Title: Genetic landscape of paediatric acute liver failure of indeterminate origin

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Authors contributions:

DL, LDS, MS, KM, CS and HP were responsible for the conception and the design of the study. Data acquisition was performed by DL, LDS, MS, AB, RB, RA, DA, IB, UB, NSB, PB, BB, PLC, EC, BD, AMD, AD, FD, AF, PF, HG, LG, NH, SH, BH, MH, SH, AH, RH, JH, RI, KIP, MJ, NJ, IK, AK, SK, SK, DK, CTK, BK, MK, HK, VK, TK, ZK, AK, MWL, EL, EL, HM, JAM, PMK, PML, VML, KM, HM, LP, NP, BP, DPR, DPA, DP, SR, NR, AR, RS, WS, NS, CS, ES, RWT, ET, VU, RU, JV, GFV, WVDW, SBW, EZ. LDS, DS, RK, TB, MB, MW, KM and TM interpreted genetic results. LDS, DS and SG were responsible for statistical analysis. DL and LDS drafted the article. MS, KM, CS, HP, GFH, TM and SK critically revised the content.

Abstract (Electronic word count: 275 (275 max))

Background & Aims: Paediatric acute liver failure (PALF) is a life-threatening condition occurring in children without known liver disease. In Europe, viral infections (12-16%) and inherited metabolic diseases (14-28%) are the main causes. Yet, in up to 50% of PALF cases the underlying aetiology remains elusive, challenging clinical management, including liver transplantation. With this PALF exome sequencing (PALFES) study we systematically studied indeterminate cases by whole-exome sequencing (WES) and uncovered unidentified genetic disorders and studied the diagnostic yield of WES in this condition. Furthermore, we investigated phenotypic or biochemical markers that could serve as predictors.

Methods: With this international, multicenter observational study, patients (0-18 years) with PALF of unknown origin were included and whole exome sequencing was performed. Data on the clinical and biochemical phenotype were retrieved and systematically analysed. **Results:** In total, 265 patients with PALF of unknown aetiology from 19 countries were recruited between 2011 and 2022, of whom 59 had recurrent PALF (RALF). WES established a genetic diagnosis in 37% of cases (99/265). The diagnostic yield was highest in children with age of onset in the first year of life (41%), and in children with RALF (66%). Thirty-seven distinct disease genes were identified. Defects in *NBAS* (n=21), *MPV17* (n=8) and *DGUOK* (n=7) were the most frequent findings. When categorising, the most frequent causes were mitochondrial diseases (44%), followed by disorders of vesicular trafficking (28%) and cytosolic aminoacyl-tRNA synthetase deficiencies (10%). One-third of patients had a fatal outcome. Fifty-six patients received liver transplantation with a fatal outcome in 9 (median follow-up time 2.2 years). No phenotypic or biochemic parameter was found to predict a monogenic disease.

Conclusions: This study elucidates a large contribution of genetics in PALF of indeterminate origin with an increasing spectrum of disease entities. The high proportion of diagnosed cases

and potential treatment implications argue for implementation of exome or in future rapid genome wide sequencing in PALF diagnostics.

LAY SUMMARY: to be included during revision

Graphical Abstract to be included during revision

Highlights to be included during revision

Introduction

Paediatric acute liver failure (PALF) is a rare, life-threatening clinical condition mainly affecting children in their first year of life [1]. In the US, PALF is mainly caused by paracetamol intoxication (13%), metabolic disorders (10%) and viral infections (8%) [2]. In Europe, a systematic collection of data on PALF aetiologies is lacking. Single-centres report inherited metabolic diseases (14-28%) and viral infections (12-16%) as main causes of PALF in Europe [3,4]. In US and European cohorts, the underlying aetiology remained unclear in about half of cases, hampering clinical management including disease-specific therapies, particularly decision-making regarding liver transplantation [1–4]. This uncertainty is critical for survival with a high burden for physicians, affected individuals and their families. Therefore, establishing a causal diagnosis is central in PALF. Although standardised approaches regarding biochemical testing and targeted Sanger sequencing in children with PALF contributed to a higher diagnostic rate and helped to elucidate some PALF aetiologies, a large fraction of PALF cases remained unclassified [5]. Access to next generation sequencing (NGS) techniques unravelled novel or uncovered genetic causes of hitherto unsolved cases of PALF. An important milestone boosting WES in acute liver failure (ALF) was the identification of biallelic variants in *NBAS* as a cause of recurrent acute liver failure (RALF) with onset in infancy (MIM: #616483) [5,6]. More recently, numerous *NBAS* cases [7] but also reports on other rare genetic diseases associated with PALF were published, such as infantile liver failure syndrome type 1 due to variants in *LARS1 (*MIM: #615438*)* [8,9], infantile liver failure syndrome type 3 due to variants in *RINT1* (MIM: #618641) [10], and transient infantile liver failure syndrome due to variants in *TRMU* (MIM: #613070) [11]. In the context of the rapidly growing knowledge of genetic causes of PALF, a single centre retrospective analysis of 148 PALF cases (between 2001 and 2011) identified the underlying genetic cause of formerly indeterminate cases in 27% using custom NGS panel, indicating the high proportion of genetic

causes among unsolved cases before the era of next generation sequencing [12]. However, a systematic whole exome sequencing (WES) study has not been performed to date in individuals with PALF of unknown aetiology.

