RESEARCH ARTICLE

The mutational landscape of genetic cholestatic diseases in Pakistani children

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Abstract

Objective: To report the mutational landscape of a clinically diagnosed cohort of paediatric patients with cholestasis liver diseases.

Method: The retrospective study was conducted at the University of Child Health Sciences, The Children Hospital, Lahore, Pakistan, from December 10, 2021, to March 31, 2022, and comprised data collected from the Paediatric Gastroenterology and Hepatology unit on demographics, clinical and laboratory findings related to children of either gender aged <12 years and diagnosed with cholestatic liver disease from July 2018 to June 2021. The diagnosis was based on clinical and biochemical findings, with no evidence of biliary atresia and metabolic liver disease. Molecular characterisation was done through whole exome sequencing.

Results: Of the 171 children evaluated, 92(53.8%) were diagnosed with genetic cholestatic disorders. There were 52(56%) boys and 41(44%) girls. The median age at presentation was 19.5 months (interquartile range: 51 months). Consanguinity was found in 82(88.1%) cases, and positive family history with one or more affected siblings was noted in 60(64.5%). Exome sequencing identified pathogenic mutations in 13 genes underlying the hereditary cholestasis; ATP8B1, ABCB11, ABCB4, TJP2, NR1H4, DCDC2, ACOX2, AKR1D1, HSD3B7, ABCC2, USP53, SLC10A1, and SLC51A. Of the 70 variants identified, 50(71.4%) were novel variants. The ABCB11-related hereditary cholestasis was the most frequent 27(29%), followed by ABCB4 (26(27.9%). Homozygosity was frequently seen in all except 8(8.6%) children, who had compound heterozygous pathogenic variants. There was no evidence of phenotypic expression in the carrier parents despite the severe nature of the respective mutations identified in the patients.

Conclusions: Genetic heterogeneity of paediatric intrahepatic cholestasis showed recurrent and novel mutations.

Key Words: Cholestasis, Progressive familial intrahepatic cholestasis, Neonatal sclerosing cholangitis, Genetic mutation, USP53, Bile acid synthetic defects, CLD.

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Introduction

Bile is mainly synthesised by hepatocytes, and is composed of bilirubin, bile acids, cholesterol, lipids and other substances that play a major role in detoxification, cholesterol homeostasis, nutrition and endocrine signalling.[1] The primary bile acids, cholic acid and chenodeoxycholic acid, are synthesised in the liver, and impairment at any steps involved in synthesis, secretion and modification of bile acids can result in accumulation of bile salts, leading to hepatic parenchymal damage and cholestasis.[2] Advanced cholestatic liver diseases, therefore, represent a highly heterogeneous group of conditions, and recent scientific progress has uncovered multiple specific responsible proteins.[3,4]

Hereditary cholestatic disorders present in infancy and childhood with an autosomal recessive inheritance.[5] The phenotypic spectrum ranges from severe progressive familial intrahepatic cholestasis (PFIC) to milder, intermittent, non-progressive benign recurrent intrahepatic cholestasis (BRIC).[6] Few studies have provided genetic and clinical spectrum in larger groups of patients without any specific genotype/phenotype correlation.[7] The genetic causes of cholestatic liver disease are more likely to present in the paediatric age group, with at least 45% having a genetic aetiology.[8]

Identifying the underlying mutation in children with cholestatic liver diseases is challenging owing to its known genetic heterogeneity, and clinical whole exome sequencing (WES) is implemented in routine cholestatic liver disease diagnostics.[9] The current study was planned to report the mutational landscape of clinically diagnosed cohort of paediatric patients.

Patients and Methods

The retrospective study was conducted at the University of Child Health Sciences, The Children Hospital (TCH), Lahore, Pakistan, from December 10, 2021, to March 31, 2022, and comprised data collected from the Paediatric Gastroenterology and Hepatology unit on demographics as well as clinical and laboratory findings related to

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children of either gender aged <12 years and diagnosed with cholestatic liver disease between July 2018 and June 2021. Diagnostic genetic testing of the patients with various phenotypes was undertaken as part of TCH initiative. After approval from the institutional ethics review board, data was retrieved using judgmental sampling technique for children with suspicion of genetic cholestasis. In infants and children, cholestasis was defined as jaundice with or without itching and deranged liver function test (LFT), while genetic cholestasis cases were defined as children with cholestasis having family history, sibling's death with similar illness and positive consanguinity, or where other causes were ruled out for neonatal cholestasis and cholestasis in older children.

