Research Article

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Individual exome analysis in diagnosis and management of paediatric liver failure of indeterminate aetiology

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Background & Aims: In children with liver failure, as many as half remain of indeterminate aetiology. This hinders timely consideration of optimal treatment options. We posit that a significant subset of these children harbour known inherited metabolic liver diseases with atypical presentation or novel inborn errors of metabolism. We investigated the utility of whole-exome sequencing in three children with advanced liver disease of indeterminate aetiology.

Methods: Patient 1 was a 10 year-old female diagnosed with Wilson disease but no detectable *ATP7B* mutations, and decompensated liver cirrhosis who underwent liver transplant and subsequently developed onset of neurodegenerative disease. Patient 2 was a full-term 2 day-old male with fatal acute liver failure of indeterminate aetiology. Patient 3 was an 8 year-old female with progressive syndromic cholestasis of unknown aetiology since age 3 months.

Results: Unbiased whole-exome sequencing of germline DNA revealed homozygous mutations in *MPV17* and *SERAC1* as the disease causing genes in patient 1 and 2, respectively. This is the first demonstration of *SERAC1* loss-of-function associated fatal acute liver failure. Patient 1 expands the phenotypic spectrum of the MPV17-related hepatocerebral mitochondrial DNA depletion syndrome. Patient 3 was found to have syndromic cholestasis due to bi-allelic *NOTCH2* mutations.

Conclusions: Our findings validate the application of whole-exome sequencing in the diagnosis and management of children with advanced liver disease of indeterminate aetiology, with the potential to enhance optimal selection of treatment options and adequate counselling of families. Moreover, whole-exome sequencing revealed a hitherto unrecognized phenotypic spectrum of inherited metabolic liver diseases.

Introduction

Liver failure whether acute or chronic is a life-threatening event leading to multi-organ dysfunction, that requires rapid clinical decision about pursuing appropriate treatment including consideration of liver transplantation, which is the ultimate effective treatment option. Paediatric liver failure is a rare disease, but the precise frequency of liver failure in children is unknown. In 1999, the Pediatric Acute Liver Failure (PALF) study group was formed with the goal of developing a database of all individuals in the United States (US) younger than 18 years of age who present with new onset of severe liver-related coagulopathy with or without concomitant encephalopathy. According to the PALF study group data, approximately half of the cases of acute liver failure in children in the US still remain of indeterminate aetiology despite comprehensive evaluation [1].

We posit that a significant subset of these children suffer from known inborn metabolic liver disorders with atypical presentations (caused by inherited and/or de novo mutations) or have novel inborn errors of metabolism that lead to liver failure. With increasing access to affordable and rapid whole-exome capture

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Abbreviations: WES, whole exome sequencing; PALF, paediatric acute liver failure; US, United States; MDS, mitochondrial DNA depletion syndrome; WD, Wilson disease; EASL, European Association for the Study of the Liver; CT, computerized tomography; MRI, magnetic resonance imaging; PTT, partial thromboplastin time; MEGDEL syndrome, methylglutaconic aciduria with sensorineural deafness, encephalopathy and Leigh-like syndrome; AASLD, American Association for the Study of Liver Diseases.

and high throughput DNA sequencing, individual genomic analysis can be used in routine clinical practice [2,3]. The exome corresponds to 1% of the human genome that represent coding regions translated into proteins, and it harbours ~85% of all disease-causing variants [4]. Recent technological advances in high-throughput DNA sequencing allow deep coverage of coding DNA sequence from as little as 0.5 μg of genomic high-quality (non-degraded) DNA. Hence, whole-exome sequencing (WES) currently presents an unprecedented balance between length of analysis, cost and information collected, making it very attractive and suitable for clinical use [2,3]. However, its successful application mandates exquisite phenotype annotation and requires adequate bioinformatics analysis to identify causal gene variant(s).

Here, we describe three children with liver failure of indeterminate aetiology despite standard evaluation, and whose diagnoses were uncovered using WES. We found rare homozygous mutations in two genes, *MPV17* and *SERAC1*, both expressed in mitochondria and implicated in mitochondrial disorders. Patient 1 revealed an atypical presentation of MPV17-related hepatocerebral mitochondrial DNA depletion syndrome (MDS); whereas patient 2 is the first report of fatal acute liver failure related to homozygous splice site mutation in the *SERAC1* gene, a genetic defect that has been predominantly associated with a neurological phenotype and more recently with transient liver dysfunction [5,6]. Patient 3 who suffers from progressive syndromic cholestasis of unclear aetiology, was found to harbour novel compound heterozygous mutations in the *NOTCH2* gene in conserved amino acid positions, establishing a presumed diagnosis of Notch2-related Alagille-like syndrome.

This study provides further evidence to recommend the clinical utility of WES as a diagnostic tool to selected infants and children with life-threatening liver diseases of indeterminate aetiology, supporting its introduction into the clinical armamentarium in the field of hepatology [7–9].