Therefore, in the present international, multicenter study, we aimed to study the proportion, type and biochemical and clinical presentation of genetic diseases identified by WES in a cohort of individuals with PALF of indeterminate aetiology.

Materials and Methods

Study design and recruitment of patients

The study was carried out as an international, multicenter study including both prospective and retrospective cases. The inclusion criterion was the clinical diagnosis of PALF of unknown aetiology in children aged 0-18 years. Diagnostic criteria vary between different countries; the clinical diagnosis of PALF was made according to local criteria, including laboratory parameters such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), bilirubin and INR. Individuals were eligible if local routine diagnostic work-up did not establish the aetiology of PALF. When there was more than one episode of PALF, cases were characterised as recurrent acute liver failure (RALF). Data on country of origin, sex, clinical signs and symptoms using the human phenotype ontology (HPO) [13] as well as laboratory and histology data were retrieved via a specific case report form. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent to participate in the study was obtained from all patients and/or from their parents in case of minor patients. The study was approved by the ethical committees of the Technical University Munich and the University Hospital Heidelberg.

Whole exome sequencing and variant prioritisation

WES of cases and unaffected parents was performed at the Helmholtz Centre Munich (Munich, Germany) for research cases and the Institute of Human Genetics, Klinikum rechts der Isar, Technical University Munich (TUM, Munich Germany) for diagnostic cases. WES data analysis was performed using an in-house pipeline of TUM [14]. WES was performed on genomic DNA extracted from blood as previously published [15]. Sequencing reads were aligned to human genome-build GRCh37/hg19 (UCSC Genome Browser) using the Burrows-

Wheeler Aligner (v.0.7.5a) [16]. Single-nucleotide variants (SNVs) and small insertions and deletions were detected using the Genome Analysis Toolkit (GATK) [17]. Copy number variants (CNVs) were detected with ExomeDepth [18]. Mitochondrial DNA (mtDNA) variants were assessed from exome data as described [19].

A special focus was given to genes previously reported to be associated with ALF. This gene list was manually created and extended through searching the OMIM database for all entries matching the keyword "liver failure". This resulted in a set of 229 distinct candidate genes (Supplementary Table 1). Variants were classified according to the guidelines of the American College of Medical Genetics and Genomics [20] using the Python package "InterVar" and the ClinVar annotation [21].

Phenotypic analysis

The following clinical variables were collected and analysed: gender, weight, height, age at the episode(s) of acute liver failure/first symptoms of liver diseases and at last assessment, patient survival, need for liver transplant (LT), and cause of death. Age ranges were defined as follows: neonatal period, age of onset at birth until day 28; infancy, age of onset before second year of life; early childhood, age of onset between second year of life and fifth year of life; childhood, age of onset between fifth and twelfth year of life; adolescence, age of onset between twelfth and eighteenth year of life. Laboratory data during the episode of liver failure were collected using the international unit system (SI). Additionally, the most prominent hepatic and extrahepatic clinical features were provided as human phenotype ontology (HPO) terms by the referring clinicians [13]. Based on the provided HPO terms, ancestral HPO terms were derived from the ontology using the R package “OntologyX” [22].

Statistical analyses

Statistical analyses were performed using R version 4.0.4. For conducting survival analyses in R the “Survival” and “Survminer” packages were used. The log-rank test was used to compare survival between different categorical variables.

Results

Study cohort

Between 2011 and 2022, a total of 265 individuals were enrolled in this study. Forty-one had been reported previously in single disease gene discoveries or phenotypic spectrum studies [5–7,9–11,23–28]. WES data were analysed at the Technical University of Munich, of which 176 were examined as singletons and 89 by trio WES.

One hundred and twenty (120/265) patients were female (45%). Patients originated from centres in 19 countries in Europe, Asia, or North America (**Fig. 1A**). The majority of patients (69%) were enrolled in Germany, Japan, and the UK, reflecting the major study sites. Fifty-nine individuals (59/265) presented with RALF. Three hundred and eighty (380) distinct, non-redundant HPO terms were reported, resulting in a median of 12 HPO terms per patient (range 2 - 29) spanning a median of 5 organ systems (**Fig. 1B**).

With respect to clinical liver involvement, 75 patients presented with hepatomegaly, 59 had cholestasis, and 52 ascites. Jaundice was present in 38 individuals. Besides the liver, main phenotypic presentations included abnormalities of metabolism (100%), the blood and blood-forming tissues (78%), the nervous system (55%), the cardiovascular system (28%), and the immune system (26%) (**Fig. 1B**).