1.The cases included for analysis were of infants and children aged <12 years who had presented with cholestatic liver disease with or without itching. Some of the follow-up patients included had chronic liver disease without jaundice or itching, with an inconclusive diagnosis.

The cases excluded were of children with any evidence of biliary obstruction or any other cause of liver injury on imaging. All neonates with cholestasis were worked up for biliary atresia by stool colour, 4h fasting hepatobiliary ultrasonography, and gamma glutamyl transferase (GGT) level. Metabolic liver diseases, like galactosaemia and tyrosinaemia, were ruled out. All children underwent detailed clinical and laboratory testing, including screening for toxoplasmosis, rubella, cytomegalovirus and herpes virus (TORCH), metabolic liver diseases, including non-glucose reducing substances in the urine and assay for galactose-1-phosphate uridyl transferase (GALT) in red blood cells, serum alpha-fetoprotein (AFP) levels, serum ferritin level and minor salivary gland biopsy in those suspected to have neonatal haemochromatosis, and Alagille syndrome using slit-lamp examination, X-ray of the spine and echocardiography. For patients having initial symptoms starting after 3 years of age, biochemical tests were performed to exclude Wilson's disease and autoimmune hepatitis.

The severity of cholestasis was defined as chronic liver disease (CLD), end-stage liver disease (ESLD) and acute liver failure of infancy (ALFI).

After taking informed consent from all the parents of the participants, genetic analyses were done for which venous blood from all patients and their parents was collected in ethylenediaminetetraacetic acid (EDTA) vacutainers. Genomic deoxyribonucleic acid (DNA) was extracted using the Gentra Puregene Blood Extraction Kit (Qiagen, Germantown, Maryland, USA).

The experimental workflow of all exomes was performed at CENTOGENE (Germany). Briefly, double-stranded DNA capture baits against approximately 36.5Mb of the human coding exome were used to enrich target exonic regions from fragmented genomic DNA with the Human Core Exome Plus kit (Twist, Bioscience), according to the manufacturer's instructions. The generated library was sequenced using an Illumina HiSeq 2500 sequencer (Vienna, Austria) with 101-bp paired end reads to obtain at least 20x coverage depth for >98% of the targeted bases. Ingenuity Variant Analysis software (2012 beta release; QIAGEN) was used to analyse the WES data. The investigation for relevant variants was focussed on coding exons and flanking +/-20 intronic nucleotides of genes with clear gene-phenotype evidence (based on OMIM® information). The standard nomenclature recommended by the Human Genome Variation Society[10] was employed to number the mutations.

All the filtered variants were validated by bi-directional Sanger sequencing, which was performed using Veriti 96-Well Fast Thermal Cycler and ABI Prism 3730 Genetic Analyser (California, USA) Finch TV (1.4.0) and Seqtrace v0.9.0 software.

In silico prediction of pathogenicity was assessed using several bioinformatics tools, including Protein Variation Effect Analyser (PROVEAN), Mutation Taster, Polyphen[2], Sorting Intolerant from Tolerant (SIFT) and Combined Annotation Dependent Depletion (CADD). Splice site variations were analysed using the Human Splicing Finder tool and Berkeley Drosophila Genome Project (BDGP).

Results

Of the 171 children evaluated, 92(53.8%) were diagnosed with genetic cholestatic disorders. There were 52(56%) boys and 40 (43%) girls. The median age at presentation was 19.5 months (interquartile range [IQR]: 51 months).

Consanguinity was found in 82(88.1%) cases, and positive family history with one or more affected siblings was noted in 60(64.5%) (Table 1).

Table-1: Patient characteristics.

Parameters	Value
1 Median age of presentations	19.5 months
2 Consanguinity	82 (88.17%)
3 Male	52 (56%)
4 Female	41 (44%)
5 Family history of affected Siblings	60 (64.5%)
6 Jaundice	61 (65.5%)
7 Pruritus	50 (53.76%)
8 Compensated chronic liver disease	62 (66.6%)
9 End-stage liver disease	12 (13%)
10 Liver failure of infancy	16 (17.2%)

Table 2: Clinical and laboratory features of patients in hereditary cholestasis groups (n = 92).

