Editorial

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Individualized medicine comes to the liver clinic

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In 2015, during his State of the Union address, President Obama announced the launch of the Precision Medicine Initiative. This is a research endeavour led by the National Institutes of Health to better understand how individual genome variability, environmental exposures, and lifestyle activities could determine the best approach to prevent and/or treat disease (https://allofus.nih.gov). The human genome contains approximately 20,000 genes and, thus far, nearly 5,000 have been implicated in disease. Moreover, every one of us harbours millions of genetic variants; many of which are rare or private (*i.e.*, unique to a person or a family). A major task in the diagnostics of genetics today is to identify those variants responsible for, or contributory to disease. Of course, the overarching scope of individualized medicine is beyond diagnosing genetic defects and includes improving the stratification of diseases for better prognostication and tailoring of therapy.

Traditionally, the diagnosis of a patient suspected of having a Mendelian disorder involved several clinical visits with providers in multiple specialties and expensive procedures to determine the most likely diagnosis. Subsequently, a medical geneticist selected a gene or a gene-panel for further testing to confirm the putative diagnosis. During the past 3 decades, the Sanger sequencing technique was the standard diagnostic approach to identify gene defects. The goal was to sequence 1 gene at a time, a cost-intensive and time-consuming method.[1] In the last decade, next generation sequencing was introduced, firstly as a research tool and then in the clinical setting, transforming the detection of genetic mutations that cause or contribute to disease. As sequencing costs are dropping quickly and technology advances rapidly, genomic tests such as whole exome sequencing (WES) have been integrated into the medical genetics clinics as a cost-effective, first-tier diagnostic test for patients on a "diagnostic odyssey". These are patients with a suspected genetic disease, who experience a protracted and arduous journey for themselves and their families as they hope to find answers about an undiagnosed medical condition they suffer from. About 25% of such patients have a gap of 5 to 20 years between the development of first symptom(s) and/or sign(s) and definitive diagnosis.

In the past 5 years, with the introduction of WES into clinical practice, we now know that the likelihood of making a specific diagnosis for a patient on diagnostic odyssey using WES ranges from 25–50%. This observed variation of the diagnostic rate depends on the age of the patient at the onset of symptoms (s)/sign(s) and/or the phenotypic heterogeneity of the disease. For example, WES testing has a higher diagnostic yield in paediatric patients than adults. The yield is also increased when there are specific phenotypes such as epilepsy[2] or neuromuscular diseases,[3] particularly when parents and/or other affected relatives are available for such testing.[4] Overall, the use of WES for the diagnosis and management of adult patients on a diagnostic odyssey is less common than in children. This is likely because of a lack of awareness among practitioners that Mendelian diseases may only become clinically apparent in early adulthood. Moreover, inadequate or no medical information and/or samples from family members of an adult proband limit the applicability of WES given that often testing of affected and unaffected family members is required as part of the WES interpretation.

In this issue of the *Journal of Hepatology*, Hakim *et al.,* evaluated the utility of WES for making a diagnosis among adult patients on a diagnostic odyssey who suffered from an idiopathic liver disease. Specifically, in this interesting study, they performed WES of germline DNA in 19 patients who were 22 to 73 years old, following an unrevealing traditional comprehensive work-up performed by an experienced hepatologist. The authors identified a monogenic disorder in 26% (5 out of 19) of the patients. The results were confirmed by Sanger sequencing in all 5 patients. Importantly, these discoveries had a direct or potential impact on the clinical management of those patients. *Patient 1* was a 33-year-old female with severe hepatic macrovesicular steatosis with periportal and pericentral fibrosis and dyslipidaemia. The patient remained undiagnosed for 18 years despite multiple extensive medical evaluations. She was found to have 1 missense variant in the peroxisome proliferator activated receptor gamma (*PPARG*) and as a result was diagnosed with autosomal dominant familial partial lipodystrophy type 3 (FPLD3). This was a *de novo* mutation of the patient since both parents were negative. Given the diagnosis of FPLD3 the authors postulated that the patient's leptin levels were low. Subsequently, leptin replacement therapy

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was initiated and led to significant improvement of dyslipidaemia, normalization of liver aminotransferases and a 50% reduction in her daily insulin dose.

Patient 2 was a 31-year-old female who presented with acute oesophageal haemorrhage in the 22nd week of her 2nd gestation and was diagnosed with benign intrahepatic cholestasis of pregnancy. *Patient 3* was a 29-year-old male who had undergone a liver transplant at age 8 for idiopathic liver cirrhosis and presented with chronic graft rejection due to medical nonadherence. In each of those 2 patients, genomic analysis detected pathogenic variants in ATP binding cassette subfamily B member 4 (*ABCB4)* consistent with the diagnosis of MDR3 deficiency known to cause intrahepatic cholestasis of pregnancy and progressive familial intrahepatic cholestasis type 3 (PFIC3) that usually leads to liver failure. Of interest, these patients can benefit from ursodeoxycholic acid treatment. Unfortunately, they have been undiagnosed and untreated for decades, thus emphasizing the importance of integrating WES analysis into clinical practice in hepatology.