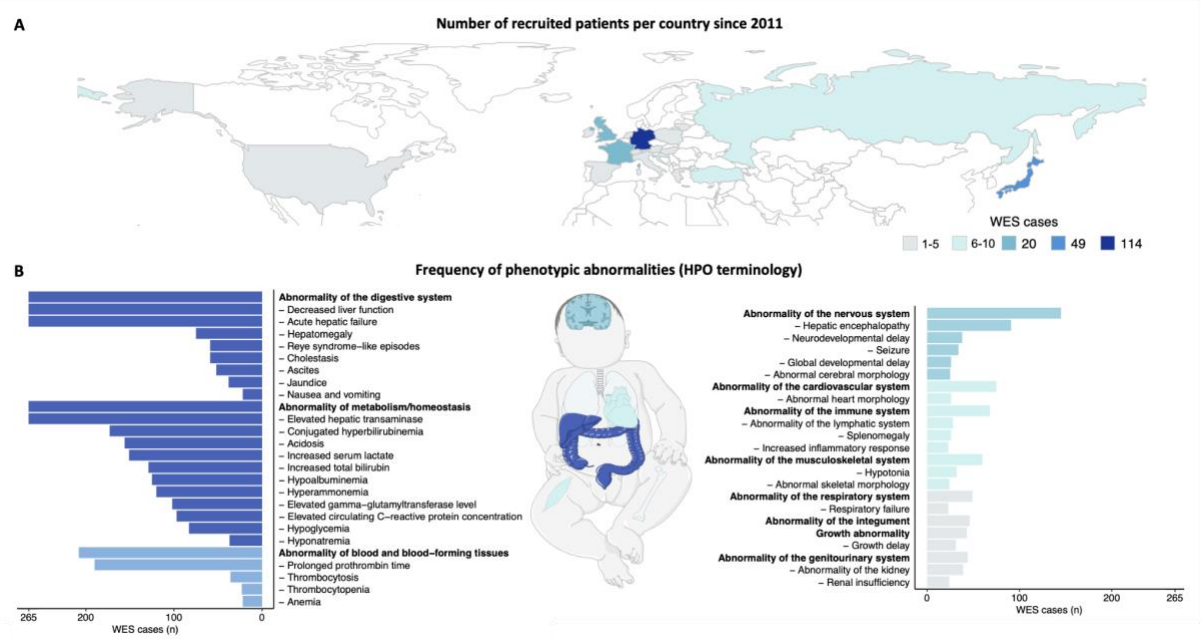
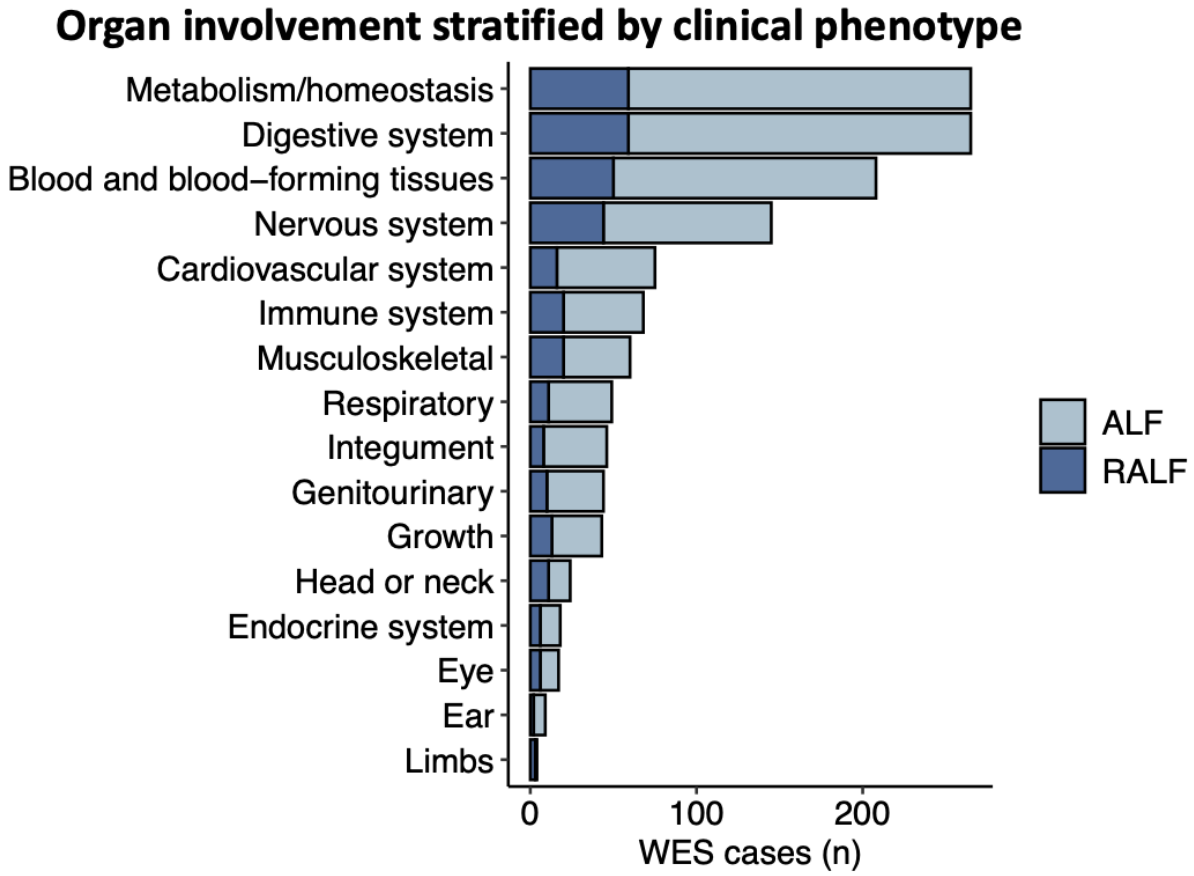


