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Changing Spectrum of Pediatric Liver Diseases: A Single-Center Retrospective Analysis of 423 Cases with Light and Electron Microscopic Findings

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Abstract

Background

To retrospectively analyze the clinical and pathological characteristics of pediatric liver diseases treated at Beijing Children’s Hospital over the past 9 years, summarize the primary features and evolutionary trends of its disease spectrum, and evaluate the diagnostic value of transmission electron microscopy (TEM) for precision diagnosis of pediatric liver disorders.

Methods

We retrospectively reviewed the clinical data of 423 patients aged ≤18 years who presented with liver disease (excluding hepatic tumors) as the primary complaint and underwent successful liver biopsy at Beijing Children's Hospital, Capital Medical University, between January 2017 and December 2025. All biopsy specimens were of adequate quality for paraffin histological and ultrastructural preparation and interpretation. Patients who underwent two biopsies (n = 10) were analyzed separately. Considering the duration of the COVID-19 pandemic and the distribution of samples, the 413 patients with a single biopsy were chronologically divided into three periods—P1 (2017–2019), P2 (2020–2022), and P3 (2023–2025)—and the histopathological types and corresponding disease spectra were compared across the three periods.

Results

The male-to-female ratio was 1.694:1; the mean age was 7.7 years and the median age was 8.2 years. The three leading diagnostic categories were metabolic liver disease, cholestatic liver disease, and hepatocellular injury. The disease spectrum shifted markedly from P1 to P3, with biopsy numbers rising steadily across periods and the spectrum evolving from a "traditional" pattern dominated by metabolic and cholestatic liver diseases in P1 to a "diversified" pattern in P2–P3, characterized by a relatively balanced distribution of metabolic disease, hepatocellular injury, cholestatic disease, and drug-induced liver injury. Electron microscopy was performed in all 423 children and contributed to the final diagnosis in 286 (67.6%), using a predefined definition of diagnostic contribution.

Conclusions

The spectrum of pediatric liver diseases changed significantly from P1 to P3, with increasing diversity of disease entities and increasingly refined diagnosis. TEM remains indispensable for the differential diagnosis of genetic-metabolic and infectious subclasses of liver disease, and its application is essential for achieving precise diagnosis and treatment of pediatric liver diseases.

Keywords: Pediatric liver diseases; Transmission electron microscopy; Pathological classification; Epidemiology

Introduction

Liver disease in children is individually rare but collectively accounts for a substantial share of pediatric morbidity and mortality. Its presentation is non-specific — jaundice, hepatomegaly and elevated aminotransferases are shared by dozens of unrelated conditions — and because a large proportion of the underlying disorders are monogenic or represent inborn errors of metabolism, reaching a specific diagnosis determines substrate restriction, enzyme replacement, transplant candidacy and family counselling, including prenatal testing[1-3].

Liver biopsy therefore remains central to the work-up. Light microscopy defines a morphological pattern, but patterns overlap between diseases and rarely name the disease itself. Molecular testing has resolved many cases that previously remained idiopathic, yet a pathogenic variant is a statement about sequence rather than a demonstration that the variant is functionally expressed in the child's hepatocytes[4-6]; turnaround time, cost and variants of uncertain significance remain practical limits precisely where a result is needed to justify treatment.

Transmission electron microscopy (TEM) addresses this gap by imaging the structures that define a large group of inherited metabolic hepatopathies: cytoplasmic glycogen, mitochondrial contour and crystalline inclusions, peroxisomes, myelinosomes, and material retained within the endoplasmic reticulum[7]. In one tertiary children's hospital, TEM disclosed key findings that directed confirmatory biochemical or genetic testing in 10 of 677 infant liver biopsies, in which ultrastructural examination was performed in only 24.7% of cases; a Chinese cohort of 72 children with suspected inherited metabolic liver disease identified characteristic ultrastructural changes in every case[8,9]. Two questions nevertheless remain open. The diagnostic contribution of TEM is almost always reported within a single disease or a narrow age band, rather than as a measured increment across a full pediatric liver service; and that service is itself changing, with non-viral liver disease rising from 9.6% of biopsies in 1983-2000 to 23.4% in 2001-2012[10] — a shift that concentrates the biopsy population on the very group in which ultrastructural examination is most informative.

We therefore reviewed all 423 children who underwent liver biopsy at a single tertiary children's hospital between 2017 and 2025, in each of whom electron microscopy was performed alongside light microscopy. Because the series was consecutive and encompassed every liver biopsy performed at the centre during the study period, and ultrastructural examination was performed in all of them, its added contribution could be assessed across the full spectrum of pediatric liver disease and without the selection bias inherent in series that request the examination selectively. We set out to characterise the current disease spectrum and its shift relative to earlier eras, the sex distribution across diagnostic categories, and in what proportion of biopsies electron microscopy contributed to the final diagnosis, using a single definition applied uniformly to all reports. We report a descriptive, single-centre series and make no claim regarding the sensitivity of TEM for any individual disease.

Materials and Methods

Study population and data collection

Data from pediatric patients who underwent liver biopsy for liver disease at our hospital between January 2017 and December 2025 were retrospectively collected, including age, sex, clinical manifestations, and pathological diagnoses. Inclusion criteria were: (1) age >28 days and ≤18 years; (2) a primary diagnosis of pediatric liver disease; (3) successful liver biopsy; and (4) adequate specimens >5 mm in length. Exclusion criteria were: (1) age ≤28 days or >18 years; (2) no primary diagnosis of pediatric liver disease; (3) refusal or non-cooperation, biopsy failure, or inadequate specimens; and (4) hepatic tumors or tumor-related diseases. Basic clinical data were collected for 423 pediatric patients. Ten patients who underwent a second liver biopsy were analyzed separately in a table, while the remaining subjects(n=413) were divided, according to the chronological order of specimen submission, into three time-coincident phases—P1 (2017–2019), P2 (2020–2022), and P3 (2023–2025)—for cross-sectional comparison of clinical and pathological data.

Pathological diagnostic criteria

Diagnoses were based primarily on the following guidelines: the 2025 AASLD Practice Statement on the Evaluation and Management of Metabolic Dysfunction-Associated Steatotic Liver Disease in Children[11]; the 2025 FISPGHAN position paper Diagnostic Approaches for Infants with Cholestatic Liver Diseases[12]; the 2025 AAP guidance for primary care providers on identifying infants with biliary atresia by 2–4 weeks of life[13]; the ESPGHAN position statement on the diagnosis and management of pediatric autoimmune liver disease[14]; and the APASL clinical practice guidance on the diagnosis and management of Wilson's disease[15,16].