Patient 4 was a 32-year-old male with persistent elevation of liver aminotransferases, hepatic steatosis, recurrent avascular necrosis, facial dysmorphic features and short stature. WES testing revealed a known rare homozygous variant in NADH: ubiquinone oxidoreductase subunit B3 (*NDUFB3*), which is the first enzyme in the electron transport chain in mitochondria. Liver electron microscopy was suggestive of a mitochondrial abnormality. Overall, it was thought that the patient had a respiratory chain disorder. He was given a mitochondrial cocktail supplement. *Patient 5* was a 23-year-old lean male with elevated alanine aminotransferase and mild macrovesicular steatosis. WES testing revealed a pathogenic heterozygous splicing variant of *APOB*, which encodes for apolipoprotein B. The variant could explain both the clinical and laboratory observations in this patient. Vitamin E supplementation was given to treat the patient.

The study by *Hakim et al.* highlights the importance of WES testing for diagnosing Mendelian diseases among adult patients with unexplained liver diseases even in the absence of family history. Of interest, all 5 patients who underwent WES testing that resulted in a specific diagnosis were <40 years old. Importantly, the study also illustrates how WES testing could lead to better understanding, classification and management of liver disease. We also acknowledge that the study by *Hakim et al.* is small in size and that larger studies are needed to confirm these promising results.

Currently, there are several recognized models for integrating WES testing into adult subspecialty clinical practice, leading to better diagnosis and treatment of patients on a diagnostic odyssey. Studies in nephrology have used WES to look for germline mutations in adults with chronic kidney disease. These investigations have identified a genetic cause in 10–25% of such patients depending on the population and disease phenotype.[5,6] Recently, a cardiovascular genetics group reported that WES has identified a genetic cause in about 26% of adult patients with a suspected inherited cardiovascular disease. This diagnostic yield was higher than commercially available gene-panels that had a diagnostic yield of 18%.[7] Lastly, in adult patients with neurological diseases, WES has an overall diagnostic rate of 30%,[8] or even higher in consanguineous populations.[9]

Since health care expenditure is a substantial financial burden, it is necessary to develop clinical decision models for any new diagnostic tool based on cost-effectiveness. Recently, a UK group published a systematic review on the health economics of using WES in clinical practice.[10] Most of the publications concluded that WES and even whole genome sequencing (WGS) were cost-effective in comparison to other tests. However, only 8 publications performed full economic evaluations. Out of these, 5 provided evidence that genomic testing is more cost-effective than traditional testing methods. Notably, all publications evaluated children with undiagnosed disorders, so we still lack evidence for the adult population in this context. More studies have to be pursued to address the cost-effectiveness of WES in clinical practice.

It is apparent that WES testing is transforming the subspecialty medical practice of undiagnosed diseases. Undoubtedly, there are several barriers that prevent further application of WES in patient care. First, there is a lack of awareness and training of the majority of health care providers about the value of WES testing for adult patients on a diagnostic odyssey or patients with a postulated genetic condition. As a result, many subspecialty providers are not comfortable with ordering WES testing or interpreting its results and/or participating in the decision making of these findings for their patients. This is especially true when variants of uncertain significance (VUS) are found as the result of WES testing. Receiving a VUS from a genomic testing might be burdensome to the patient. We recommend that patients should provide consent for such research studies and receive pre- and post-test genetic counselling, regardless of whether the testing is part of a research study or clinical care, due to the psychosocial impact such an uncertainty can cause. Thus, there is a need to develop multidisciplinary teams as proposed by Hakim *et al.*, and reported by others, to assist providers interpreting and returning the relevant genetic findings to patients.[11] Second, as practitioners we are obligated to better inform our patients about the expectations and limitations of WES testing. For example, we know that a negative report does not necessarily exclude a genetic disease. As well, WES testing could reveal other actionable inherited or *de novo* conditions that are incidental and may require further clinical care and follow-up. Thus, the active participation of certified genetic counsellors in this effort is a must. Third, there are third party payers who do not cover WES testing in adult patients on a diagnostic odyssey because of scepticism regarding the clinical impact of such testing.[12] This impression is likely going to change in the future when we, as the academic community, publish more studies to emphasize the cost savings of medical evaluations that incorporate WES testing early in the diagnostic process. Of note, the current cost of clinical grade WES testing and interpretation is comparable to other tests/procedures we already use in practice (*i.e.*, MRI, endoscopy, *etc.*).

Based on the experience of Hakim *et al.*, the work of others in different sub-specialties, and our own work within the Mayo Clinic Center for Individualized Medicine, we advocate the use of WES testing in the clinic for adult patients with idiopathic liver disease. We reserve this suggestion for patients who have onset of symptom(s)/sign(s) at <40 years of age. Moreover, discoveries from such testing could also assist the individualized management and follow-up of these patients. Finally, the work of Hakim *et al.* raises several important questions for future studies. How can we improve the diagnostic rate, beyond the 26%, for these patients who have an unrevealing WES result? Is WGS going to provide the answer(s) for such patients? Or, is it time to start using genomics along with other Omics data (*i.e.*, transcriptomic, epigenomic, metabolomic, *etc.*) as well as artificial intelligence to elaborate the causes of idiopathic liver

disease? As practitioners and investigators in the field of hepatology we are obligated to address these questions in patients with undiagnosed or idiopathic liver disease.

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Both authors conceived the content and worked on the text of the editorial.

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