Figure 1: **Geographical origin, clinical phenotypes and affected organ systems.** (**A**) Geographic origin: the number of patients recruited within the different countries is indicated by colour. (**B**) Frequency of phenotypic abnormalities within the whole cohort using the Human Phenotype Ontology (HPO). HPO terms with a frequency of 20 and higher are displayed. WES, whole exome sequencing.

All patients had abnormal blood homeostasis or abnormalities of metabolism (according to the HPO terminology), including elevated aminotransferases, hyperbilirubinemia, acidosis, increased serum lactate, hypoalbuminemia, hyperammonemia and hypoglycemia. Concerning blood and blood-forming tissue, the most frequent clinical features besides prolonged prothrombin time were thrombocytosis, thrombocytopenia and anaemia. Frequently reported neurological abnormalities were hepatic encephalopathy, neurodevelopmental delay and seizures. Organ involvement stratified by clinical phenotype revealed only few differences between ALF and RALF cases, but abnormalities of the nervous and musculoskeletal system were more common in the RALF than in the ALF group (nominal p value < 0.05; **Sup Fig. 1A).**



Suppl. Figure 1: Organ system involvement in affected patients stratified by the clinical phenotype using the Human Phenotype Ontology. ALF, acute liver failure; RALF, recurrent acute liver failure.

Biochemical characterisation of the whole cohort showed broad ranges for plasma ALAT activity with a median of 907 U/l (range: 20 U/l - 19,200 U/l) and ASAT activity with a median of 1.770 (range: 36 U/l - 31,800 U/l) (**Fig. 2A, 2B**). When analysed separately, patients with RALF had a significantly higher ALAT activity with a median of 3,900 U/l (range: 167 U/l - 19,200 U/l) (p-value $1.5x10^{-6}$, Welch Two Sample t-test) and ASAT activity with a median of 4,800 (range: 74 U/l - 31,800 U/l) (p-value $1.473x10^{-6}$, Welch Two Sample t-test) compared to the activity levels in the ALF group with ALAT activity with a median of 592 U/l (range: 20 U/l - 15,528 U/l) and ASAT activity with a median of 1395 (range: 36 U/l - 21,227 U/l). Median

maximal INR was 2.98 (range: 0.9 - 15.7), and median maximal total bilirubin was 133.6 µmol/L (range: 4.62 - 1,219.2 µmol/L). (**Fig. 2C, 2D, 2E)**. Bilirubin and INR levels did not differ significantly between patients with ALF and RALF.