Liver biopsy and specimen processing

All patients underwent ultrasound-guided percutaneous liver biopsy. Coagulation function tests, liver ultrasound, and other relevant examinations were performed preoperatively to exclude contraindications; informed consent was obtained from the parents or guardians, and cooperation from the child was obtained or general anesthesia was administered. Each specimen was divided into two portions: one fixed in formalin, paraffin-embedded, and routinely sectioned for hematoxylin-eosin (HE) and immunohistochemical staining for light microscopy; the other fixed in glutaraldehyde, re-fixed for electron microscopy, dehydrated, embedded, and processed into semi-thin and ultra-thin sections for ultrastructural observation under a transmission electron microscope

Definition of diagnostic contribution

Electron microscopy was recorded as having contributed to the final diagnosis when it provided a finding that was not evident on light microscopy and that was incorporated into the integrated final diagnosis — whether by establishing a diagnosis not reached on light microscopy, by identifying a specific aetiology within an established morphological pattern, or by confirming a diagnosis that had been suspected but not established. Examinations whose ultrastructural findings were non-contributory, non-specific, or did not alter the integrated diagnosis were recorded as not contributing. Reports were reviewed by two pathologists(Jiatong Xu, Xingfeng Yao), with disagreements resolved by consensus.At our institution, electron microscopy is performed

routinely on all pediatric liver biopsies as part of the standard diagnostic work-up.

Statistical analyses

Analyses were performed using SPSS 26.0. Quantitative data are expressed as mean ± standard deviation and compared between groups using t-tests; qualitative data are presented as frequency and percentage (%), compared using chi-square tests and Fisher's exact test. Temporal distributions of disease categories across the three periods were compared using Pearson's chi-square test when all expected cell counts were ⩾5 and Fisher's exact test otherwise. Two-sided $P < 0.05$ was considered statistically significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

Results

Temporal Trends in the Overall Disease Spectrum

Among the 413 pediatric patients enrolled in this study, 260 (62.95%) were male and 153 (37.05%) were female. Throughout the study period, male patients consistently outnumbered female patients across all phases, reflecting the genuine influence of genetic mechanisms in certain liver disease subtypes and sex hormone-mediated differences in metabolic regulation **(Fig 1A, Table 1).** The youngest patient was 9 months old, while the oldest was 17 years and 1 month, with a mean age of 7.72 years. The median age, quartiles, and maximum age all increased progressively across phases **(Fig 1B)**. Notably, the quartiles and median age were slightly higher in male patients than in female patients **(Fig 1C, 1D)**.The number of liver biopsy specimens increased from 72cases in phase P1 to 134cases in phase P2 and 207 cases in phase P3, representing an approximately 3-fold increase (187.5% growth) **(Fig 1E)**. This upward trend reflects advancements in four key areas. First, enhanced clinical awareness and diagnostic vigilance—pediatric physicians demonstrated an increased demand for pathological diagnosis of challenging liver diseases, with a greater inclination to confirm diagnoses through biopsy. Second, technological advances in procedural techniques—the widespread adoption of ultrasound-guided precision localization improved the safety profile of biopsy procedures. Third, improved parental awareness and acceptance of liver biopsy—the popularization of minimally invasive techniques, increased accessibility of medical information, and improved health literacy significantly enhanced parental willingness to consent to biopsy. Fourth, the subspecialization and institutional development of our hospital's pediatric hepatology service enabled systematic evaluation and effective management of an increasing number of complex and challenging cases.

Fig.1

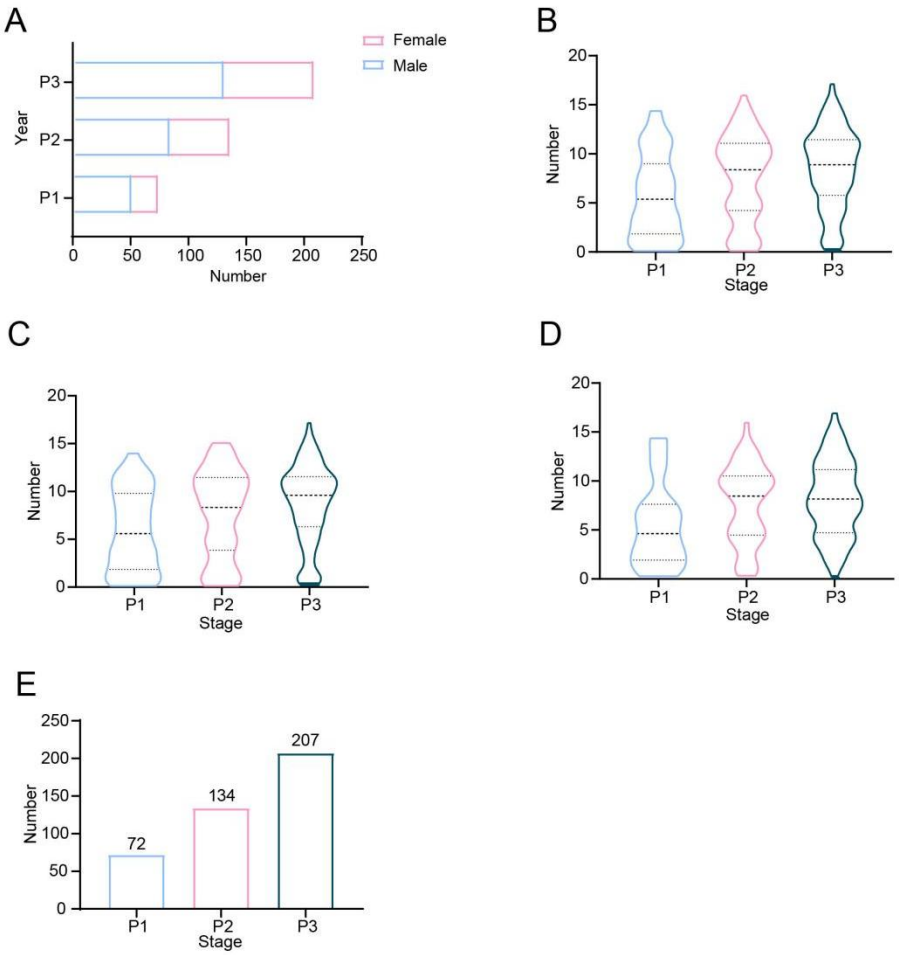


Fig. 1 Statistics of sex, age, and case number in pediatric patients undergoing percutaneous liver biopsy

A. Sex distribution of pediatric liver biopsy patients by year. B. Age structure of pediatric patients at different stages. C. Age structure of male patients at different stages. D. Age structure of female patients at different stages. E. Changes in the number of percutaneous liver biopsies across different stages.

Table 1. Sex distribution of pediatric patients with liver disease across three time periods

Stage	Boys,n（%）	Girls,n（%）	Total,n	Boy-to-Girls ratio
P1	49 (68.1)	23 (31.9)	72	2.30 : 1
P2	82 (61.2)	52 (38.8)	134	1.58 : 1
P3	129 (62.3)	78 (37.7)	207	1.65 : 1
Total	260 (63.0)	153 (37.0)	413	1.70:1

Note: The male-to-female ratio was calculated as the number of male cases divided by the number of female cases in each time period. The original Chinese table listed the 2017–2019 ratio as 2.30 : 1, but recalculation from the

counts (49 / 23) gives 2.13 : 1. This table uses the recomputed ratio. Percentages may not sum to 100.0% due to rounding.

