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**Original Research Article**

# Clinical and genetic spectrum of monogenic liver diseases in children diagnosed using next generation sequencing: a single centre experience from Kerala

**Prasanth Kunjan Nadar Sobhan[1]*, Bindu Sarojam[2], Sankar Vaikom Hariharan[2]**

[1]Department of Gastroenterology, [2]Department of Paediatrics, Government Medical College, Thiruvananthapuram, Kerala, India

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***Correspondence:**
Dr. Prasanth Kunjan Nadar Sobhan,
E-mail: drprasanthksobhan@gmail.com



## ABSTRACT

**Background:** Children presenting with chronic liver disease have a high likelihood of an underlying genetic disorder. There is a delay in establishing a diagnosis of monogenic liver diseases if relied on typical clinical phenotypes and conventional laboratory investigations or imaging studies alone. Early diagnosis improves patient outcome through timely and adequate therapy.
**Methods:** This study retrospectively analyzed the clinical and genetic spectra of monogenic liver disease in children diagnosed using next-generation sequencing (NGS) in a tertiary care teaching hospital in Kerala. Patients were classified into five groups according to their clinical presentation: neonatal/infantile cholestasis, hepatomegaly/ hepatosplenomegaly, progressive cholestasis (beyond infancy), acute liver failure and decompensated chronic liver disease.
**Results:** There were 31 children enrolled, 14 (45.16%) males and 17 (54.84%) females. The median age at genetic diagnosis was 25.74 months. NGS identified 20 distinct genes related to varying clinical presentation. Six genes were identified in Group A, nine genes were identified in Group B, three genes were identified in Group C and two genes each in Group D and E. JAG1, ABCB4 and PYL1 (13 % each) were the top three genes related to monogenic liver disease in this study.
**Conclusions:** Patients with hepatomegaly or hepatosplenomegaly constituted the major clinical presentation of genetic disorders followed by neonatal/infantile cholestasis in our study. Genetic cholestatic disorders and glycogen storage disorders were the most common monogenic liver diseases. NGS has an important role in the diagnosis of monogenic liver disease in children and can facilitate early medical treatment and predict the prognosis.

**Keywords:** Monogenic, Liver disease, Children

## INTRODUCTION

Nearly 50% of chronic liver disorders presenting in childhood have a genetic cause, and approximately 20% of liver transplantation (LT)s in children are performed as a consequence of hepatic monogenic disease (MD)s. Excluding biliary atresia (the most frequent indication for pediatric LT) and autoimmune liver disease, most of the remaining conditions causing progressive liver disease in childhood have a genetic basis.[1] The majority of cases belong to the rare diseases and, frequently, there is a delay in establishing a diagnosis, which relies on typical clinical phenotypes supported by conventional laboratory investigations or radiological analysis. To improve patient outcome through timely and adequate therapy, an early diagnosis is desirable. Next generation sequencing (NGS)

has revolutionized the analysis of human genetic variations, offering a highly cost-effective way to diagnose hepatic monogenic diseases (MDs). Among the NGS strategies, the use of targeted gene panels has proven useful to rapidly and reliably confirm a clinical suspicion, whereas the whole exome sequencing (WES) with variants filtering has been adopted to assist the diagnostic workup in unclear clinical scenarios. WES is powerful but challenging because it detects a great number of variants of unknown significance that can be misinterpreted and lead to an incorrect diagnosis.[2-5]

In pediatric hepatology, NGS can be very valuable tool to discriminate neonatal/infantile cholestatic disorders, disclose genetic causes of acute liver failure, and diagnose the subtype of inborn errors of metabolism presenting with a similar phenotype (such as glycogen storage disorders). The inclusion of NGS in diagnostic processes will lead to a paradigm shift in medicine, changing our approach to the patient as well as our understanding of factors affecting genotype-phenotype match. NGS has revolutionized the analysis of human genetic variations, offering a highly cost-effective way to diagnose monogenic diseases including pediatric liver diseases.[6]

We have conceptualized this study on real world experience of next generation sequencing (NGS) as a tool in establishing diagnosis of monogenic liver diseases (MLDs) in children.

## METHODS

We performed this retrospective analysis of prospectively collected data to study the clinical and genetic spectrum of monogenic liver diseases in children (less than or equal to 12 years of age) diagnosed using next generation sequencing (NGS) and also to describe their immediate outcome. This study was approved by the Human Ethics Committee (HEC) of our institute (No.06/19/2020/MCT/ 13.11.2020).