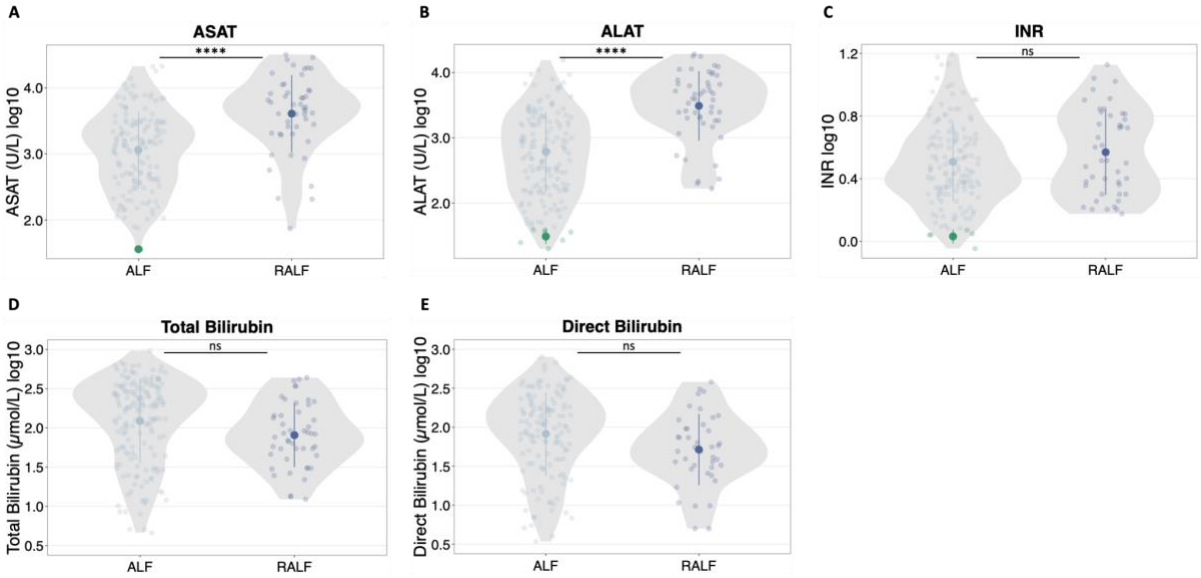


Figure 2: Biochemical characterization of the cohort. Laboratory characterisation of ALF and RALF including ASAT, ALAT, liver function (INR), total bilirubin as well as direct bilirubin using violin plots. Bold dots indicate the median, bars indicate the 25th to 75th percentile; green dots represent values within the reference range for normal values according to a consensus of Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin (DGKL) and Verband der Diagnostika- und Diagnostikageräte-Hersteller (VDGH) [29]. ****$P \leq 0.0001$ by Welch Two Sample t-test. ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; INR, international normalised ratio.

At the time point of data analysis, 186 patients were alive (139 with native liver survival, 47 with liver transplantation), while 79 children had died, 9 of those after liver transplantation (**Fig. 3A**). Age of onset of ALF (first episode in patients with a RALF phenotype) ranged from neonatal to juvenile, with the majority of patients presenting within the first year of life (data

available for 118 patients) (**Fig. 3B**). The majority of deaths due to ALF (77%) occurred within the first year of life (**Fig. 3C)**. Comparison of the overall survival rate in the two clinical subgroups revealed a significantly higher native liver survival rate in the RALF subgroup as compared to the ALF subgroup **(Fig. 3D).** When stratified by age of ALF onset, decreased native liver survival probability was associated with a younger age of onset **(Fig. 3E)**. A trigger for ALF was reported in 83 cases, with febrile infections being the most prominent one (51 cases).

