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

Keywords: Inherited metabolic liver diseases; Whole-exome sequencing; Genetic diagnosis; Liver failure of indeterminate aetiology; Germline mutations.
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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).

Table 1. Summary of the demographics, clinical features and genetic alterations of the three patients.

Patient No.	Ethnicity (consanguinity)	Age of onset	Age at the time of transplant	Age of death/ current age	Presenting features	Gene (NM_number)	Mutation	Homozygous/ Heterozygous	Amino acid or nucleotide change (coding DNA nomenclature)
1	Indian (yes)	5 yr	9 yr	9 yr/n.a.	Bilateral lower extremities spasms and abnormal LFTs	*MPV17* (NM_002437.4)	Missense	Homozygous	R50W (c.148T >C)
2	Pakistani (yes)	2 d	n.a.	12 d/n.a.	Shock, coagulopathy, lactic acidemia, hyperammonemia, elevated transaminases, hyperbilirubinemia and hypoalbuminemia	*SERAC1* (NM_032861.3)	Splice site	Homozygous	IVS13 + 1G >C (c.1403 + 1G >C)
3	Hispanic (no)	3 mo	n.a.	n.a./8 yr	Jaundice and pruritus	*NOTCH2* (NM_024408.3)	Missense	Compound heterozygous	S1741L (c.5222C >T) and H1882Y (c.5644C >T)

yr, years; d, days; mo, months; n.a., not applicable; LFTs, liver function tests.

The patient recovered rapidly after liver transplant and returned to school achieving high academic performance. However, nine months post-liver transplant, the patient presented with new onset of dystonia and tremor. Brain magnetic resonance imaging (MRI) revealed new bilateral basal ganglia masses with surrounding oedema (Fig. 1F). Initially, radiological findings were thought to be consistent with copper deposition in basal ganglia but her symptoms rapidly progressed to involve the white matter (Fig. 1G and H). She was found to have EBV viremia in blood and cerebral spinal fluid (CSF). The patient was treated for presumed central nervous system post-transplant lymphoproliferative disorder (not biopsy proven). Despite chemotherapy, the patient developed progressive neurodegenerative disease, including seizures, which culminated with patient's death at ten-years of age. Autopsy was not performed in accordance with family wishes. WES of the proband and her parents revealed a homozygous missense mutation, R50W (c.148T>C), in the *MPV17* gene (NM_002437.4) (Fig. 1I), which was subsequently confirmed by Sanger sequencing (Fig. 1J). Both parents were heterozygous carriers for the R50W variant (Fig. 1J). R50W occurs at an amino acid position that is 100% conserved between evolutionarily distant humans and flies (Fig. 1K). Upon review of the literature, the variant R50W has been previously reported in two patients, in one of the patients reported as a compound heterozygous [12] and in another patient in a homozygous state [13]. The R50W homozygous patient is a Hispanic girl who presented with failure to thrive at 8 months of age and died of liver failure with concomitant neurological symptoms when she was only 19 months old [13].

Patient 2: Neonatal fatal liver failure in a patient with homozygous splice site mutation in SERAC1 gene