Core Shift in Disease Spectrum: Transition from "Metabolic/Cholestatic Predominance" to "Coexistence of Multiple Injury Patterns"

Based on the characteristics of various pediatric liver diseases and a classification approach integrating hepatic pathomorphology with etiology, this study integrated etiology, pathology, and developmental stage to subclassify the liver diseases in enrolled pediatric patients into thirteen categories: metabolic liver disease, cholestatic liver disease, hepatocellular injury, hepatic fibrosis, infectious diseases, systemic diseases involving the liver, granulomatous liver disease, congenital and hereditary structural abnormalities, neonatal-specific liver diseases, drug-induced liver injury (DILI), autoimmune liver disease, vascular diseases, severe liver injury, and neoplasms.Across the three phases included in the analysis, metabolic liver disease (129 cases, 31.2%) and cholestatic liver disease (77 cases, 18.6%) were the two predominant categories. In addition, hepatocellular injury, drug-induced liver injury, infectious diseases, and autoimmune liver disease each accounted for more than 20 cases, which together constituted the six major subtypes，whereas all other liver disease subtypes comprised fewer than 10 cases each **(Fig 2A,2B)**.

Of the 413 patients with a single liver biopsy, the distribution of disease categories across the three periods differed significantly for four categories . The proportion of hepatocellular injury increased significantly over time, rising from 4.2% (3/72) in P1 to 11.2%in P2 and 23.7%in P3. Conversely, metabolic liver disease—although remaining the largest single category—declined significantly in proportion, from 47.2% in P1 to 29.9% in P2 and 26.6%in P3. Infectious diseases were rare in P1 but increased markedly in P2 before declining slightly in P3(Fisher's exact test, P = 0.0358)(**Fig 2B, 2C**). The two biopsies without histological abnormality both occurred in P1 (Fisher's exact test, P = 0.030).Although not reaching statistical significance, increasing trends were observed for drug-induced liver injury (6.9% → 10.4% → 15.0%; P = 0.153) and autoimmune liver disease (1.4% → 5.2% → 6.3%; P = 0.267), whereas cholestatic liver disease showed a non-significant decline (26.4% → 20.9% → 14.5%; P = 0.059). The remaining categories showed no significant temporal variation（**Table 2**）.An analysis of the stage distribution across six major categories of liver diseases—including metabolic liver disease, cholestatic liver disease, hepatocellular injury, drug-induced liver injury, infectious diseases, and autoimmune liver disease—reveals that the absolute number of all disease subtypes in Stage P3 was higher than in either Stage P1 or Stage P2; however, their proportions varied significantly across different stages **(Fig 2B, 2C)**. This clearly demonstrates that the disease spectrum gradually shifted from being “predominantly metabolic and cholestatic” to one characterized by “coexisting multifaceted damage.” Regarding gender characteristics, the gender ratios differed across the six major liver disease subtypes. Among them, metabolic diseases maintained a significantly higher incidence among males across the P1, P2, and P3 time periods: the overall male-to-female ratio was 3.30:1 (99 cases vs. 30 cases), and during the 2023–2025 period, the ratio reached 3.58:1 (43

cases vs. 12 cases) **(Fig 2D) (Table 3)**. This is highly consistent with the inheritance patterns of genetic metabolic disorders; for example, Wilson's disease and hemochromatosis are often associated with X-linked or autosomal recessive inheritance, making male heterozygotes and homozygotes more susceptible to the disease. In addition, infectious diseases also exhibit a higher incidence among male pediatric patients. In contrast, female pediatric patients accounted for a higher proportion of cases in autoimmune liver disease and drug-induced liver injury.

Fig.2

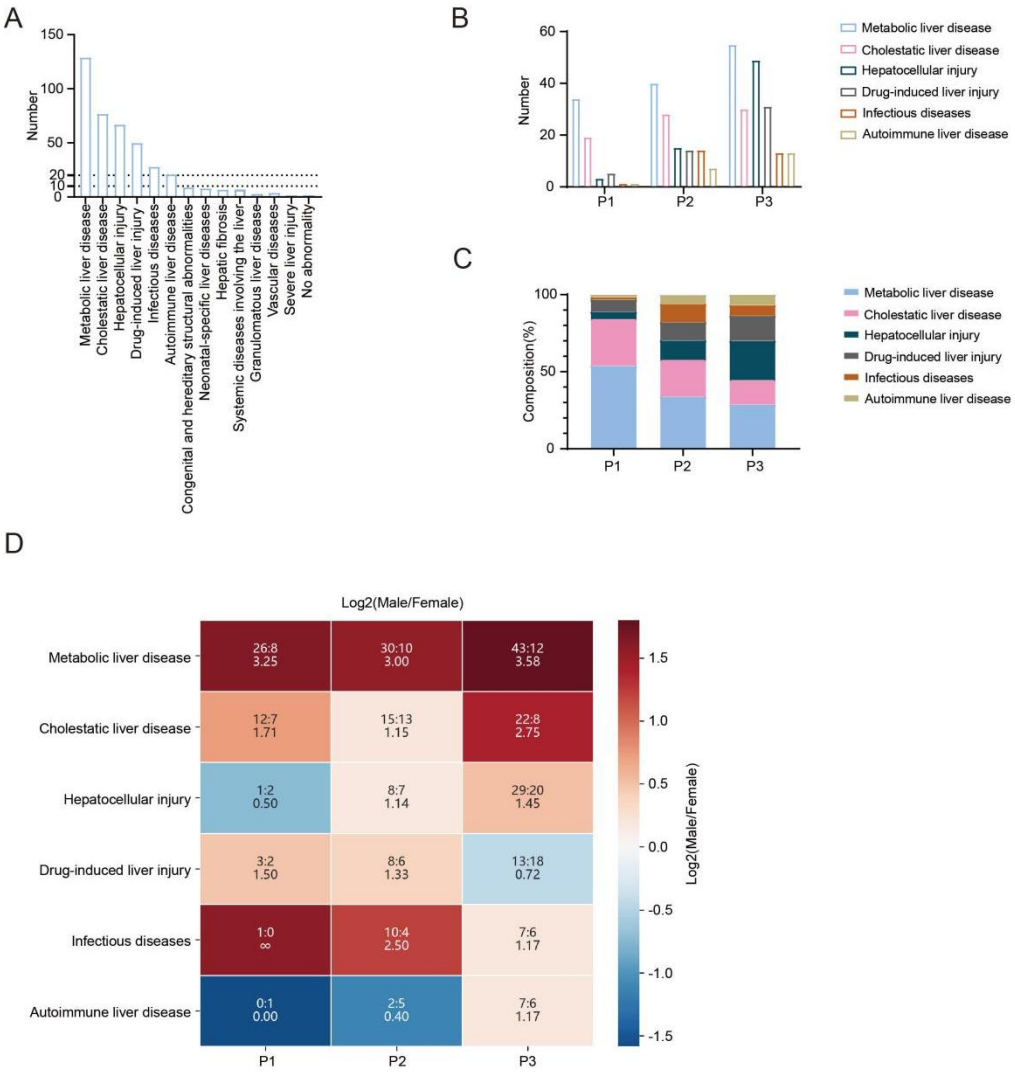


Fig. 2 Number of Cases and Gender Distribution Across Subtypes of Pediatric Liver Disease at Different Stages

A. Bar chart showing the number of pediatric cases for each liver disease subtype included in the study. B. Bar chart showing the number of pediatric cases for major liver disease subtypes at different stages. C. Pie chart showing the percentage of pediatric cases for major liver disease subtypes at different stages. D. Heat map showing differences in the gender ratio of pediatric cases for major liver disease subtypes at different stages.