	Age at presentation (months) Median(IQR) Mean±SD	History of consanguinity n(%)	Family History of affected siblings n(%)	Intense pruritus n(%)	STB mg/dL Median(IQR) Mean±SD	Serum AST (U/L) Median(IQR) Mean±SD	Serum ALT (U/L) Median(IQR) Mean±SD	Serum GGT (U/L) Median(IQR) Mean±SD	TLC Number/ul Median(IQR) Mean±SD
ATP8B1N=7	6(26.50)	7 (100)	3 (50)	5 (71.4)	6.5(0)	216.50±24.74 199-234	177.00±76.36 (123-231)	66.00±14.1 (56-76)	3400(0)
ABCB11N=27	6(6)1-12	24 (92.3)	20 (74)	17 (65)	22.99±5.52	872.40±240.12 (324-1250)	1349.13±515.02 (432-1998)	68.26±19.56 (45-112)	19170.00 (928)
ABCB4N=26	30.1 (51)1-72	22 (91.6)	10 (38)	10 (38)	6.21±3.32	135.50±50.7066 -231	213.62±56.90 (112-324)	223.43±72.9 (145-324)	4500(3600)
TJP2N=5	9.5(54)1-72	5 (100)	1 (20)	3 (60)	8.7±2.17	243.75±99.54	545.50(248.50 (215-546)	83.25±12.86 (65-95)	7700(4625)
NR1H4N=2	5(0)2-8	2 (100)	1 (50)	-	28.30±8.76	993.00±165.46	448.66±116.66 (231-898)	71.00±22.60 (55-87)	19120(0)
DCDC2(NSC)N=7	8.0±2.0	7 (100)	7 (100)	4 (57)	3.2(0)	189.33±	181.00±41.2 (134-211)	266±50.10 (206-295)	4300(0)
HSD3B7/AKR1D1 /ACOX2(CBAS) N=11	4.5(26)(1-36)	11 (100)	4 (36.3)	2 (18)	12.35(13.10)	318.33(306.00)	0	82.50±17.54 (65-114)	1200(6302)
USP53N=2	54(0)36-72	2 (100)	0	2 (100)	6.6±2.9	1228.50(0)	(338.50)	72.50±7.77 (67-78)	10900(0)
ABCC2 (DBJ)N=3	3624-84	3 (100)	1 (33.3)	0	6.7±0.73	0	110.33±15.88 (92-120)	54.66±9.07 (45-63)	8900(0)
SLC10A1(NTCP) N=1	1.0	1 (100)	1 (100)	0	18.8	231	345	65	10245
SLC51A(ASBTi) N=1	18.0	1 (100)	0	0	2.3	112	126	104	9400

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TLC: Total leukocyte count, STB: Serum total bilirubin, ASBT: Apical sodium-dependent bile acid transporter, GGT: Gamma glutamyl transpeptidase, NTCP: Sodium taurocholate co-transporting polypeptide, TJP2: Tight junction protein, DBJ: Dubin Johnson syndrome, CBAS: Congenital bile acid synthesis defects, IQR: Interquartile range.

Clinical and biochemical characteristic of each genetic cholestatic group was noted (Table 2).

Exome sequencing identified pathogenic mutations in 13 genes underlying the hereditary cholestasis; Adenosine Triphosphate 8B1 (ATP8B1), ATP binding cassette subfamily B member 11 (ABCB11), ATP binding cassette subfamily B member 4 (ABCB4), Tight junction protein 2 (TJP2), Nuclear receptor subfamily 1 group H member 4 (NR1H4), Doublecortin domain containing protein 2 (DCDC2), Acyl CoA oxidase 2 (ACOX2), Aldo reductase 1 (AKR1D1), Hydroxy delta 5 steroid dehydrogenase 3 beta (HSD3B7), ATP binding cassette subfamily C member 2 (ABCC2), Ubiquitin specific peptidase 53 (USP53), Solute carrier family 10 member 1 (SLC10A1), and Solute carrier family 51 subunit alpha (SLC51A) (Figure 1). Of the 70 variants identified, 50(71.4%) were novel variants. The ABCB11-related hereditary cholestasis was the most frequent 27(29%), followed by ABCB4 26(27.9%).