The case records of children (less than or equal to 12 years of age) diagnosed with MLD using NGS {clinical exome studies (CES)} during February 2014 to July 2020 at Pediatric Gastroenterology (PGE) services, Department of Pediatrics, SAT Hospital Govt. Medical College, Thiruvananthapuram were extracted, reviewed and analyzed. Children (less than or equal to 12 years) with suspected monogenic liver disease were classified into five categories based on clinical presentation: Group A- neonatal/infantile cholestasis; Group B- hepatomegaly/hepatosplenomegaly; Group C- progressive cholestasis (beyond infancy); Group D- acute liver failure and Group E- decompensated chronic liver disease and the relevant details were extracted for enrolment in the study.

Cholestasis was defined in presence of serum conjugated bilirubin level >1 mg/dL when the total bilirubin was <5 mg/dL or a conjugated component >20% of the total when the total bilirubin was >5 mg/dL. Newborns with prematurity, sepsis, parenteral nutrition lasting 2 weeks, and endocrine abnormalities were excluded.

The operational definitions used for chronic liver disease (CLD) was persistent elevation of liver enzymes more than twice the normal, for a period of 3-6 months or more and /or other biochemical evidence and / or radiological evidence (ultrasound and /or transient elastography /CT /MRI) and/or histological evidence of chronic liver disease obtained from liver biopsy. Pediatric acute liver failure (PALF) was diagnosed on the basis of evidence of liver dysfunction within 8 weeks of onset of symptoms (neonates may have only deranged liver functions without overt symptoms) , uncorrectable (6-8 hours after administration of one dose of parenteral vitamin K) coagulopathy with International Normalized Ratio (INR) >1.5 in patients with hepatic encephalopathy, or INR >2.0 in patients without encephalopathy and no evidence of chronic liver disease either at presentation or in the past respectively.

The study variables included demographic factors – age and gender; clinical factors: age of onset of symptoms, symptoms at presentation, family history- consanguinity, h/o liver diseases in family, clinical examination findings, investigations: haemoglobin, platelet count, serum bilirubin (total and conjugated),serum transaminases, PT INR, total protein, albumin, ALP, GGT; bile acid level; imaging – ultrasound abdomen and or CECT abdomen/MRI abdomen/transient elastography; upper GI endoscopy; liver biopsy and the enrolled subjects were categorized into the following clinical phenotypes: neonatal/ infantile cholestasis, hepatomegaly/ hepatosplenomegaly, progressive cholestasis (beyond infancy), acute liver failure and decompensated chronic liver disease. All children with clinically suspected monogenic liver disease based on the clinical phenotype and other investigations underwent next generation sequencing (NGS) for confirmation of diagnosis and prognostication as per department protocol when other investigations including liver biopsy remained inconclusive.

Genomic DNA was extracted by automated method from peripheral blood samples of the patients in the genetic laboratory and sent to standardized private lab externally for clinical exome studies. The children with their parents were given pre-test and post-test counselling by a trained medical geneticist in our genetic clinic. The candidate causal mutations were then confirmed by Sanger sequencing, and co segregation analyses among the families were also conducted. Interpretation of variants was based on recommended standards from the American College of Medical Genetics and Genomics, and all variants were categorized as pathogenic, likely pathogenic, variants of unknown significance, likely benign or benign. The variants classified as pathogenic or likely pathogenic related to disease were defined as pathogenic variants.

Standard of care (SOC) was given to the study population and only retrieval of data pertinent to the study protocol was done and waiver of consent was granted by the HEC.

Categorical variables are presented as numbers (percentages). Continuous variables with a normal distribution are presented as the mean ± SD or as the median and interquartile range (IQR).

## RESULTS

### *Demographic features of the patients*

31 children across five heterogenous clinical presentations as mentioned above were enrolled in this study during the study period. There were 14 (45.16%) males and 17 (54.84%) females. A total of 93.8% of the patients were within six years of age at the time of enrollment, and 56.3% of the patients were within two years of age. The median age at genetic diagnosis was 25.74 months (IQR: 2, 144 months). 4 patients died, with a mortality rate of 12.9%, and three patients (9.68%) underwent liver transplantation during follow-up.