Table 2. Demographic and clinical characteristics of 413 children who underwent liver

biopsy in China from 2017-2025

Subtypes of Liver Disease	Number	Stage				
	Total n=413	P1	P2	P3	χ^2	P
Metabolic liver disease	129	34 （26.36%）	40 （31.01%）	55 （42.64%）	10.7847	0.0046**
Cholestatic liver disease	77	19 （24.68%）	28 （36.36%）	30 (38.96%)	5.6469	0.0594
Hepatocellular injury	67	3 (4.48%)	15 (22.39%)	49 (73.13%)	18.6439	8.94×10^{-5}****
Drug-induced liver injury	50	5 (10.00%)	14 (28.00%)	31 (62.00%)	3.7511	0.1533
Infectious diseases	28	1 (3.57%)	14 （50.00%）	13 (46.43%)	-	0.0358*
Autoimmune liver disease	21	1 (4.76%)	7 (33.33%)	13 (61.90%)	-	0.2671
Congenital and hereditary structural abnormalities	9	2 (22.22%)	0 （0.00%）	7 (77.78%)	-	0.0561
Neonatal-specif ic liver diseases	8	2 (25.00%)	5 (62.50%)	1 (12.50%)	-	0.0520
Hepatic fibrosis	7	1 (14.29%)	5 (71.43%)	1 (14.29%)	-	0.0738
Systemic diseases involving the liver	7	2 (28.57%)	3 (42.86%)	2 (28.57%)	-	0.3981
Granulomatous liver disease	3	0 (0.00%)	2 (66.67%%)	1 (33.33%)	-	0.5835
Vascular diseases	4	0 (0.00%)	1 (25.00%)	3 (75.00%)	-	0.8278
Severe liver injury	1	0 (0.00%)	0 (0.00%)	1 (100.00%)	-	1.0000
No abnormality	2	2 (100.00%)	0 (0.00%)	0 (0.00%)	-	0.0300*

Note:P values were calculated by Pearson's chi-square test for hepatocellular injury and drug-induced liver injury, and by Fisher's exact test for the remaining categories owing to small expected counts.Data are presented as n (% of row total). χ^2 values (df = 2) are reported for Pearson's chi-square test; "—" indicates Fisher's exact test (statistics not applicable).

Table 3.Sex Distribution of Major Diseases

Subtypes of Liver Disease	Stage	2017-2019		2020-2022		2023-2025	
	Total n=372	Sex		Sex		Sex	
		Boys, n=43	Girls, n=20	Boys, n=73	Girls, n=45	Boys, n=121	Girls, n=70
Metabolic liver disease	129	26	8	30	10	43	12
Cholestatic liver disease	77	12	7	15	13	22	8
Hepatocellular injury	67	1	2	8	7	29	20
Drug-induced liver injury	50	3	2	8	6	13	18
Infectious diseases	28	1	0	10	4	7	6
Autoimmune liver disease	21	0	1	2	5	7	6

Declining Proportions of Metabolic and Cholestatic Liver Diseases

Metabolic liver disease ranked first among the fourteen liver disease subtypes, encompassing metabolic dysfunction-associated steatotic liver disease (MASLD), inherited metabolic disorders (e.g., glycogen storage diseases [GSD] and lipid metabolism disorders), copper and iron metabolism disorders, porphyria, and other rare metabolic diseases. Its evolution across P1–P3 was distinctive in two respects: the absolute number of cases continued to rise, while its proportion within each phase declined progressively (from 47.2% in P1 to 26.6% in P3) **(Fig 2B, 3A)**. Thus, although absolute cases increased by 62% (from 34 to 55), the growth rate lagged behind that of the overall biopsy volume, indicating more rapid expansion of other subtypes.

The metabolic liver diseases in this cohort comprised primarily non-alcoholic fatty liver disease (NAFLD), GSD, and Wilson disease, with a small number of Gilbert syndrome, Dubin–Johnson syndrome, and mitochondrial disorder cases. NAFLD showed the most remarkable change, increasing from 10 cases in P1 to 37 in P3 (a 3.7-fold rise) **(Fig 3B)**, and—unlike GSD and Wilson disease—a markedly higher proportion of male patients **(Fig 3C)**. This increase is consistent with the rising prevalence of childhood obesity, the 2023 international consensus renaming NAFLD as MASLD with clarified diagnostic criteria, and the wider adoption of non-invasive screening tools such as FibroScan and MRI-PDFF.

In contrast, GSD—substantial in P1—declined markedly in proportion during P2 and P3, possibly attributable to effective carrier screening, prenatal diagnosis, and early intervention, as well as reclassification of some cases into other inherited metabolic disorders (e.g., mitochondrial and lysosomal diseases) with increasing availability of genetic testing. Wilson disease showed only a modest increase in absolute case numbers, reflecting improved genetic testing, standardized

biochemical assays, active screening of high-risk populations, and enhanced clinical awareness.

Cholestatic liver disease likewise exhibited a sustained, albeit smaller, decline in proportion across P1–P3. Approximately 20% of these patients had cholestasis caused by biliary atresia or obstruction, and 4 cases had progressive familial intrahepatic cholestasis (PFIC). The absolute number of cases increased slightly in P2 and stabilized in P3, consistent with the expected incidence trend of this predominantly congenital and hereditary disease category **(Fig 3A)**.