Patient 2 is the third child of consanguineous parents (first-degree cousins) born in Pakistan (Table 1). The patient was a full term infant born via normal spontaneous vaginal delivery without any birth or immediate complications, with hospital discharge 32 h after delivery. At birth, he weighed six pounds 10 ounces and pertinent birth history includes negative group B *Streptococcus* disease and negative serology. Few hours after discharge, the patient was noted to be hypothermic, cyanotic and lethargic. In the emergency department, he was found to have a monomorphic wide complex tachycardia with weak pulses for which he required cardiac compressions, and ventilation and vasopressor support. Laboratory tests were remarkable for severe coagulopathy (INR of 7.37, normal range: 0.8–1.15; and partial thromboplastin time (PTT) >120 sec, normal range: 23.2–31.6 sec), lactic acidemia (lactic acid of 7.0 mmol/L, normal range: 0.5–2.2 mmol/L), hyperammonemia (ammonia of 176 micromol/L, normal range: 11–35 micromol/L), elevated transaminases (AST of 322 U/L, normal range: 0–79 U/L; and ALT of 91 U/L, normal range: 0–34 U/L), hyperbilirubinemia (total bilirubin of 6.08, normal is less than 1.20 mg/dl; and direct bilirubin of 0.80, normal range is less than 0.20 mg/dl) and hypoalbuminemia (albumin of 2.2 g/dl, normal range: 3.5–5.0 g/dl). Newborn screening revealed elevated tyrosine levels, but this was thought not to be the primary disorder given the lack of reducing substances in the urine. At 3 days of age, qualitative urine analysis for organic acids was performed and revealed marked elevation of lactate, pyruvate and ketones. In addition, there was a mild elevation of unrelated metabolites

