leading to the update of public database will therefore be required to accurately interpret the WES results in clinical practice.

In conclusion, we propose that additional studies are carried out to evaluate the impact of WES or more targeted resequencing approaches in selected categories of liver disease patients older than 40 years, due to the possible large impact on liver disease management and screening in family members. In addition, *APOB* mutations are likely an underdiagnosed factor contributing to the development of advanced fatty liver disease, even in those with increased adiposity, which may even represent a triggering factor for the development of hepatocellular damage.[7]

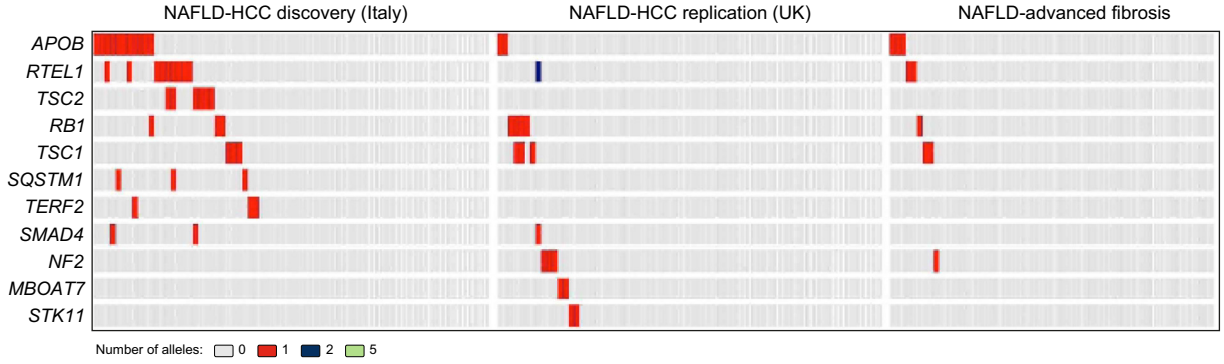

Fig. 1. Computation plot showing the distribution of likely pathogenic inherited variants enriched in patients with NAFLD-HCC compared to controls in patients with severe liver disease related to NAFLD. HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease. p <0.0001 at Fisher's exact test for the overall enrichment of rare pathogenic variants in patients with NAFLD-HCC *vs.*controls.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.06.008.

References

[1] Hakim A, Zhang X, DeLisle A, Oral EA, Dykas D, Drzewiecki K, et al. Clinical utility of genomic analysis in adults with idiopathic liver disease. J Hepatol 2019;70:1214–1221.

[2] Pinto EVF, Lazaridis KN. Individualized medicine comes to the liver clinic. J Hepatol 2019;70:1057–1059.

[3] Pelusi S, Baselli G, Pietrelli A, Dongiovanni P, Donati B, McCain MV, et al. Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. Sci Rep 2019;9:3682.

[4] Noto D, Cefalu AB, Cannizzaro A, Mina M, Fayer F, Valenti V, et al. Familial hypobetalipoproteinemia due to apolipoprotein B R463W mutation causes intestinal fat accumulation and low postprandial lipemia. Atherosclerosis 2009;206:193–198.

[5] Peloso GM, Nomura A, Khera AV, Chaffin M, Won HH, Ardissino D, et al. Rare protein-truncating variants in APOB, lower low-density lipoprotein cholesterol, and protection against coronary heart disease. Circ Genom Precis Med 2019;12 e002376.

[6] Youssefian L, Vahidnezhad H, Saeidian AH, Pajouhanfar S, Sotoudeh S, Mansouri P, et al. Inherited non-alcoholic fatty liver disease and dyslipidemia due to monoallelic ABHD5 mutations. J Hepatol 2019;71:366–370.

[7] Di Filippo M, Moulin P, Roy P, Samson-Bouma ME, Collardeau-Frachon S, Chebel-Dumont S, et al. Homozygous MTTP and APOB mutations may lead to hepatic steatosis and fibrosis despite metabolic differences in congenital hypocholesterolemia. J Hepatol 2014;61:891–902.

Luca Valenti[1,2,*]
Serena Pelusi[1,2]
Guido Baselli[1,2]

[1]*Translational Medicine, Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*

[2]*Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milano, Italy*

*Corresponding author. Address: Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Padiglione Marangoni, Ospedale Policlinico, via F Sforza 35, 20122 Milano (MI), Italy. Tel.: +39-02-50320278.

E-mail address: luca.valenti@unimi.it

Reply to: "Whole exome sequencing for personalized hepatology: Expanding applications in adults and challenges"

Exome sequencing advances precision medicine in adult hepatology

To the Editor:

It is with enthusiasm that we read the letter by Dr Valenti *et al.* entitled "Whole exome sequencing for personalized hepatology: expanding applications in adults and challenges" on the application of whole exome sequencing (WES) in the evaluation of 201 patients with advanced liver fibrosis attributed to non-alcoholic fatty liver disease (NAFLD), including individuals with hepatocellular carcinoma.

In alignment with our group's experience,[1] the authors identified a Mendelian disease in a significant subset of patients (11%), which had been undiagnosed until genomic analysis was performed in adulthood. Interestingly, the authors found that approximately 7.5% of their cohort harbor heterozygous deleterious variants in apolipoprotein B encoded by *APOB*. In order to validate the genetic findings in these patients, the authors assessed the patients' lipid profiles and found that they were consistent with the genotype, thereby uncovering the diagnosis of hypobetalipoproteinemia in 15 of these patients. This study illustrates the relevance of incorporating a forum such as Genome Rounds, where genetic findings are discussed and integrated with phenotypic features. It is important to recognize that a genetic diagnosis alone does not establish a definitive diagnosis unless supported by clinical, laboratory, imaging and/or histological findings.[1,2] Specifically, in patients with liver steatosis and rare heterozygous variants predicted to be deleterious in *APOB*, a circulating lipid profile and APOB levels should be obtained to functionally validate the clinical relevance – in other words, the pathogenicity – of uncharacterized mono-allelic loss-of-function variants in this gene. Moreover, our data and the Dr Valenti *et al.*'s study underscore that WES has the highest potential to yield actionable information when integrated in the appropriate clinical context. Specifically, patients with NAFLD typically have hepatic steatosis in the context of hyperlipidemia and low high-density lipoprotein cholesterol; when this pattern deviates to hypolipidemia, it hints at a genetic defect of apolipoprotein function and the potential utility of genetic testing. It will be very interesting to see the long-term natural history of the patients in Dr Valenti's cohort, as it is likely to differ from classical NAFLD and therefore may have implications in clinical management. Ultimately, we anticipate that a genomic approach in adult hepatology will assist to inform diagnosis, prognosis and treatment across a wide spectrum of molecular liver disease subtypes.[1–3]

Financial support

S.V. is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number K08DK113109. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.