Fig.3

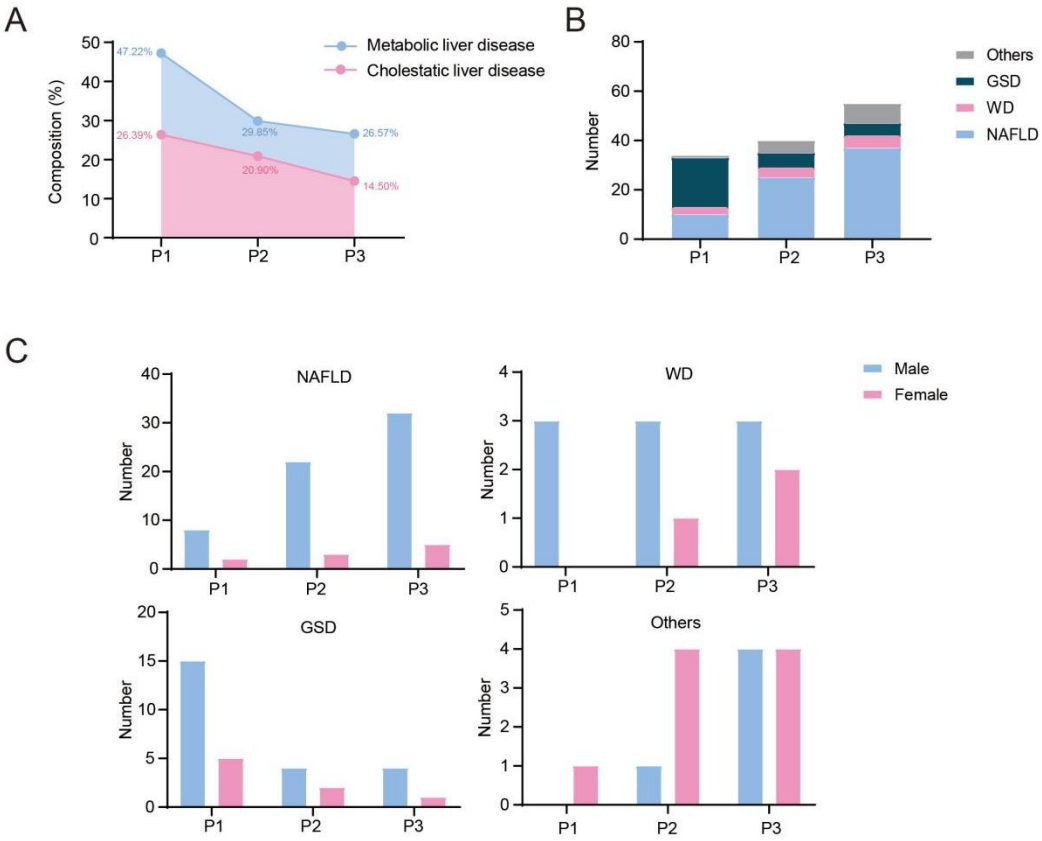


Fig. 3 Proportions of Metabolic Liver Disease and Cholestatic Liver Disease, and Distribution of Specific Metabolic Liver Diseases

A. Line chart showing the proportion of metabolic liver disease and cholestatic liver disease at different stages. B. Distribution of case numbers for specific categories of metabolic liver disease among pediatric patients at different stages. C. Chart showing the distribution of case numbers by gender for specific categories of metabolic liver disease at different stages.

Temporal Evolution of Liver Disease Subtypes with Rising Proportions

In contrast to the declining trends described above, hepatocellular injury increased sharply over the study period. Its proportion rose from less than 4% to over 22%, making it the third most common indication for biopsy, with case numbers increasing 16-fold—from 3 cases in P1 to 49 in P3. This surge is closely associated with the expanding range of medications used in children, the

misuse of health supplements and herbal products, and heightened clinical awareness of adverse drug reactions **(Fig 4A, Table 4)**.

Drug-induced liver injury (DILI) also showed rapid growth, with its proportion more than doubling (6.41% → 14.03%) (Figure 4A, Table 4). Notably, DILI was the only major disease category with a female predominance: the male-to-female ratio declined progressively from 1.5:1 in P1 to 0.7:1 in P3. Possible explanations include sex-related differences in drug metabolism (e.g., for antibiotics and anticonvulsants), differences in healthcare-seeking behavior, and sex-specific drug exposures such as weight-loss products and supplements **(Table 3)**.Infectious diseases and autoimmune liver disease likewise emerged as increasingly important categories, rising from only 1 case each in P1 to a substantial proportion by P3. Infectious diseases peaked in P2 (14 cases), possibly reflecting intensified viral infection screening during the COVID-19 pandemic, before declining slightly to 13 cases in P3—suggesting that this peak was partly a transient phenomenon **(Fig 4A)**.

Taken together, the disease spectrum of pediatric liver biopsies at our institution underwent a profound structural transformation between 2017 and 2025. The proportional increases of hepatocellular injury, infectious diseases, and autoimmune liver disease all exceeded 10-fold from P1 to P3, whereas the traditionally dominant metabolic and cholestatic liver diseases grew more slowly. Meanwhile, biopsies without histological abnormality gradually disappeared, and granulomatous, vascular, and severe liver injuries emerged de novo **(Fig 4B, 4C)**. Overall, the spectrum shifted from a "traditional" pattern dominated by metabolic and cholestatic liver diseases to a more "diversified" pattern in which metabolic liver disease, hepatocellular injury, cholestatic liver disease, and DILI were relatively balanced. This evolution reflects both advances in diagnostic techniques and a genuine change in the etiology of pediatric liver disease, with important implications for clinical management, pathological diagnostic priorities, and public health interventions.