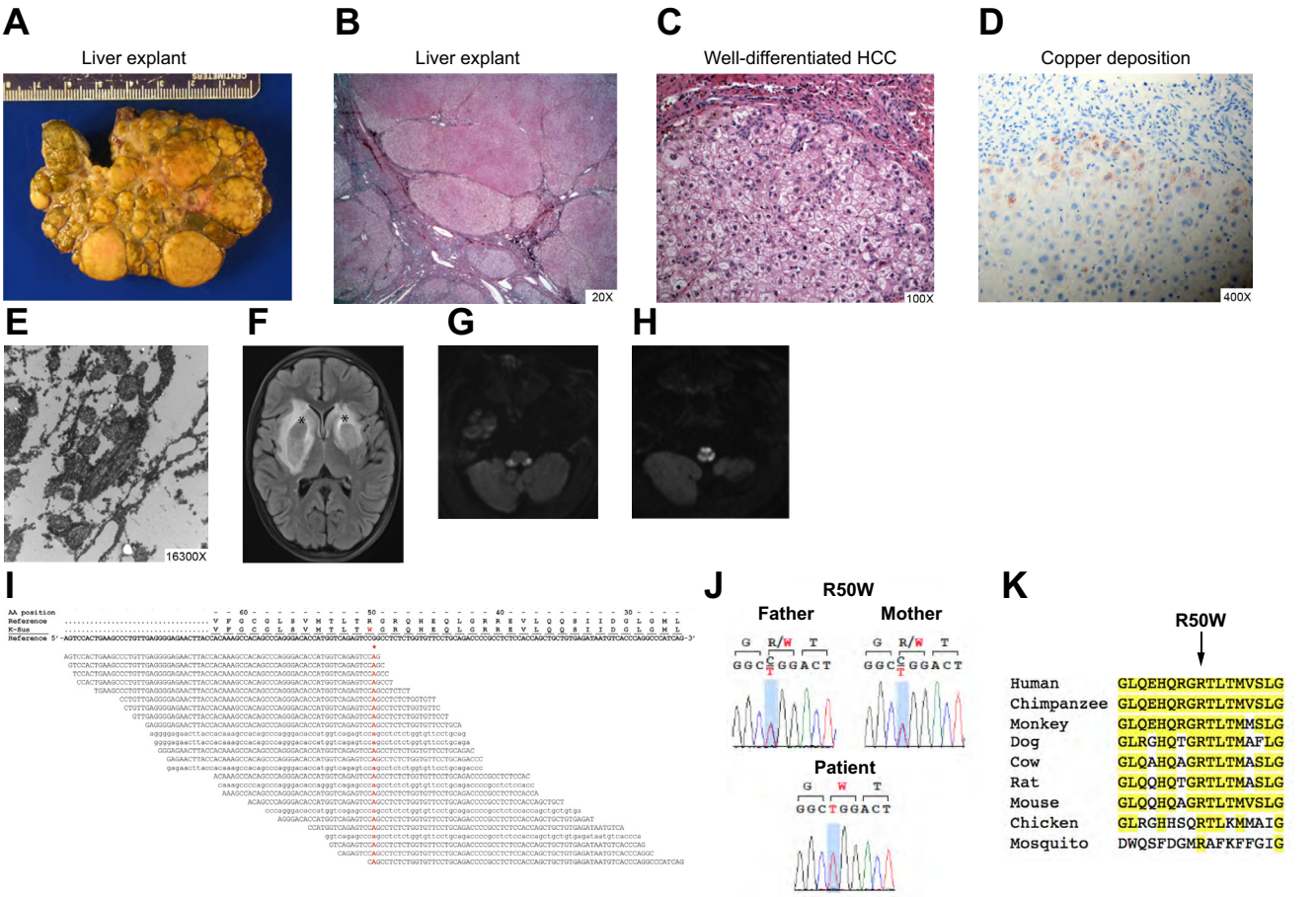

Fig. 1. Liver histological, radiological and genetic findings in patient 1. (A and B) Liver explant with macroscopic nodular cirrhotic appearance at low and high (20×) power, respectively. (C) Well-differentiated hepatocellular carcinoma (100×). (D) Significant copper deposition in many of the hepatocytic nodules, predominantly in the periseptal hepatocytes (400×). (E) Pleomorphic mitochondria with dilated cristae with occasional granular-dense deposits and crystalloid inclusions. (F) Brain magnetic resonance imaging shows bilateral basal ganglia masses with surrounding oedema as depicted by asterisks. (G and H) Representative images of brain magnetic resonance showing white matter abnormalities in the brain stem. (I) Missense homozygous mutation in *MPV17* (R50W) detected by massively parallel sequencing. (J) Sanger sequence traces of codons 49–51 are shown. Patient 1 is homozygous for a single base change (CGG>TGG, in red) in *MPV17*, resulting in a R50W (c.148T>C) missense mutation. Proband's parents are heterozygous for the same mutation. (K) Conservation of R50 across species. The amino acid sequence of 42–58 of human MPV70 is shown and compared to the corresponding sequences of nine vertebrate and invertebrate orthologs and paralogs. Amino acid positions identical to the human genome are highlighted in yellow.

(3-hydroxyisovaleric acid, 3-methylglutaconic acid, 2-ethyl-3-hydroxypropionic acid and fumaric acid). The pattern was not diagnostic of any specific disorder. Genetic testing for known neonatal liver failure causing genes (*FAH*, *ALDOB*, *ATP7B*, *GALT*, *GALE*, *GALK1*, *TRMU*, *SERPINA1*, *HPD*) was unremarkable. The patient passed away on day 12 of life.

Post-mortem, high throughput WES revealed a homozygous splice site mutation (IVS13+1G>C, c.1403+1G>C) in the *SERAC1* (serine active site containing 1, NM_032861.3) gene, which was confirmed by Sanger sequencing (Fig. 2). Interestingly, this patient had a two year-old brother who is alive, but had transient acute liver failure during his first few days of life that remained of unknown aetiology. Applying WES, the patient's brother was found to have the same homozygous splice site mutation (data not shown). *SERAC1* encodes a phosphatidylglycerol remodelling protein that is essential for mitochondrial oxidative phosphorylation and intracellular cholesterol trafficking. This damaging mutation has been recently identified in two unrelated patients with type IV MEGDEL syndrome (Methylglutaconic Aciduria with sensorineural Deafness, Encephalopathy and Leigh-like syndrome) [5] with predominant neurological phenotype and no reference of underlying liver dysfunction. One of the reported cases is a child of consanguineous parents originally from Pakistan, suggesting it may be a founder mutation.