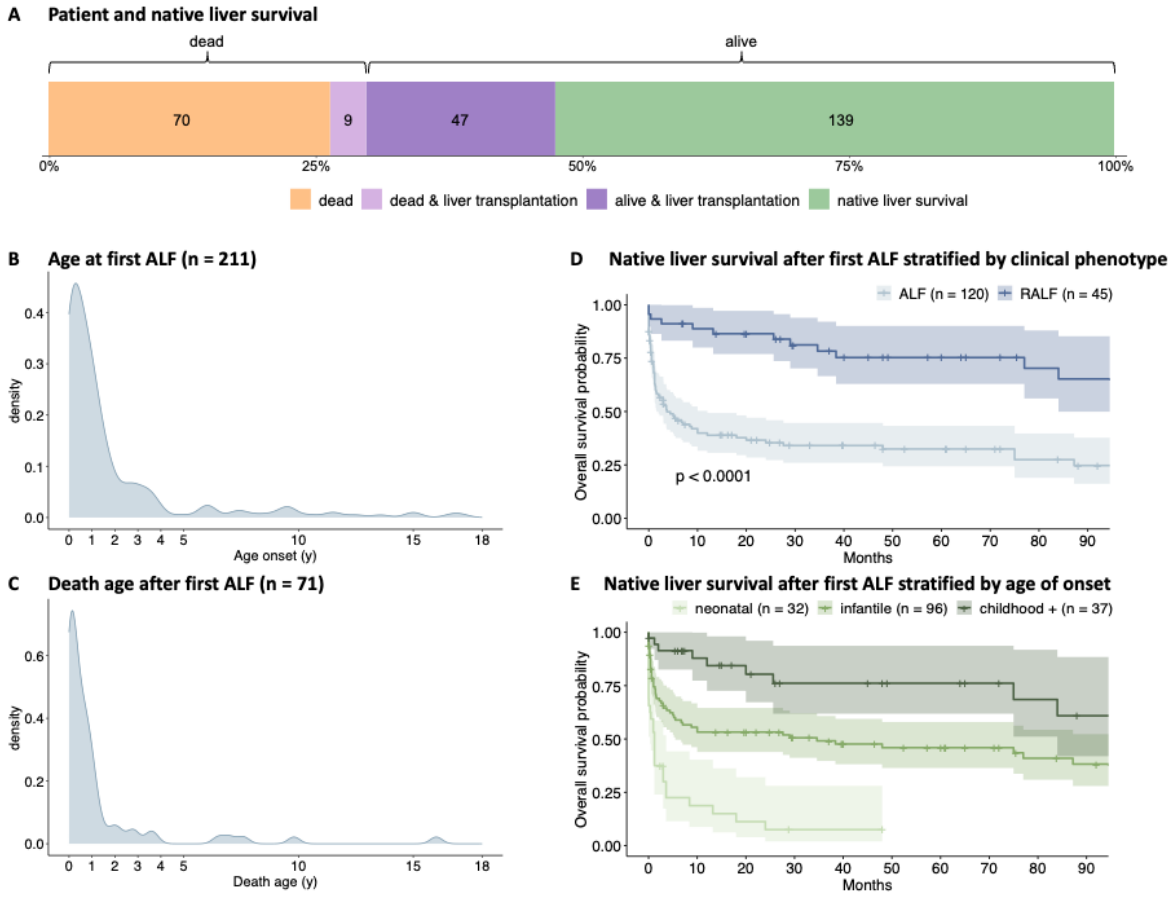


Figure 3: Outcome of PALF patients. (**A**) Patient and native liver survival. (**B**) Timeline for age at first ALF in years. (**C**) Timeline for age of death after the first ALF in years. (**D**) Kaplan Meier plot for the native liver survival after the first ALF stratified by the clinical phenotype in months. P-value calculation by log-rank test. (**E**) Kaplan Meier Plot for the native liver

survival after the first ALF stratified by the age of onset. ALF, acute liver failure; RALF, recurrent acute liver failure.

Genetics

WES analysis established a genetic diagnosis in 99/265 previously indeterminate cases (37%). Twenty patients remained unsolved with variants of uncertain significance in OMIM disease genes or candidate disease genes. In the remaining 146/265 patients (55%), no variant could be prioritised. Pathogenic and likely pathogenic variants were detected in 37 distinct disease genes, of which more than half (22/37, 59%) were reported in single cases only (**Fig 4A**). Defects in *NBAS* (21%), *MPV17* (8%) and *DGUOK* (7%) occurred most frequently. In 94/99 cases, the causative genetic defect was inherited in an autosomal recessive fashion; among these, 54/94 were homozygous for disease-causing variants (Suppl. Table 2). In total, 111 distinct variants classified as "likely pathogenic" or "pathogenic" according to ACMG criteria within the 37 genes were found. Of the 111 variants 56 (50%) were already reported as 'likely pathogenic' or 'pathogenic' in ClinVar at the time point of WES analysis. Missense variants formed the major proportion of all disease-causing variants. When categorising the disease genes to functional groups, most of them are associated with mitochondrial diseases (44%), followed by disorders of vesicular trafficking (28%) and cytosolic aminoacyl-tRNA synthetase deficiencies (10%) (**Fig 4A**). Diagnostic yield was higher in cases with ALF within the first five years of life (40%; n=188) compared to children $\geq$ 6 years of age (13%; n=23) (**Suppl. Fig 3**). Highest yield was achieved in infancy (41%; n=127) and in children with RALF (66%; n=59) (**Fig 4B**). The majority of cases with disorders of vesicular trafficking developed RALF. NBAS deficiency was the most frequent diagnosis in this group. In the majority of mitochondrial diseases (88%), ALF occurred within the first six months of life (median 0.3

years), whereas disorders of vesicular trafficking and cytosolic aminoacyl-tRNA synthetases were characterised by an age of onset of half a year and older (**Fig 4C**).

Patients with disorders of vesicular trafficking rarely died, while patients with mitochondrial disease showed a high mortality. For the other disease groups patient numbers were too low for analyses of survival (**Fig 4D**).