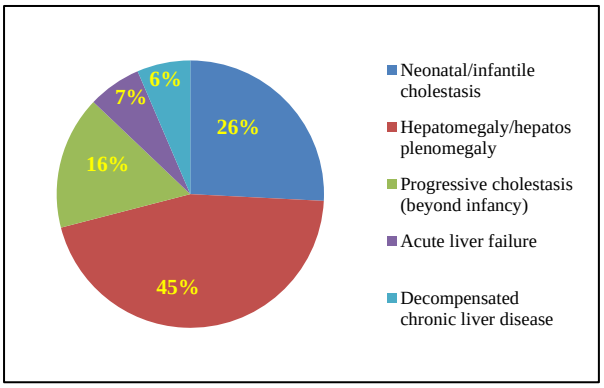


**Figure 1: Distribution on monogenic liver disease based on clinical presentation.**

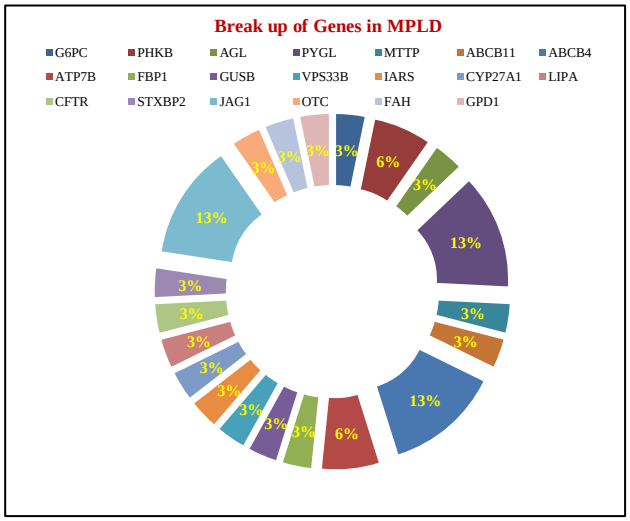


**Figure 2: Gene distribution among monogenic liver disease in children.**

### *Diagnostic profile by next generation sequencing (clinical exome sequencing)*

In this study, there were 20 genes related to genetic liver disease. Six genes were identified in Group A, nine genes were identified in Group B, three genes were identified in Group C and two genes each in Group D and E. The clinical presentation, gene distributions and numbers of identified gene mutations are shown in Figures 1, 2 and 3 respectively. JAG1, PYL1 and ABCB4 were the top three genes related to monogenic liver disease in this study.

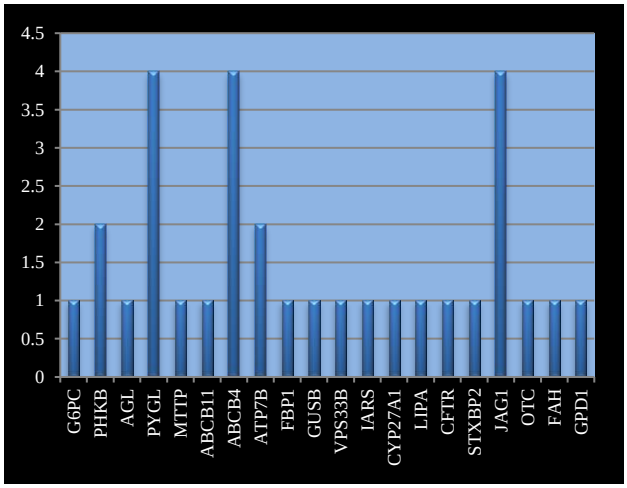


**Figure 3: Number of identified gene mutations among monogenic liver disease.**

Among the mutations identified in patients with monogenic liver disease, 21 patients carried homozygous mutations, 2 patients harboured compound heterozygous mutations, and 8 patients had heterozygous mutations, which included 10 hemizygous mutations and eight autosomal dominant mutations. Twenty patients carried pathogenic variations, and the remaining ten had VUS which had phenotype consistent with the genotype.

### *Group A: Neonatal/infantile cholestasis*

Cholestasis (as defined above) persisting for >6 weeks with onset in the first 12 months of age was included in this group. There were eight subjects in this group and various genes identified were JAG 1, ABCB4, GUSB, CFTR, VPS33B and CYP27A1.