Patient 3: A case of chronic cholestasis and NOTCH2 gene mutation

Patient 3 is the third child of non-consanguineous Puerto Rican parents. She is a full term infant, born from uneventful spontaneous vaginal delivery. She was healthy up to three months of age when she first presented with jaundice. Liver biopsy revealed bile duct paucity (Fig. 3B and C) in background of liver parenchyma with preserved lobular architecture and lack of significant fibrosis (Fig. 3A), suggestive of the Alagille syndrome. She is currently an 8 year-old girl with known bile duct paucity, progressive cholestasis, short stature (persistently on the third percentile for height), and dysmorphic facies. Evaluation for classic phenotypic features of Alagille syndrome was negative, namely cardiac,

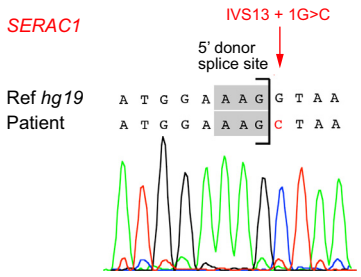

Fig. 2. *SERAC1* homozygous splice site mutation detected in patient 2 using WES. Sanger sequence trace of the 5′ donor splice site of codon 13 is shown. Patient 2 is homozygous for a base change (G>C, red arrow) in *SERAC1*, resulting in a homozygous splice site mutation: IVS13+1G>C (c.1403+1G>C).

vertebral or ophthalmological abnormalities. Moreover, full *JAG1* DNA sequencing was negative for disease-causing mutations. There was no family history of liver disease, including two healthy older siblings, a 15 year-old brother and a 10 year-old sister. She developed progressive hyperbilirubinemia and liver fibrosis, and is currently awaiting liver transplant. WES revealed novel compound heterozygous variants in *NOTCH2* (NM_024408.3), one inherited from her father (S1741L, c.5222C>T) and the other from her mother (H1882Y, c.5644C>T) (Fig. 3D and F). Both mutation variants are in the *NOTCH2* intracellular domain, in amino acid residues that are conserved across several species (Fig. 3E and G), and probably damaging according to the Polyphen-2 prediction scores. The clinical, histological and genetic findings together, suggest it is most likely that this patient has Notch2-related Alagille-like syndrome.

Discussion

It was only in 2009, that the potential utility of WES in establishing a genetic diagnosis of an unanticipated congenital disease was demonstrated [4,15]. In a short time, significant technological advances have occurred and WES is now available in the clinical setting at affordable cost and with the results available in a timely manner. Hence, WES is a powerful clinical diagnostic tool to investigate selected clinical conundrums that have remained unsolved, as exemplified by our cases. Children and young adults with advanced liver disease represent a population enriched for underlying inherited disorders. Several algorithms for biochemical testing to facilitate genetic diagnosis of common inherited diseases have been developed, including a 'jaundice chip' that screens for five common genes and known mutations [16,17]. Thus, there is a significant unmet need in our diagnostic armamentarium that impacts directly on the management of these patients. Our report highlights the impressive potential of applying unbiased exomic analysis in evaluating these patients, predicting a new era where hepatology and the field of paediatric hepatology will fully embrace this technology.