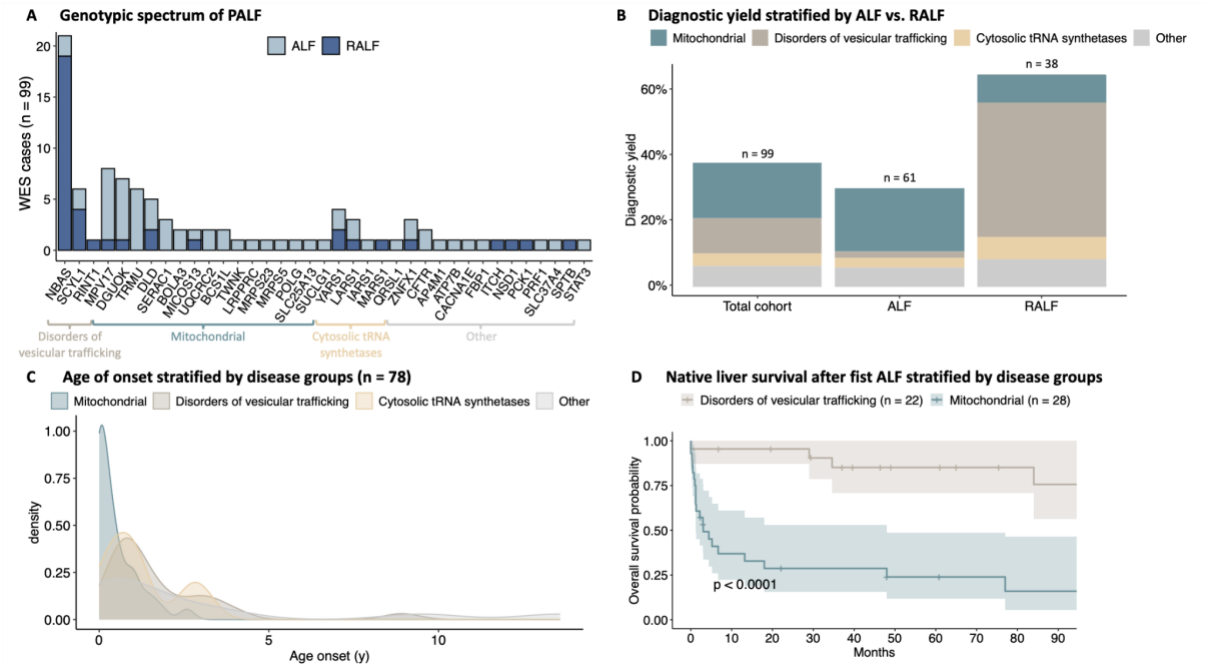
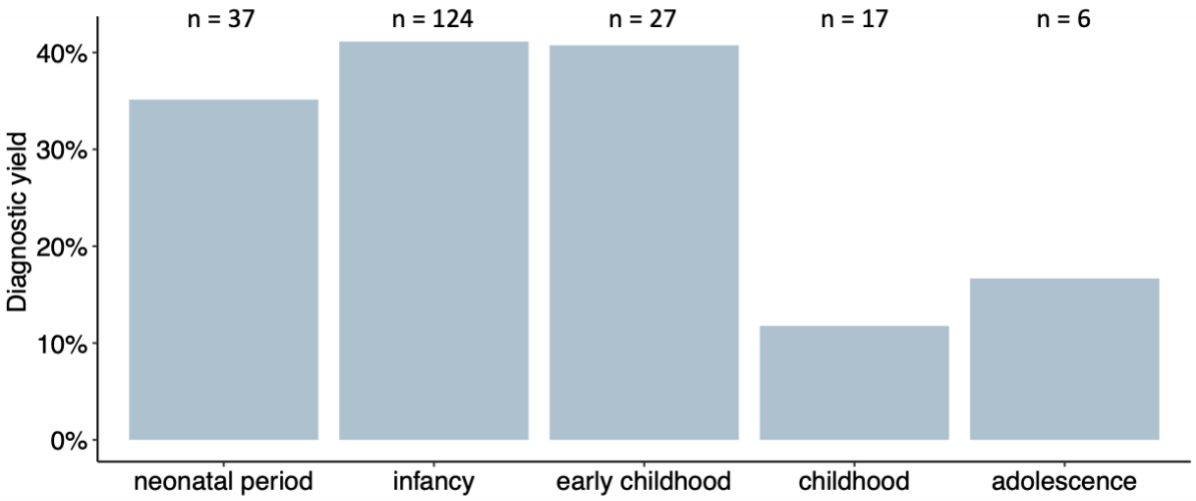
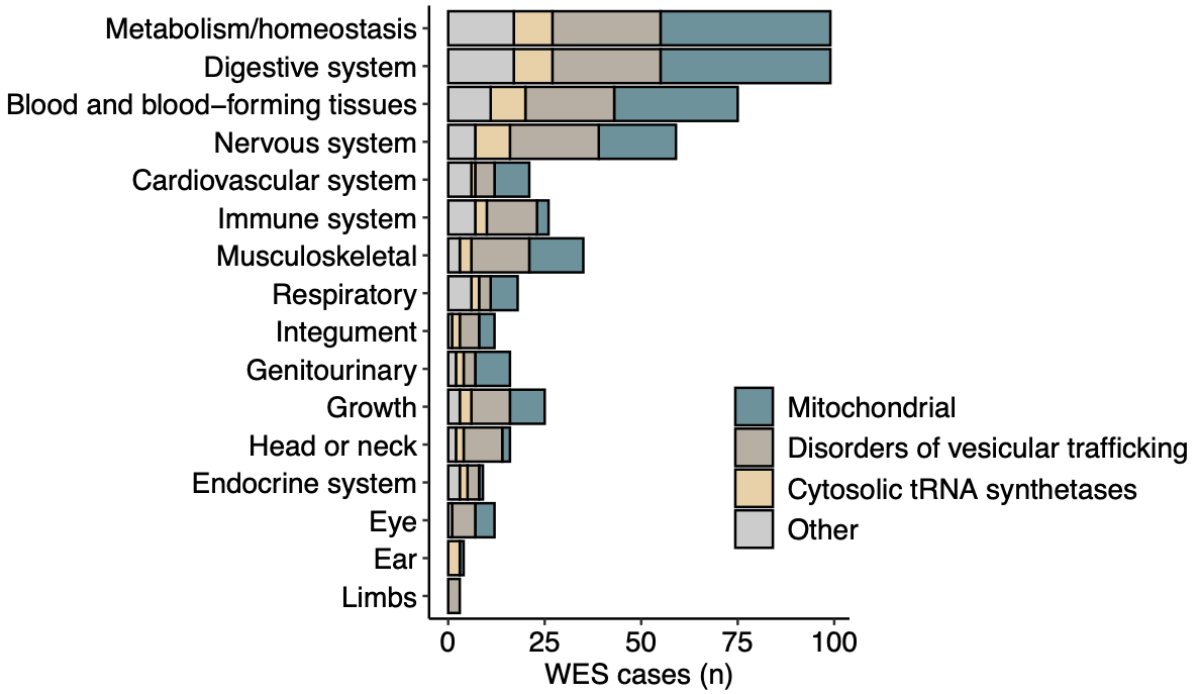