The most common etiology of this group identified in this study was Alagille Syndrome due to JAG1 gene mutation in three infants with cholestasis. Among these, two children were diagnosed as biliary atresia and had undergone Kasai porto-enterostomy (KPE) from elsewhere. Both presented to us for pruritus, persisting jaundice and coagulopathy post KPE. Upon re-evaluation both were harbouring pathogenic mutations in JAG1 gene. Both underwent living donor liver transplantation (LDLT) during the follow up period of this study. Third one was diagnosed to have Alagille syndrome at 5 months of age

on evaluation for neonatal cholestasis and is under follow up.

There was a case of Sly syndrome or Mucopolysaccharidosis VII (MPS VII) due to homozygous pathogenic mutation in GUSB gene. Our case had cholestasis as predominant presentation, which is an unusual manifestation of MPS VII. There was a case of progressive familial intrahepatic cholestasis (PFIC) type 3 due to ABCB4 gene heterozygous mutation with variant of uncertain significance. But the clinical phenotype was correlating and liver biopsy had shown absent MDR3 staining.

There was a case of neonatal cholestasis with acholic stools due to cystic fibrosis resulting from CFTR gene homozygous mutation which is pathogenic (delta 508).This infant had meconium ileus and severe pancreatic exocrine insufficiency and expired at 5 months of age.

There were 2 cases of low gamma glutamyl transferase (GGT) cholestasis identified in this group. One was a case of cerebrotendinous xanthomatosis due to homozygous pathogenic mutation in CYP27A1 gene who presented with neonatal cholestasis and another was a case of arthrogryposis renal cholestasis (ARC) syndrome classical phenotype due to homozygous pathogenic mutation in VPS33B gene. The child with cerebrotendinous xanthomatosis had improvement in cholestasis during follow up. However he developed acute lymphatic leukaemia at 12 months of age during the follow up and is currently undergoing chemotherapy. The infant with ARC syndrome expired at 7 months of age.

***Group B: Hepatomegaly/hepatosplenomegaly***

Majority of the subjects (n=14) had hepatomegaly or hepatosplenomegaly at presentation. The various genes identified in this group were ATP7B/ G6PC / PHKB / AGL / PYGL / GPD 1/ FBP 1 / MTTP /IARS.

Glycogen storage disease (GSD) was the predominant disease in this group. Eight patients were diagnosed with GSD. Among them, 4 patients had type VI GSD caused by a PYGL mutation and two patients had GSD type IXb caused by a PHKA2 mutation. One patient had GSD type IIIa caused by an AGL mutation with pathogenic variant, and developed advanced fibrosis and portal hypertension during follow-up in the study period. One patient had type Ia caused by a G6PC mutation with pathogenic variant. All GSD patients were under dietary treatment with corn starch.

There were 2 cases of Wilson disease caused by an ATP7B mutation. Pathogenic variant with homozygous mutation was observed in one case which was detected on evaluation for hepatomegaly and history of elder sibling demise due to acute liver failure. A variant of uncertain significance with heterozygous mutation compatible with the clinical phenotype was observed in another case who presented with hepatomegaly and elevated aspartate (AST) and alanine (ALT) aminotransferases.

In one case, child had presented with hepatosplenomegaly and clinically suspected GSD correlating with biochemical investigations. Liver biopsy had demonstrated macrovesicular steoatohepatitis with advanced fibrosis and hepatocytes show intense staining on PAS with weak staining on PAS with diastase. But clinical exome studies revealed homozygous mutation in GPD 1 gene causing transient infantile hypertriglyceridemia. The child had improvement in lipid profile and hepatomegaly upon follow up as described in the literature. Based on American College of Medical Genetics (ACMG) classification, this was classified as variant of undetermined significance. In yet another case, an infant for whom clinically suspected GSD, had hepatomegaly. On evaluation this child had fatty hepatomegaly sonologically, elevated AST and ALT and lactate levels. But clinical exome studies demonstrated a homozygous pathogenic mutation in FBP1 gene causing fructose1,6 bisphosphatase deficiency.

Abetalipoproteinemia resulting from homozygous pathogenic MTTP gene mutation was confirmed in a 5year girl who presented with failure to thrive; hepatomegaly and coagulopathy corrected with vitamin K and no luminal gastrointestinal symptoms like diarrhoea.

A 9 month old infant was diagnosed to have GRIDHH (growth retardation, impaired intellectual development, hypotonia and hepatopathy) syndrome. This infant had presented with failure to thrive, alopecia and massive hepatomegaly.On evaluation was found to have hyperammonemia, elevated lactate and triglyceride levels and liver biopsy showed marked macrovesicular steatosis and increased fibrosis with suggestion of metabolic liver disease. Clinical exome studies showed compound heterozygous variant of uncertain significance for IARS gene causing GRIDHH syndrome.