Fig.4

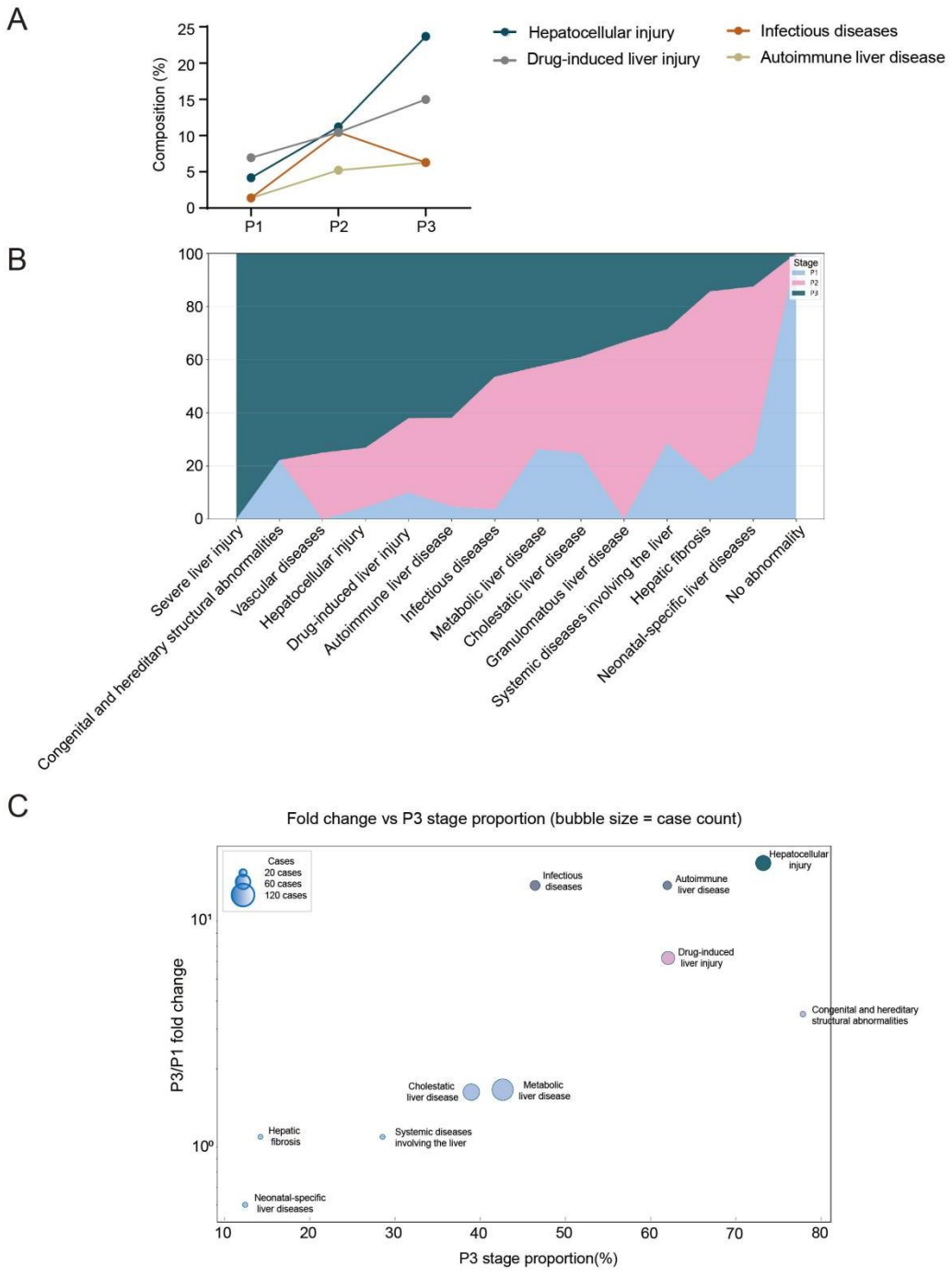


Fig.4 Growth Rates and Proportions of Each Disease Subtype Across Stages

A. Line chart showing the proportions of hepatocellular injury, infectious diseases, drug-induced liver injury, and autoimmune liver disease at different stages B. Stacked area chart showing the cumulative proportions of each liver disease subtype across the three stages C. Bubble chart showing the growth multiples of each liver disease subtype versus their proportions in Stage P3 (bubble size = number of cases)

Table 4. Comparison of the Number, Proportion, and Rate of Increase of Children with Hepatocellular Injury and Drug-Induced Liver Injury Across three time periods

Subtypes of Liver Disease	P1	P2	P3	Growth Multiple（P3/P1）
Hepatocellular injury	3(3.85%)	15(10.71%)	49(22.17%)	16.3
Drug-induced liver injury	5(6.41%)	14(10.00%)	31(14.03%)	6.2

Changes in Special Categories of Liver Diseases

Among pediatric patients, a particularly noteworthy special group is the neonatal population. Neonatal-specific liver diseases (Neonatal Liver Diseases) are defined as age-specific liver diseases occurring primarily during the neonatal period (within 28 days after birth) or early infancy, representing a distinctive feature that differentiates pediatric liver diseases from adult liver diseases. This study revealed that neonatal liver diseases exhibited a transient peak during phase P2, followed by a subsequent decline, which may be attributable to the unique clinical environment during that period (including diagnostic and therapeutic delays caused by the COVID-19 pandemic and the circulation of specific pathogens). A similar temporal pattern was also observed for hepatic fibrosis.Furthermore, vascular diseases emerged as a novel category during phase P2 **(Table 5).** No cases were documented in phase P1, whereas 1 case was identified in phase P2 and 3 cases in phase P3. This observation suggests enhanced diagnostic awareness of pediatric vascular liver diseases (such as Budd-Chiari syndrome and hepatic sinusoidal obstruction syndrome), which may be associated with increased exposure to specific medications (including chemotherapeutic agents and herbal medicines).

Concurrently, biopsies showing no histological abnormality were no longer observed. While 2 cases (2.56%) in phase P1 demonstrated normal histology, no such cases were identified in phases P2 and P3. This finding indicates that with the continuous advancement of clinical awareness, both biopsy indications and preoperative evaluation modalities (such as imaging and serological assessments) have become increasingly precise.

Table 5. Distribution of the Number of Pediatric Cases of Specific Categories of Liver Disease Across three time periods

Subtypes of Liver Disease	P1	P2	P3
Neonatal-specific liver diseases	2	5	1
Vascular diseases	0	1	3

Subtypes of Liver Disease	P1	P2	P3
No abnormality	2	0	0

Pathological findings in children who underwent a second liver biopsy

Liver biopsy has the advantage of being a relatively less invasive procedure, and it can be used to assess disease progression, monitor treatment response, and re-confirm the etiology. Of the 423 children whose data were collected, 10 underwent a second liver biopsy and were analyzed separately; the remaining 413 children constituted the cross-sectional cohort. Based on the diagnosis established at the first biopsy, the disease subtypes were cholestatic disease in 6 children, parasitic infection in 1, and autoimmune hepatitis in 3. Of these 10 children, 4 underwent the second biopsy within 6 months of the first procedure, and the remaining 6 underwent the second biopsy 1 – 3 years later. In addition to routine light microscopy, all second biopsy specimens from these children were processed for electron microscopy and evaluated in detail at the ultrastructural level; the findings indicated that the disease had improved relative to the previous biopsy **(Table 6)**.

Table 6. Comparison of Basic Clinical Information and Electron Microscopic Findings in Children Undergoing Repeated Liver Biopsies

No.	Sex	Age (years)	Interval between two biopsies	Subtypes of Liver Disease	Comparison of ultrastructural changes between two biopsies under electron microscopy
1	boy	2.80	1year	Cholestatic liver disease	Disappearance of the needle-like crystals within mitochondria and a decrease in cytosolic bilirubin granules compared with the first biopsy
2	boy	7.59	6 months	Cholestatic liver disease	Decrease in lipofuscin granule-like structures in the cytoplasm compared with the first biopsy
3	boy	7.89	1year	Cholestatic liver disease	Improvement of cholestatic changes with mild fibrosis, with decreased bile thrombi, mild hepatocyte swelling, mild steatosis and perisinusoidal fibrosis
4	girl	9.87	3year	Cholestatic liver disease	Hepatocellular injury changes with a decrease in glycogen granules compared with the first biopsy

5	boy	13.16	6 months	Cholestatic liver disease	Mild decrease in pigment granules compared with the first biopsy
6	girl	3.24	1.2year	Autoimmune hepatitis	Alleviation of liver injury and steatosis compared with the first biopsy
7	girl	8.55	6 months	Autoimmune hepatitis	Decrease in hepatocellular glycogen compared with the first biopsy
8	girl	10.81	1.5year	Autoimmune hepatitis	Hepatocellular injury with cholestasis improved to hepatocellular injury with mild fibrosis
9	boy	10.64	1.2year	Autoimmune hepatitis	Liver cirrhosis improved to hepatocellular injury changes with mild steatosis
10	boy	1.31	6 months	Hepatic parasitic granulomatous inflammation	Decrease in hepatocyte swelling compared with the first biopsy

Note: The interval between two biopsies is presented as the time elapsed from the first to the second liver biopsy.