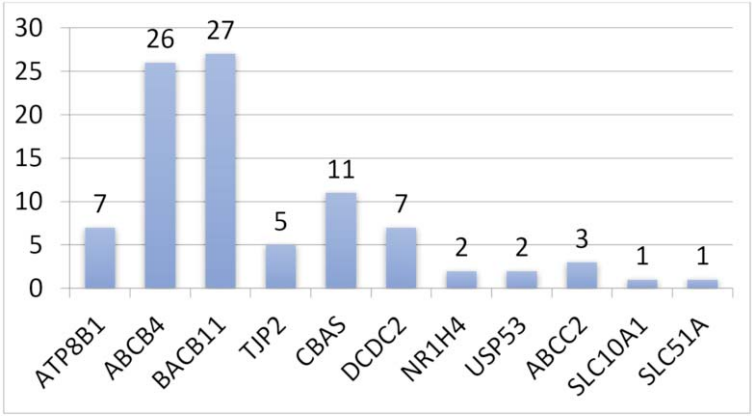

Figure: Groups of patients with hereditary cholestasis in the study sample.

Table-3: Characteristic features of hereditary cholestasis in the study sample (n=92).

Gene	Patient ID	Zygosity	Nucleotide Change	Provean	Mutation Taster	FATHMM	Polyphen-2	CADD	Genome AD	Phenotype	Reference
ATP8B1 (P1-P6, 86)	P1 & P2	Hom	Exon7:c.589_592delGGAGinsCTCCA:p.Gly197Leufs*10	-	1.00 D	-	-	-	-	J,P,L,S, ESLD	Reported
	P3 & P5	Hom	Exon16:c.1804C>T:p.Arg602*	*-14.44 D	1.00 D	-	-	43.0 D	3.98E-06	J,P,L,S, CLD	Reported
	P4	Hom	Exon25:c.3040C>T:p.Arg1014*	*-10.44 D	1.00 D	-	-	41.0 D	3.98E-06	J,P,L,S, CLD	Reported
	P6	Hom	Intron 6:c.555-1G>C	-	1.00 D	-	-	-	-	J,P,L,S, ESLD	Novel
	P86	Hom	Exon15:c.1573C>T:p.Arg525*	*-7.31 D	1.00 D	-	-	34.0 D	7.96E-06	J,P,L,S, CLD	Reported
ABCB11 (P7-P33)	P7 & P9	Hom	Exon 15:c.1760C>A:p.Ser587*	*-12.39 D	1.00 D	-	-	47.0 D	-	J,P,L,S, ALFI	Novel
	P8 & P28	Het/Hom	Intron 3:c.98+1G>A	-	1.00 D	-	-	32.0 D	-	J,P,L,S, CLD	Novel
	P10	Hom	Intron 7:c.611+1G>A	-	1.00 D	-	-	34.0 D	1.21E-05	J,P,L,S, CLD	Reported
	P11 & 16	Hom	Intron 18:c.2178+2T>C	-	1.00 D	-	-	33.0 D	-	J,P,L,S, ALFI	Novel
	P12	Hom	Exon11:c.1156G>T:p.Gly386*	*-9.15 D	1.00 D	-	-	42.0 D	1.21E-05	J,P,L,S, CLD	Reported
	P13, P14 &P15	Hom	Intron 9:c.908+1G>C	-	0.99 D	-	-	34.0 D		J,P,L,S, ESLD	Novel

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Gene	Patient ID	Zygosity	Nucleotide Change	Provean	Mutation Taster	FATHMM	Polyphen-2	CADD	Genome AD	Phenotype	Reference
	P8 & P17	Het/Hom	Exon 26:c.3510_11insT:p.Met1171Tyrfs*29	-	1.00 D	-	-	-	2.03E-04	J,P,L,S, CLD	Novel
	P18, P19 &P20	Hom	Exon 2:c.22C>T:p.Arg8*	*-2.00 N	1.00 D	-	-	37.0 D	1.20E-05	J,P,L,S, ALFI	Reported
	P21	Het	Intron 22:c.2815-2A>G	-	1.00 D	0.98367	1.00 D	31.0 D	-	J,P,L,S, CLD	Novel
	P21	Het	Exon 25:c.3382C>G:p.Arg1128Gly	*-6.616 D	0.99 D	*-2.82 D	1.00 D	28.0 D	4.20E-06	J,P,L,S, CLD	Reported
	P22	Het	Exon 7:c.583G>A:p.Val195Met	*-1.412 N	0.99 D	*-1.35 T	0.832 D	24.8 D	1.61E-05	J,P,L,S, CLD	Novel
	P22	Het	Exon 25:c.3254T>G:p.Phe1085Cys	*-7.301 D	0.99 D	*-3.02 D	1.00 D	33.0 D	-	J,P,L,S, CLD	Novel
	P23	Hom	Exon 27:c.3691C>T:p.Arg1231Trp	*-7.515 D	0.99 D	*-3.74 D	1.00 D	31.0 D	2.01E-05	J,P,L,S, ALFI	Reported
	P24	Hom	Exon 15:c.G1708A:p.Ala570Thr	3.628 D	0.99 D	*-3.92 D	1.00 D	27.8 D	4.03E-06	J,P,L,S, CLD	Reported
	P25	Het	Exon 14:c.1484G>C:p.Arg495Thr	5.17 D	0.99 D	*-3.50 D	1.00 D	30.0 D	-	J,P,L,S,ALFI	Novel
	P25	Het	Exon 8: c.722_723delinsC:P.Leu241Profs*21	-	1.00 D	-	-	-	-	J,P,L,S, ALFI	Novel
	P26&P27	Hom	Exon 25:c.3382C>T:p.Arg1128Cys	*-7.561 D	0.99 D	*-3.03 D	1.00 D	32.0 D	1.68E-05	J,P,L,S, ESLD	Reported