Patient 1 highlights the limitations of current algorithms for the diagnosis of Wilson disease. The patient fulfilled criteria for the diagnosis of Wilson disease per EASL and American Association for the Study of Liver Diseases (AASLD) clinical practice guidelines [11,18] but did not harbour *ATP7B* gene mutations. It is highly relevant in this regard that in several series, it has been reported that approximately 15% of patients with a clinical and biochemical phenotype of WD do not have detectable *ATP7B* mutations [8]. The diagnosis of WD in patient 1 was questioned as her disease phenotype unfolded following successful liver transplant; in particular the finding of HCC in the liver explant and neurodegenerative disease post-liver transplant. WES revealed a homozygous germline defect consistent with a diagnosis of MPV17-related hepatocerebral MDS. *MPV17* encodes a mitochondrial inner-membrane protein of unknown function. Bi-allelic mutations in *MPV17* underlie hepatocerebral MDS. To date, some 35 affected individuals have been described in the literature with MPV17-related hepatocerebral MDS. Combined data from these cases show that all had liver dysfunction, and 90% developed liver failure during infancy or early childhood. A quarter of the patients were found to have liver cirrhosis and two patients developed hepatocellular carcinoma [19]. Patient 1 did not present with lactic acidosis or early-onset hypoglycemia, which occur in approximately 75% and 50% of patients suffering from MPV17-related hepatocerebral MDS, respectively. Approximately 90% of affected individuals have neurological symptoms, which includes developmental delay (83%), hypotonia and muscle weakness (66%), white matter abnormalities in brain MRI (34%), peripheral neuropathy (28%), and seizures, microcephaly and ataxia in less than 10% of the cases [19]. Most other multisystem mitochondrial disorders with prominent neuromuscular involvement, exhibit liver dysfunction as late feature in the natural history; in contrast, in MPV17-related hepatocerebral MDS, liver disease occurs early in the natural history. The mutation identified in our proband, R50W, has only been reported in two other affected individuals, one in an equivalent homozygous state [13] and the other as a compound heterozygous [12]. Interestingly, the only other case of R50W homozygosity in the literature had a much earlier clinical presentation with failure to thrive (at eight months of age) and rapid progression (death at 19 months of age from liver failure) compared to our patient. If the correct diagnosis had been made prior to liver transplantation, the procedure would not have been undertaken due to the high likelihood of the development of neurological disease.

Interestingly, MPV17-related MDS phenocopies WD mitochondrial membrane crosslinking and mitochondrial destruction, as well as oxidative-phosphorylation defects [20,21]; notably deletions of mitochondrial DNA have been described in *ATP7B* deficiency [22]. Thus, our findings suggest genetic heterogeneity in patients with phenotypic findings of WD, and the utility of WES to investigate patients with Wilsonian liver disease but no *ATP7B* mutations. Taken together, our findings suggest that the *MPV17* gene should be examined in patients with WD phenotypes that are *ATP7B* mutation negative, and raises the possibility that MPV17 may be involved in the regulation copper homeostasis.

Genetic analysis in patient 2 uncovered an uncharacterized clinical presentation of fatal liver failure in a patient with a homozygous splice site mutation in the *SERAC1* gene. Using an mRNA transcript analysis, Wortmann and colleagues demonstrated that exon 13 splice site mutations result in skipping of this exon, which encodes part of the lipase domain of the SERAC1 protein [5]. While preparing this manuscript, Sarig and colleagues reported the first four cases of infantile hepatopathy in patients with decreased or absent expression of *SERAC1*, a gene which was previously thought to be exclusively associated with a neurological phenotype. Two novel homozygous mutations in

SERAC1, distinct from the one detected in our patient, have been identified in two pairs of brothers of two consanguineous unions who presented with hypotonia and transient liver failure within the first 48 h of life [14]. In contrast to our patient 2 who succumbed to liver failure, the four patients reported by Sarig *et al.* had transient liver dysfunction in infancy that resolved spontaneously. Thus, our case broadens the genotype – phenotype correlation for patients with *SERAC1* loss of function mutations and supports the suggestion recently made by Sarig and colleagues to rename this rare disorder to MEGDHEL (methylglutaconic aciduria with sensorineural deafness, hepatopathy, encephalopathy and Leigh-like) syndrome in order to reflect liver involvement [14].

Patients 1 and 2 had unsuspected mitochondrial hepatopathies, which were only diagnosed using unbiased WES. Thus, the sequencing of the entire mitochondrial DNA, in addition to the exons of 1034 nuclear genes encoding mitochondrial proteins, also known as MitoExome [23], might also be considered in the investigation of children with liver failure of indeterminate aetiology. However, WES has the potential to reveal other aetiologies of liver disease as illustrated by patient 3. In contrast to patient 1 and 2, patient 3 was the offspring of a non-consanguineous union and the case illustrates the utility of WES, even in this more complex setting [24].