***Group C: Progressive cholestasis (beyond infancy)***

There were five cases under this group and the various genes identified were JAG 1, ABCB 4 and ABCB11. One patient who presented with pruritus and progressive cholestasis and high GGT levels and elevated bile acids at 2 years of age and history of neonatal hepatitis was confirmed to harbour pathogenic heterozygous mutation in JAG1 gene for Alagille syndrome. There were 3 cases of PFIC 3 resulting from ABCB4 gene harbouring homozygous mutation with variant of uncertain significance. All these children were older than 12 months of age and had presented with pruritus and progressive cholestasis. The clinical phenotype was correlated with PFIC type 3 and liver biopsy had shown absent MDR3 staining. One of them underwent LDLT at six years of age in view of decompensated cirrhosis with progressive jaundice, coagulopathy and growth failure with pediatric end stage liver disease (PELD) score 27 and is presently on follow up. There was one case of PFIC type 2

confirmed in a 12 year boy who presented with progressive jaundice and pruritus and had conjugated hyperbilirubinemia with low GGT and elevated bile acids. He had homozygous pathogenic mutation in ABCB11 gene.

***Group D: Acute liver failure***

There were two cases in this group. One was a 4 year old girl with recurrent episodes of acute liver failure. She was evaluated extensively and finally diagnosed to harbour heterozygous mutation in OTC gene with variant of uncertain significance resulting in ornithine transcarbamylase deficiency. Another one was a 2 month old infant girl with acute liver failure and history of elder sibling demise due to acute liver failure in infancy due to unknown etiology. This infant on evaluation had features of acute liver failure with pancytopenia, hyperferritinemia and hypertriglyceridemia and possibility of hemophagocytic lymphohistiocytosis (HLH) was clinically considered and bone marrow examination demonstrated histiocytes with phagocytocis.Clinical exome sequencing confirmed homozygous STXBP2 gene pathogenic mutation consistent with Familial HLH type 5.The latter child expired.

***Group E: Decompensated chronic liver disease***

This last group had two cases who were less than 12 months of age. First one was a 3 month old infant with progressive jaundice ascites and coagulopathy. On evaluation this case had bilateral adrenal calcifications. Clinical exome sequencing confirmed homozygous LIPA gene pathogenic mutation consistent with Wolman disease. The second one was an eight month old infant with hepatosplenomegaly and ascites and coagulopathy out of proportion to jaundice. She had bilateral nephromegaly also. Clinical exome sequencing confirmed homozygous FAH gene pathogenic mutation consistent with Tyrosinemia type 1. Both the children expired during the study period.

## DISCUSSION

In human beings, the liver is the vital metabolic organ of the body, which carries out more than 500 varied functions that range from general detoxification to protein synthesis, bile production, metabolism of fats, carbohydrates, proteins, bilirubin, vitamin and mineral storage and it even subserve immune functions. With such a multitude of functions to perform, it is not surprising that more than 400 rare monogenic disorders of hepatic origin have been described. Nearly half of the chronic liver disorders presenting in childhood have a genetic basis, and around 20% of liver transplantations in children are performed as a consequence of liver-based monogenic disease. Most of the chronic progressive liver diseases in children except biliary atresia and autoimmune liver disease are inherited liver diseases. In different genetic backgrounds, the disease spectrum is completely different. Due to the lack of specific laboratory examinations, most of these diseases could not be precisely diagnosed according to their clinical manifestations before the advent of NGS.[7]

Next generation sequencing (NGS), massively parallel or deep sequencing are related terms that describe a DNA sequencing technology which has revolutionised genomic research. NGS can be used for clinical diagnostics in different ways depending on the number and type of target regions that are sequenced. Targeted approaches include sequencing whole single genes in cases where individual variants would previously have been genotyped; sequencing panels of disease-specific genes, with panels ranging in size from 2 to >2,000 genes; and sequencing all exons of approximately 4,000 genes currently associated with monogenic disease, which are also known as the clinical exome. Sequencing all ~20,000 protein-coding genes is known as WES (whole exome sequencing) and entire genomes is called whole genome sequencing (WGS). Next-generation sequencing (NGS) technologies have revolutionized genomic and clinical genetic research. NGS offers comprehensive sequencing of multiple known causative or associated genes in highly heterogeneous diseases.[8]