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Gene	Patient ID	Zygosity	Nucleotide Change	Provean	Mutation Taster	FATHMM	Polyphen-2	CADD	Genome AD	Phenotype	Reference
	P29	Het	Exon22:c.2633_2636delTGATinsAGAG:p.Met878_Ile879delinsLysArg	*-8.426 D	1.00 D	-	-	-	-	J,P,L,S, CLD	Novel
	P29	Het	Exon 28:c.3772C>T:p.Gln1258*	*-13.80	1.00 D	-	-	53.0 D	8.11E-06	J,P,L,S, CLD	Novel
	P30	Het	Exon 14:c.1546A>G:p.Ile516Val	*-0.810 D	0.99 D	*-3.03 D	0.996 D	26.1 D	-	J,P,L,S, ESLD	Novel
	P30	Het	Exon 28:c.3803G>A:p.Arg1268Gln	*-6.473D	0.99 D	*-1.91 D	1.00 D	34.0 D	-	J,P,L,S, ESLD	Reported
	P31	Hom	Exon 19:Homozygous deletion encompassing exon 19	-	-	-	-	-	-	J,P,L,S, ESLD	Novel
	P32	Hom	Exon15:c.1789insGG:p.Val597Glyfs*7	-	1.00 D	-	-	-	-	J,P,L,S, ALFI	Novel
	P33	Het	Exon 24:c.3129_3130delAGinsT p.(Lys1043Asnfs*54)	-	1.00 D	-	-	-	-	J,P,L,S, CLD	Novel
	P33	Het	chr2:169850221-169853248, encompassing exon 6-8	-	-	-	-	-	-	J,P,L,S, CLD	Novel
ABCB4 (P34-P58, 85)	P34,P57	Hom	Exon 16:c.1906C>T:p.Gln636*	*-3.28 D	1.00 D	-	-	35.0 D	-	J,P,L,S, CLD	Reported
	P35	Hom	Exon 14:c.1571C>A:p.Thr524Asn	*-4.577 D	0.99 D	*-2.43 D	1.00 D	27.3 D	-	J,P,L,S, CLD	Novel