This proband had an atypical presentation of advanced liver disease with bile duct paucity, cholestasis, short stature and dysmorphic facies, suggestive of Alagille-like syndrome, but with negative *JAG1* gene mutations. Alagille syndrome is thought to follow an autosomal dominant inheritance associated with haploinsufficiency, and with known incomplete penetrance [25]. Mutations in the *JAG1* gene are found in 94% of cases of Alagille syndrome. In <1% of cases, mutations in the JAG1 receptor, *NOTCH2* gene, have been described and are associated with the disease [26]. Interestingly, patient 3 was found to have novel *NOTCH2* compound heterozygous mutations. Each parent carried one of the variants, in the absence of a liver phenotype. However, as the parents were evaluated for living liver donors, these genetic alterations in *NOTCH2* will likely negatively impact their donor candidacy. Kamath and colleagues [26] also reported one patient with cholestasis, bile duct paucity and cataracts with two *NOTCH2* missense variants. Otherwise, the remaining cases of Notch2-related Alagille syndrome reported in the literature show heterozygous *NOTCH2* mutations. All *NOTCH2* mutations related to Alagille syndrome are unique and are found across the entire gene. Previous study suggested that the spectrum of clinical features associated with *NOTCH2* mutations differs from *JAG1* mutations, with lower frequency of vertebral abnormalities