Patients and methods

Human subjects

The study protocol was approved by the Yale Human Investigation Committee, and informed consent was obtained in accordance with institutional review board standards. Three children with advanced liver disease of indeterminate aetiology despite comprehensive evaluation underwent further analysis using whole-exome sequencing.

Exome capture and sequencing

DNA was extracted from peripheral blood total leukocytes by standard procedures. We used 1 microgram of genomic DNA per patient for exome capture and sequencing. DNA fragments containing exonic sequence were captured using the Roche/NimbleGen SeqCap EZ Human Exome Library v1 (for patient 1) or v2 (for patients 2 and 3), and sequenced on the Illumina HiSeq platform. Mean coverage of the exome was greater than 100× with 96% of the exome covered at least 8 times and 90% covered at greater than 20×. The resulting sequence was analysed for single nucleotide variants and small insertions and deletions (indels) differing from the reference genome (Human Genome 19, HG19).

At our institution, we are able to identify genetic liver disease using WES within 7 days of starting the genetic analysis at a cost of $1700 per exome, including DNA extraction, sequencing, quality control plus data analysis and report writing.

Exome sequencing analysis

We used the whole-exome sequencing analysis pipeline described earlier in [4]. The variant filtering strategy for patients 1, 2, and 3 is outlined in Supplementary Figs. 1–3, respectively. In brief, patient's variants passing quality filters were filtered against public (NHLBI, 1000 Genome, dbSNP) and in-house (Yale 2500 exomes) databases for minor allele frequency (MAF) < 1%. Then, protein-altering variants were selected. For patients 1 and 2, which were offspring of consanguineous families, homozygous variants were selected. Additionally, for patient 1, since both parents' exomes were sequenced, a variant found in the homozygous state in the parents was excluded. For patient 3, the offspring of unrelated parents, the protein-altering variants were selected if they were related to a disease listed in the Online Mendelian Inheritance in Man (OMIM) database. The variants (Supplementary Tables 1–3 for patients 1, 2, and 3, respectively) derived from the variant filtering strategy were then prioritized based on their likelihood to have damaging functions to the protein using public algorithms such as Polyphen-2 and SIFT and/or matching totally or partially the patient's phenotype. Variants identified by exome sequencing that were potentially diagnostic for the patient's condition were confirmed by Sanger sequencing as depicted in the main Figures.

Orthologues

Full-length orthologous protein sequences from both vertebrate and invertebrates were obtained from GenBank. Protein sequences were aligned using the ClustalW algorithm.

Results

Patient 1: Recessive MPV17 mutation in a child with clinical diagnosis of Wilson disease

Patient 1 is the only child of consanguineous parents from India (Table 1). She was in good state of health until age five years, when she developed incapacitating leg cramps, and was found to have abnormal liver function tests during workup. She was diagnosed with Wilson disease (WD) based on low serum ceruloplasmin (18 mg/dl, normal range: 22–58 mg/dl), elevated 24 h urine copper excretion of 64 micrograms/24 h (normal range: 15–60 micrograms of urinary copper/24 h), and elevated liver copper content of 468.1 mcg/g liver dry weight (normal range = 10–35 mcg/g dry weight), which attributes a total score of 4 and establishes the clinical diagnosis of WD according to the scoring system adopted by the European Association for the Study of the Liver (EASL) clinical practice guidelines [10,11]. Pre-transplant liver biopsy showed mild micro- and macrovesicular steatosis and the copper stain showed hepatocellular accumulation (1–2/3+) predominantly in periportal areas with some staining within the lobules. However, sequencing of the coding regions of the *ATP7B* gene did not reveal any disease causing mutations. She was started on copper-chelation therapy but progressed to end-stage liver disease (ESLD) with severe ascites, malnutrition and jaundice. During pre-transplant evaluation, the patient reported no new extra-hepatic signs or symptoms, and there were no ophthalmologic, renal or neurological findings. Baseline head computerized tomography (CT) was normal. She underwent uncomplicated deceased donor liver transplant for decompensated ESLD at nine years of age. Liver explant confirmed cirrhotic liver (Fig. 1A and B) and revealed two nodules of well-differentiated hepatocellular carcinoma of 1.9 and 1.5 cm of maximum dimension, which were incidentally found (Fig. 1C). In addition to multiple macronodules and dense fibrous septa, the explant showed focal steatosis, extensive oncocytic change, cholestasis, and focal areas of ballooning degeneration with Mallory's hyaline. There was heavy copper deposition in many of the hepatocytic nodules, predominantly in the periseptal hepatocytes (Fig. 1D). Electron microscopy revealed pleomorphic mitochondria with dilated cristae with occasional granular-dense deposits and crystalloid inclusion (Fig. 1E).