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Gene	Patient ID	Zygosity	Nucleotide Change	Provean	Mutation Taster	FATHMM	Polyphen-2	CADD	Genome AD	Phenotype	Reference
	P36, P40, P45 &P46	Hom	Exon 14:c.1714C>T:p.Gln572*	*-13.37 D	1.00 D	-	-	43.0 D	3.19E-05	J,P,L,S, CLD	Novel
	P37,P38, P39 & P85	Hom	Exon 15:c.1783C>T:p.Arg595*	*-12.53	1.00 D	-	-	37.0 D	1.59E-05	J,P,L,S, CLD	Reported
	P41, P43, P44 & P56	Hom	Exon23:c.2861G>A:p.Gly954Asp	*-3.237 D	0.99 D	*-2.58 D	0.830 D	27.2 D	-	J,P,L,S, CLD	Novel
	P42	Hom	Exon 25:c.3242T>C:p.Leu1081Pro	*-6.662 D	0.99 D	*-3.53D	1.00 D	31.0 D	3.98E-06	J,P,L,S, CLD	Novel
	P47	Hom	Exon 13:c.1369G>A:p.Gly457Arg	*-6.380 D	0.99 D	*-3.02 D	1.00 D	32.0 D	-	J,P,L,S, CLD	Novel
	P48	Hom	Exon 4:c.149A>T:p.Asp50Val	*-5.022 D	0.99 D	*-2,33 D	0.957 D	26.0 D	-	J,P,L,S, CLD	Novel
	P49	Hom	Exon13:c.1453A>G:p.Thr485Ala	*-4.383 D	0.99 D	*-3.29 D	0.411 B	24.1 D	-	J,P,L,S, CLD	Novel
	P50	Hom	Exon 23:c.2860G>A:p.Gly954Ser	*-1.347 N	0.99 D	*-2.49 D	0.932 D	28.8 D	3.98E-05	J,P,L,S, CLD	Reported
	P51	Hom	Exon 28:c.3859C>T:p.*1287Argext*2	-	0.99 D	-	-	-	-	J,P,L,S, CLD	Novel
	P52, P53, P54 &P55	Hom	Exon 4:c.153G>A:p.Trp51*	*-9.73 D	1.00 D	-	-	40.0 D	-	J,P,L,S, CLD	Novel
	P58	Hom	Exon 9:c.874A>T:p.Lys292*	*-12.84 D	1.00 D	-	-	38.0 D	-	J,P,L,S, CLD	Reported
TJP2 (P59-P63)	P59	Hom	Exon 5: c.782_782delA:p.Tyr261Serfs*50	-	1.00 D	-	-	-	4.63E-06	J,P,L,S, CLD	Novel
	P60 NM_004817.3	Hom	Intron 3:c.239+1G>A	-	1.00 D	-	-	13.25 D	-	J,P,L,S, CLD	Novel
	P61	Het	Exon 5: c.721C>T:p.Arg241Cys	*-2.04 N	0.84 D	*-2.57D	0.978 D	22.5 D	-	J,P,L,S, CLD	Novel

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Gene	Patient ID	Zygosity	Nucleotide Change	Provean	Mutation Taster	FATHMM	Polyphen-2	CADD	Genome AD	Phenotype	Reference
	P61	Het	Intron 10:c.1521-2_1521-1insTAA	-	1.00 D	-	-	-	3.18E-05	J,P,L,S, CLD	Novel
	P62	Hom	Exon 6: c.975T>A:p.Ser325Arg	*-4.31 D	0.99 D	1.71 T	0.993 D	23.6 D	-	J,P,L,S, CLD	Novel
	P63	Hom	Exon 16:c.2327_2327delT:p.Leu776*	-	1.00 D	-	-	-	-	J,P,L,S, CLD	Novel
NR1H4 (P64-P65)	P64	Hom	Exon 9:c.1268_1268delA:p.Gln423Argfs*15	-	1.00 D	-	-	-	-	J,P,L,S, ALFI	Novel
	P65 NM_005123.3	Hom	Exon 7:c.964G>C:p.Gly322Arg	*-5.160 D	0.99 D	*-4.088 D	1.00 D	28.1 D	-	J,P,L,S, ALFI	Novel
DCDC2 (P66-P70, P72, P93)	P66 & P67	Hom	Exon 9:Deletion chromosome 6:24205090_24205614	-	-	-	-	-	-	J,P,L,S, CLD	Novel
	P68 & P69	Hom	Exon 5: c.559C>T:p.Leu187Phe	*-3.44 D	0.99 D	*-3.63 D	1.00 D	-	-	J,P,L,S, CLD	Novel
	P70, P93 & P72	Hom, Hom/Het	Exon 3:c.380_381insT:p.Ser128Valfs*5	-	1.00 D	-	-	-	-	J,P,L,S, CLD	Novel
ACOX2 (P71)	P71	Hom	Exon 4:c.461_464delCAGA:p.Thr154Serfs*25	-	1.00 D	-	-	-	2.35E-03	J,P,L,S, CLD	Reported
AKR1D1 (P73, P88, P89)	P73	Hom	Exon 2:c.158A>T:p.Asp53Val	*-8.208 D	0.99 D	*-0.25 T	1.00 D	23.8 D	-	J,P,L,S, ALFI	Novel
	P88	Hom	Exon 2:c.157G>A:Asp53Asn	*-4.560	0.99 D	*-0.15 T	1.00 D	24.2 D	1.19E-05	J,P,L,S, ALFI	Novel
	P89	Hom	Exon2:c.158A>G:Asp53Gly	*-6.384	0.99 D	*-0.24 T	0.999 D	24.0 D	-	J,P,L,S, ALFI	Reported
HSD3B7 (P74-P77, P87, P90-P92)	P74& P91	Hom	Intron 2:c.167-6G>A	-	0.99 D	-	-	16.84 D	-	J,P,L,S, ALFI	Novel