Diagnostic Value Assessment of Electron Microscopy

Electron microscopy plays a crucial role in the diagnosis of various liver diseases, providing subcellular evidence beyond histology to help stratify the causes of complex pediatric liver diseases. An analysis of routine light microscopy and electron microscopy reports from the 423 pediatric patients included in this study revealed that electron microscopy played a critical role in the diagnosis of 286 patients (67.61%). The core value of electron microscopy in the diagnosis of pediatric liver disease lies in its ability to visualize organelles invisible under light microscopy, such as mitochondria, peroxisomes, and lysosomal contents. The identification of characteristic tubular or lamellar inclusions within lysosomal-stored macrophages or hepatocytes, abnormal mitochondrial cristae and matrix densities, endoplasmic reticulum dilation, and changes such as glycogen/ lipid accumulation, abnormalities in the structure of the capillaries and bile duct plates, and suspected viral particles. These findings suggest conditions such as Gaucher disease, Niemann-Pick disease, mitochondrial/peroxisomal disorders, glycogen or amino acid metabolic disorders, and cholestatic biliary tract lesions, and provide targeted guidance for further enzymatic assays, metabolite analysis, and genetic sequencing. This study found that among 129 cases of inherited metabolic disorders, electron microscopy provided a basis for differential diagnosis in at least 83 cases (64.3%), confirming the diagnosis. For example, in glycogen storage disease type I, glycogen was observed floating freely in the cytoplasm, whereas in type IV, glycogen was observed aggregated into large vacuoles surrounded by membranes **(Fig 5A)**; in Gaucher disease, dense bodies and medullary concentric lamellar bodies were observed **(Fig 5B)**; in Niemann-Pick disease, lipid-rich lamellar and myelin-like bodies were observed **(Fig 5C)**; and in

hepatolenticular degeneration, mitochondria were observed **(Fig 5D)**, among others. In addition, electron microscopy can identify characteristic ultrastructural inclusions, such as tubular fibers, myelin-like lamellar bodies, and crystalline structures **(Fig 5E)**. It is worth noting that in clinical practice, due to factors such as the representativeness of biopsy samples, the timing of fixation, and the observer's experience, electron microscopic findings should often be incorporated into a comprehensive evaluation that includes clinical, biochemical, imaging, light microscopic, and molecular evidence, rather than being relied upon solely as the basis for a definitive diagnosis.

Fig.5

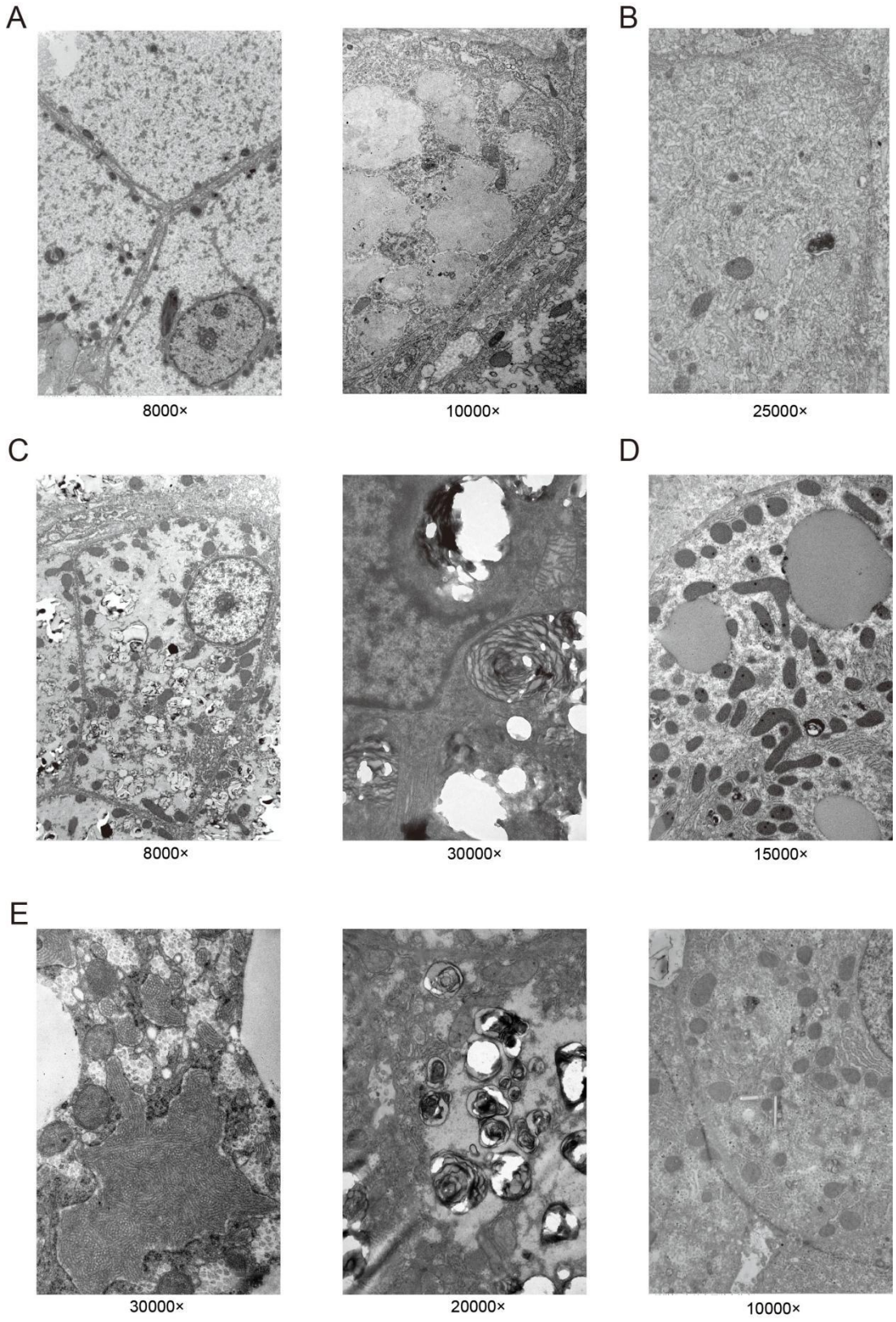


Fig.5 Ultrastructural features of liver cells from pediatric patients as observed by electron microscopy

A. Glycogen in liver cells from pediatric patients with glycogen storage disease (left: Type I glycogen storage

disease; right: Type IV glycogen storage disease) B. Fibrillar inclusions in lysosomes of liver cells from a child with Gaucher disease C. Abundant electron-dense mitochondria in liver cells from a child with Niemann-Pick disease (left) and tightly stacked concentric lamellar structures in lysosomes of liver cells from a child with Niemann-Pick disease (right) D. Deposit granules in liver cells of a child with hepatolenticular degeneration. E. Tubular fibers within giant membranous inclusions in liver cells of a child with hepatic hypofibrinogenemia (left); myeloid lamellar bodies in liver cells of a child with cerulocytosis (middle); and crystalline structures in liver cells of a child with impaired liver function (right).