Monogenic diseases that involve the liver represent a heterogeneous group of disorders. In conditions associated with predominant liver parenchymal damage (i.e., genetic cholestatic disorders like PFIC and Alagille syndrome, Wilson's disease, tyrosinemia, alpha 1 antitrypsin deficiency), hepatic complications are the major source of morbidity. A second group includes liver-based genetic disorders characterised by an architecturally near-normal liver (urea cycle disorders, Crigler-Najjar syndrome). In these defects, extrahepatic complications are the main source of morbidity and mortality while liver function is relatively preserved. In a third group of monogenic diseases (organic acidurias except maple syrup urine disease, cystic fibrosis, familial hemophagocytic lymphohistiocytosis) the underlying genetic defect is expressed at a systemic level and liver involvement is just one of the clinical manifestations.[1]

Togawa et al performed the study on usefulness of NGS panel containing 18 genes in children with neonatal/infantile intrahepatic cholestasis. Out of 109 patients recruited, 31 (28%) were molecularly diagnosed. This low diagnostic yield could be related to the patient's selection criteria, because besides the patients clinically diagnosed with known genetic cholestasis, there were included children with probable non-genetic causes such as prematurity, infections, metabolic, and endocrinological abnormalities. Other cause of low diagnostic yield could be using NGS panel with only 18 genes. The positive molecular diagnosis rate in the group clinically diagnosed with known genetic cholestasis syndromes such as Alagille syndrome, PFIC, neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) and Dubin–Johnson syndrome was 71% (22/31).[9]

In the study by Chen et al 102 patients presenting with various forms of cholestasis or idiopathic liver disease were recruited and subsequently underwent a molecular analysis by NGS panel containing 52 genes. The overall diagnostic yield of genetic diagnoses was 33 of 102 (32%) patients. A greater diagnostic yield was noted in 25 of 37 (68%) patients with suspected genetic diseases including PFIC (16/27, 59%), Wilson disease (5/5, 100%), or syndromic cholestasis (4/5, 80%).[10]

In the study by Nicastro et al to measure the detection rate of underlying monogenic diseases on a prospective cohort of 50 children with neonatal/ infantile cholestasis who underwent NGS, the predominant diagnosis was Alagille Syndrome, followed by PFIC 2, alpha – 1 antitrypsin deficiency, 3 and 1 in the order of decreasing prevalence.[11]

Fang et al identified 25 genes related to different phenotypes, including 46 novel disease-related pathogenic mutations. They had classified their study population into three groups viz. cholestasis, liver enzyme elevation and hepato/splenomegaly and compared the diagnostic rates which were 46.0% (29/63), 48.6% (34/70), and 84.6% (33/39) respectively. ATP7B, SLC25A13, and G6PC were the top three genes related to monogenic liver disease in their study.[12]

In the study by Lipinski et al 21 various pathogenic variants (including 11 novel) in 5 different genes (including ABCB11, ABCB4, TJP2, DGUOK, CYP27A1) were identified. The molecular confirmation was obtained in 15 out of 22 patients (68%).[13]

In the recent study on hepatic glycogenoses among children by Korula et al, clinical exome studies have shown that type III and type VI were the most common (3 each) and 1 each of type Ia, IXa, and IXc in their cohort.[14]

This study from a pediatric gastroenterology service in a public sector tertiary care centre in Kerala reports on the clinical utility of NGS in the diagnosis of monogenic liver diseases in children. To the best of our knowledge, there is no published data on the role and utility of NGS in diagnosing pediatric liver diseases from South India. 86.67% of the patients diagnosed with monogenic liver disease in this study were before six years of age. Thus, a proper diagnostic step using NGS would help identify monogenic liver disease at an early age.

## CONCLUSION

In our study CES identified 20 distinct genes related to different compatible clinical presentations among 31 subjects. Patients with, hepatomegaly or hepatosplenomegaly constituted the major clinical presentation of genetic disorders followed by neonatal/infantile cholestasis in our study. JAG1, ABCB4 and PYL1 (13 % each) were the top three genes related to monogenic liver disease in this study. Genetic cholestatic disorders and glycogen storage disorders were the most common monogenic liver diseases. In conclusion, a large number of pediatric liver diseases are caused by monogenic disorders. This study has added to the clinical utility of NGS in characterizing the molecular basis of monogenic liver diseases in children with heterogenous clinical manifestations.

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