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Gene	Patient ID	Zygosity	Nucleotide Change	Provean	Mutation Taster	FATHMM	Polyphen-2	CADD	Genome AD	Phenotype	Reference
	P75& P87	Hom	Exon 6:c.592G>A: p.Gly198Ser	*-2.983 D	0.99 D	*-2.12 D	0.975 D	24.5 D	1.99E-05	J,P,L,S, ESLD	Novel
	P76	Hom	Exon 5-7:c.458_972 del	-	-	-	-	-	-	J,P,L,S, CLD	Novel
	P77	Hom	Exon 6:c.689A>G: p.Tyr230Cys	*-8.788 D	0.99 D	*-3.45 D	1.00 D	32.0 D	1.22E-05	J,P,L,S, CLD	Novel
	P90, & P92	Hom	Exon 2:c.45_46de lAG:p.Gly17L eufs*26	-	1.00 D	-	-	-	3.72E-05	J,P,L,S, CLD	Reported
USP53 (P78-P79)	P78	Hom	Exon 14&15:Delch r4:120190845_120193189	-	-	-	-	-	-	J,P,L,S, CLD	Novel
	P79	Hom	Exon 11:c.774_774delG:p.Thr259Profs*8	-	1.00 D	-	-	-	-	J,P,L,S, CLD	Novel
SLC10A1 (P80)	P80	Hom	Exon 3:c.745C>T: p.Arg249Trp	*-1.075 D	0.733 P	*-1.23 T	0.999 D	27.3 D	-	J,P,L,S, CLD	Novel
ABCC2 (P81-P83)	P81	Hom	Exon22:c.2914C>T:p.Gln972*	*-10.44 D	1.00 D	-	-	36.0 D	-	J,L	Novel
	P82	Hom	Exon 4:c.398C>T: p.Ser133Phe	3.621 N	0.937 D	1.78 T	0.001 B	17.77 D	-	J,L	Novel
	P83	Hom	Exon 7 :c.807_808delTGinsCT:p.Gly270*	0.67 N	1.00 D	-	-	-	-	J	Novel
SLC51A(P84)	P84	Hom	Exon 6: c.556C>T:p.Gln186*		1.00 D	-	-	-	-	J,P,L,S, CLD	Reported

Hom: Homozygous, Het: Heterozygous, J: Jaundice, P: Pruritis, L: Liver (hepatomegaly), S: Spleen (splenomegaly), CLD: Chronic liver disease, ESLD: End-stage liver disease, ALFI: Acute liver failure of infancy.

Homozygosity was frequently seen in all except 8(8.6%) children, who had compound heterozygous pathogenic variants (Table 3). There was no evidence of phenotypic expression in the carrier parents despite the severe nature of the respective mutations identified in the patients.

Discussion

Developments in genomic technologies and identification of genes responsible for Mendelian liver diseases have played a significant role in human understanding, identification and elaboration of pathophysiological processes, which have indicated the need for reclassification of liver diseases and their management.[12] The current study evaluated the utility of WES for genetic diagnosis of 92 children diagnosed with cholestatic liver diseases. The patients represented a group for which exhaustive clinical evaluation and investigations were undertaken without any conclusive result.

Cholestasis related to ABCB11 was the most common type followed by ABCB4, which is in accordance with an earlier report.[13] All groups of hereditary cholestasis are characterised by normal serum GGT cholestasis except for ABCB4 & DCDC2 related groups which has high GGT, which was the case in the current study.[14] Pruritus is believed to be the most obvious symptom in cholestasis, but the current study found it a non-pathognomonic symptom and majority of the groups presented either without pruritus or as a late manifestation, which can happen in all sorts of ESLDs.[15] All groups of cholestatic liver diseases showed a wide spectrum of presentation from mild elevated transaminases and hepatomegaly to frank liver failure or advance liver disease. ABCB11, ABCB4 and TJP2 related hereditary cholestasis pose a greater risk for development of liver tumour, but in the current cohort there was no liver tumour in any of the groups, and even in patients who were in second decade of life.[16] Age of presentation in ABCB11 and NRIH4 related cholestasis were lower compared to other groups, and patients in these two groups had rapid progression to liver failure.[17] The current study observed high serum alanine aminotransferase (ALT), total leukocyte count (TLC) and alpha-fetoprotein (AFP) levels in ABCB11 and NRIH4 groups, which is in line with recent literature.[18]