Discussion

The classification systems for pediatric and adult liver diseases are rather complex; clinically, comprehensive classification is currently based on multiple dimensions including etiology, pathological morphology, pathogenesis, and clinical characteristics[17,18]. This study innovatively proposes a three-dimensional integration concept of "etiology – pathology – developmental stage." Based on a classification approach combining pathological morphology with etiology, the liver diseases in enrolled pediatric patients were classified into fourteen categories: metabolic liver disease, cholestatic liver disease, hepatocellular injury, hepatic fibrosis, infectious diseases, systemic diseases involving the liver, granulomatous liver disease, congenital and hereditary structural abnormalities, neonatal-specific liver diseases, drug-induced liver injury, autoimmune liver disease, vascular diseases severe liver injury and no abnormality. Compared with traditional simplified classifications, this approach not only more accurately reflects the particularities of pediatric liver diseases but also achieves bidirectional anchoring of etiology and pathology while retaining sensitivity to changes in the disease spectrum, thereby avoiding therapeutic strategy homogenization resulting from crude classification and providing robust support for future precision medicine and personalized treatment. Liver biopsy combined with pathological examination is the "gold standard" for clarifying the etiology and pathological classification of pediatric liver diseases[8,9]. Among these, transmission electron microscopy (TEM), with its ultrastructural resolution capability, possesses irreplaceable advantages in the differential diagnosis of inherited metabolic liver diseases, infectious diseases, and certain rare liver disease subtypes, providing clinicians with decisive diagnostic information that is difficult to obtain through conventional light microscopy[19,20]. However, large-sample studies on long-term trends in the spectrum of pediatric liver diseases remain relatively scarce domestically, and the practical diagnostic value of electron microscopy in the precise diagnosis of pediatric liver diseases particularly requires further evidence-based data support.

Through simultaneous routine light microscopic and electron microscopic examinations of liver biopsy specimens at our institution, this study clearly revealed significant changes in the spectrum of pediatric liver diseases over the past decade, primarily characterized by "one rise and one fall"—namely, a significant increase in the proportion of inherited metabolic liver diseases and NAFLD, accompanied by a marked decrease in viral hepatitis. Inherited metabolic diseases accounted for nearly one-third of cases, with a significant increase in the latter five years compared with the former five years (24.2% vs. 38.7%). Specifically, the proportion of pediatric

NAFLD increased from 6.5% to 13.4%, more than doubling. This is attributable, on the one hand, to the widespread availability and application of electron microscopy and genetic testing technologies, enabling precise diagnosis of a greater number of previously indeterminate cases. For example, the observation of characteristic glycogen, lipid, or lysosomal inclusions within hepatocytes under electron microscopy often serves as the primary clue suggesting specific metabolic disorders, thereby guiding subsequent targeted genetic testing[21-23]. On the other hand, this also reflects the effective control of infectious liver diseases following the implementation of public health policies such as neonatal hepatitis B vaccination in China, with the relative importance of hereditary diseases consequently becoming increasingly prominent[24,25]. Simultaneously, this mirrors the profound changes in lifestyle and environmental factors among children in China. Steatohepatitis has transformed from an adult disease into a common pediatric condition. Although NAFLD is reversible in its early stages, approximately 20–30% of pediatric patients with NAFLD will progress to non-alcoholic steatohepatitis (NASH) without intervention, with some eventually developing cirrhosis and hepatocellular carcinoma[26,27]. This situation alerts us that the focus of pediatric liver disease prevention and treatment has shifted from infectious to metabolic etiologies, urgently necessitating the establishment of early screening, diagnosis, and intervention systems for pediatric fatty liver disease. Comprehensive intervention involving the healthcare system, families, schools, and policy-level initiatives is required. In brief, this trend represents a direct manifestation of the impact of modernized lifestyles on children's health, signifying that pediatric liver diseases have entered the "era of metabolic diseases." Therefore, clinicians and pathologists should fully recognize this transformative trend, actively and standardize the performance of liver biopsy in children suspected of having inherited metabolic diseases or NAFLD, and incorporate electron microscopic examination as a routine procedure to improve the overall diagnostic and therapeutic standards for pediatric liver diseases in China.

The importance of electron microscopic examination in pathological diagnosis is self-evident. The necessity of observing the microstructure of pathological tissues has transformed electron microscopy from an auxiliary diagnostic tool into a key modality for precise diagnosis, with its value becoming increasingly prominent particularly in the field of inherited metabolic liver diseases[28,29]. Since Partin first described the hepatic ultrastructural changes of Reye syndrome by electron microscopy in 1971, establishing the central role of mitochondrial toxicity in the diagnosis of this disease, electron microscopy has become a critical component in the pathological diagnosis of liver diseases[30]. With the advancement and popularization of electron microscopy technology, its diagnostic spectrum has expanded from a limited number of diseases to the majority of liver diseases, including relatively common conditions such as non-alcoholic steatohepatitis (NASH), glycogen storage disease (GSD), and Wilson disease (WD), as well as rarer disorders such as Niemann-Pick disease (NPD)[31,32]. Technically, it has evolved from standalone observation to combined light microscopy–electron microscopy approaches and

electron microscopy – molecular diagnostic integration[33,34]. The evolution of electron microscopy in pediatric liver diseases reflects a developmental trajectory from morphological description to mechanistic elucidation, and from single-modality application to multidisciplinary integration. Today, as inherited metabolic liver diseases have become the predominant category of non-viral liver diseases in children, electron microscopy has transitioned from an "optional examination" to a "recommended routine diagnostic procedure"; however, maximizing its value still requires integration with light microscopy, immunohistochemistry, and genetic testing to form a comprehensive diagnostic chain. Although electron microscopy currently still has limitations in certain diseases, including sampling constraints, non-specific findings, high technical thresholds, and inability to independently establish a definitive diagnosis, it is believed that with further technological advances, electron microscopy will undoubtedly provide valuable clues for the diagnosis of pediatric liver diseases and an even broader range of disorders.

Declarations

Ethics approval and consent to participate. This retrospective study was approved by the Ethics Committee of Beijing Children's Hospital, Capital Medical University (approval number: 2026-259-D), and was conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived by the committee because the study used only archived pathology specimens obtained during routine diagnostic work-up, with no additional procedures or risk to patients.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y Zheng designed the study and drafted the manuscript. J Xu performed the histological and ultrastructural examinations. X Kang collected and analysed the clinical data. X Yao and L He critically revised the manuscript. All authors read and approved the final manuscript.

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