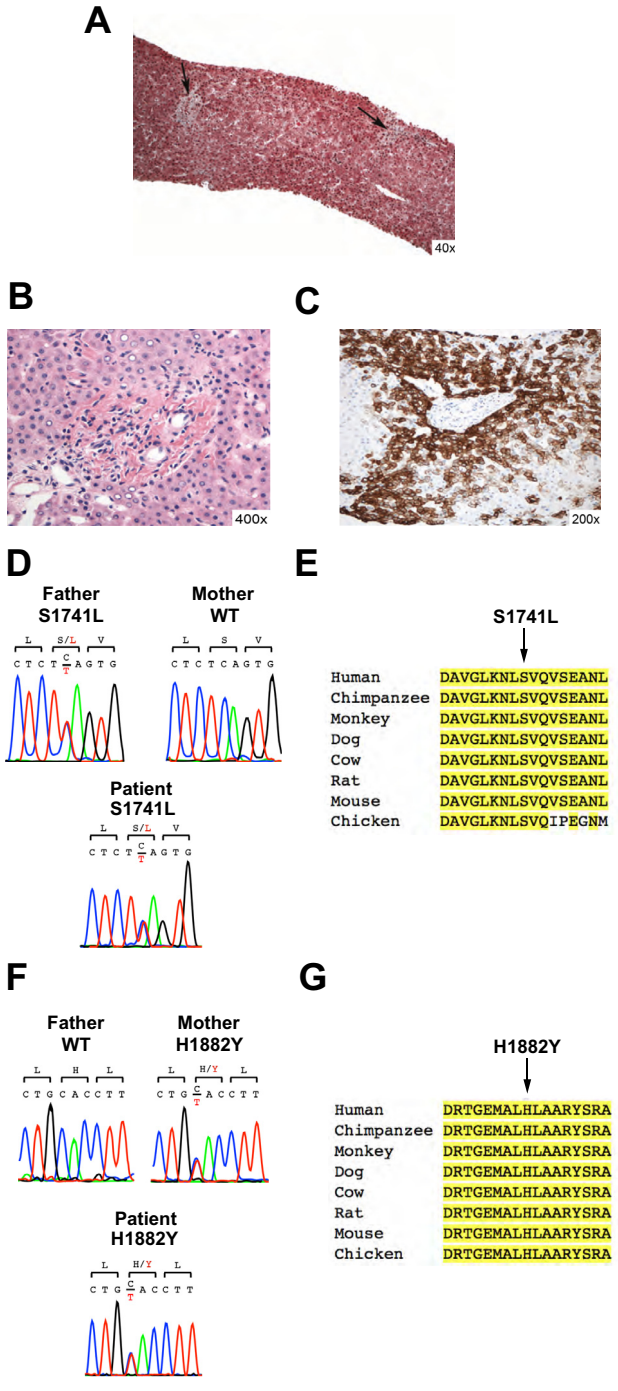

Fig. 3. Liver histological findings and *NOTCH2* compound heterozygous mutations detected in patient 3. (A) Low magnification showing preserved lobular architecture of the liver parenchyma and lack of significant fibrosis (Trichrome stain, 40×). The portal tracts (arrows) seen at this magnification fail to show either significant inflammation or any obvious pathology. (B) Higher magnification (400×) of the portal tract shows absence of the bile duct and also any lack of ductular reaction. Occasional lymphocytes or histocytes can be seen in the portal tract. Few glycogenated hepatocytic nuclei can be seen in the periportal region (H&E stain). (C) Immunostain for CK7 highlights the absence of the native bile duct and shows intense staining of the periportal hepatocytes (200×). (D) Sanger sequence trace of codons 1720–1722 are shown. Patient 3 is heterozygous for a base change (TCA>TTA, in red) in *NOTCH2*, resulting in a S1741L (c. 5222C>T) missense heterozygous mutation. The patient's father is heterozygous for the same mutation whereas the patient's mother is wild type (WT). (E) Conservation of S1741 across species. The amino acid sequence of 1733–1749 of human NOTCH2 is shown and compared to the corresponding sequences of eight vertebrate orthologs and paralogs. Amino acid positions identical to the human genome are highlighted in yellow. (F) Sanger sequence trace of codons 1881–1883 are shown. Patient 3 is heterozygous for a base change (CAC>TAC, in red) in *NOTCH2*, resulting in a H1882Y (c.5644C>T) missense heterozygous mutation. The patient's mother is heterozygous for the same mutation whereas the patient's father is wild type (WT). (G) Conservation of H1882Y across species. The amino acid sequence of 1874–1890 of human NOTCH2 is shown and compared to the corresponding sequences of eight vertebrate orthologs and paralogs. Amino acid positions identical to the human genome are highlighted in yellow. H1882 is conserved among all species examined.

and facial features [26], which is only partially concordant with our case. Similar to the study by Kamath *et al.* [26], Marchetti and Iascone reported two siblings affected by severe cholestasis who were found to have *NOTCH2* frameshift mutations in exon 18, but who did not show any cardiac disease, skeletal, ocular abnormalities or facies typical of ALGS. Thus, in accordance to both studies, from Kamath *et al.* [26] and Marchetti and Iascone, our study provides further clinical and genetic evidence that classical clinical diagnostic criteria no longer capture the heterogeneity of this disorder, and a genetic and clinical redefinition of Alagille syndrome is warranted.

As WES is less expensive and data can be generated and analysed in a timely fashion, its use lends itself to further investigate cases who fulfil partial or full classical criteria of Alagille syndrome [27]. Identification of gene variants at the exome level has the potential to provide valuable information to improve our understanding of this heterogeneous disorder. For example, in murine models the same mutation may have distinct phenotypes, depending on the genetic backgrounds [28]. Thus, similar findings likely occur in humans, and therefore a comprehensive characterization and integrative analysis of individual exomes has the potential to enhance our understanding of the impact of single nucleotide variants in the clinical phenotype.

In summary, we provide evidence to recommend the utility of WES in diagnosing patients with severe liver disease of indeterminate aetiology. This study is a proof of concept that inborn errors of metabolism will likely feature more prominently in clinical practice with greater access to WES in the clinic. Hence, WES may help to improve diagnostic and management algorithms in rare instances of unexplained acute and chronic liver disease in children. Our data also generate new information on genotype-phenotype correlation, and it is expected that the phenotypic spectrum of inherited metabolic liver diseases will expand beyond the current understanding with this technology.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.06.038.

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