The study found 70 different mutations in the current cohort of hereditary cholestasis, and missense mutations were the commonest. The phenotypes in all groups were not clearly correlated with mutation types. As expected, genetic heterogeneity was observed in all types of hereditary cholestasis, but there was a big number of novel genomic variations which differed from those reported in other populations, such as G308V, D554N and 1661T in ATP8B1 gene which have been documented frequently in Caucasian cohorts.[19] It is reported in literature that in ATP8B1-related cholestasis, type of mutation determines the clinical severity of genetic cholestasis and nonsense, frameshifting and large deletion mutations usually are disease-causing.[20] The current findings are similar. In other groups of cholestasis, missense mutations were pathogenic. The current study found no correlation of any specific mutation with either chronic liver disease or severe cholestasis rapidly progressing to ESLD and acute liver failure. It means environmental factors also play important role in disease progression.

Congenital bile acid synthesis defects (CBAS) constitutes up to 1-2% of cases of neonatal cholestasis, and early diagnosis with prompt treatment with cholic acid has an excellent long-term prognosis.[21] The current study found it to be the third most common group of hereditary cholestasis (11.8 %) after ABCB11 (29%) and ABCB4 (27.9%). Among them, CBAS type 1 (HSD3B7 mutation) was the commonest, followed by type 2 (AKR1D1 mutation) and only one patient of type 6 (ACOX2 mutation) was found. All of them presented as neonatal cholestatic liver disease and steatorrhea. However, in CBAS type 2, age at diagnosis was lower and disease progression was more rapid and severe compared to type 1 and 6 CBAS, and the highest mortality without an early treatment. The study found that CBAS type 6 was benign though there was just a single patient in the cohort.

The highly variable phenotype of ABCB4 documented in literature was quite unique in the current cohort. Reported cases observed appearance of signs of cholestasis within the first year of life, although in the current cohort the median age of presentation was 30 months, and jaundice with pruritus was not the presenting complaints among the patients who, instead, presented with cirrhotic liver with or without portal hypertension. Some of the patients presented as biliary atresia, but no specific mutations were found responsible for it. Also, the study did not find other clinical spectrum of ABCB4 deficiency-associated diseases documented in literature.[22]

In the current cohort, 6 patients from three consanguineous families had neonatal sclerosing cholangitis; two of them underwent orthotropic liver transplant in the second decade of life, while the rest of them were doing well on ursodeoxycholic acid and vitamins ADEK and this is in accordance to the published literature.[23]

Two female patients in the current study had USP53-related cholestasis with clinical spectrum similar to literature.[24] The older patient had history of transient cholestasis that resolved in the first year of life, and currently she was aged 6 years without jaundice and pruritus, while the second patient was a 9- month-old girl

who had neonatal cholestasis, and developed advance liver disease with portal hypertension and pruritus. Unlike an earlier study[25], none in the current cohort was deaf.

Besides, 3 patients had ABCC2-related cholestasis with mild conjugated hyperbilirubinaemia and mildly elevated transaminases. All patients were found to be stable and doing well with milder tinge of icterus without any medications, which is in line with literature.[26] Two of the current patients suffered with rare defects of bile acid transport; Sodium taurocholate co-transporting polypeptide (NTCP) and Apical sodium-dependent bile acid transporter inhibition (ASBTi). It was observed that transporter defects could damage the liver and lead to significant cholestasis and hepatic fibrosis, as mentioned in literature.[27,28]

The current study has its limitations as it was a retrospective study done at a single centre, which means the findings are not generalizable.

Conclusion

Genetic testing aimed at establishing a precise diagnosis in patients with genetic cholestatic diseases is extremely important in order to alleviate the disease burden. An accurate diagnosis will guide clinical decisions, allowing for personalised medical management, monitoring and more accurate prognosis. In addition, the proactive genetic counselling on reproductive choices can be offered to the couples. Functional characterisation of each novel missense variants are warranted to gain further insight in the disease mechanism on the individual level.

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