Figure 4: Molecular aetiology of PALF. (**A**) Genetic spectrum of PALF; (**B**) Diagnostic yield, stratified by ALF vs. RALF; (**C**) Age of onset stratified by disease groups, and (**D**) native liver survival after first ALF stratified by the two most frequent disease groups. ALF, acute liver failure; RALF, recurrent acute liver failure.



Suppl. Figure 3: Diagnostic yield stratified by age of onset. Neonatal period, age of onset at birth until day 28; infancy, age of onset before second year of life; early childhood, age of onset between second year of life and fifth year of life; childhood, age of onset between fifth and twelfth year of life; adolescence, age of onset between twelfth and eighteenth year of life.

The three most frequent disease groups in this cohort presented a broad phenotypic spectrum with multiple organs involved (**Suppl. Figure 4**).



Suppl. Figure 4: Organ involvement stratified by disease groups using the Human Phenotype Ontology.

Discussion

PALF is a life-threatening event with very heterogenous aetiologies. Despite ongoing efforts of standardising diagnostic approaches, in one third to half of cases no causal diagnosis is achieved [30]. In 2015, variants in *NBAS* were identified as a novel cause of PALF [5], the number of individuals diagnosed with NBAS deficiency rose rapidly [6,7] and motivated to further explore the genetic landscape of PALF of previously unknown aetiology. Hence, the PALFES study was started including up to now 265 individuals with PALF of unknown aetiology from three different continents. This represents by far the largest cohort of unresolved PALF cases studied by WES reported in the literature to date. A genetic diagnosis was established in 37% of previously unresolved PALF cases; in cases with RALF the diagnostic yield was even higher (66%).

With the elucidation of about 40% of hitherto unexplained PALF cases, genetic diseases likely represent the largest aetiological group of PALF. Nevertheless, it is noteworthy that 55% of patients in this series had no evidence of a genetic disorder identified. Transcriptome or proteome analyses may further increase the diagnostic rate of genetic diseases by providing functional evidence on inconclusive candidate variants detected or by discovery of functional relevant variants missed by the genetic analysis of our cohort. Independently, a substantial part of indeterminate PALF is likely of non-genetic origin [31]. According to Squires *et al.* (2022) [32], the fraction of PALF cases with unknown aetiology is particularly high in the first three years of life, which is the age range in which our study demonstrates the highest molecular diagnostic yield by WES.

Three main groups of genetic diseases underlying previously indeterminate PALF have been identified: mitochondrial disorders, disorders of vesicular trafficking and cytosolic aminoacyl-tRNA synthetase deficiencies (**Fig. 5**). Mitochondrial genetic diagnoses were noticeably more frequent within our study than had previously been reported [32]. Nevertheless, it should be considered that there might be a selection bias in the PALFES cohort, as a paediatric mitochondrial disease cohort was collected in parallel [33]. Moreover, due to our expertise in genetics of rare liver disease and mitochondrial disorders [5–7,9,10,27–31], our study group may have received a higher proportion of individuals suspected to have an underlying genetic cause, specifically mitochondrial diseases and genetic diseases associated with RALF. Hence, an unbiased diagnostic yield might possibly be lower, with a potentially lower rate of mitochondrial disorders and genetic causes of RALF cases. One further limitation is the lack of an internationally uniform definition of PALF, which is reflected in our cohort. Most of the individuals included in our cohort (204/265) fulfilled the inclusion criteria of the longitudinal studies on PALF performed by the "PALF study group" USA [1], which are commonly used as a definition of PALF. When analysing cases fulfilling the PALF study group criteria

compared to the overall cohort of our study, no differences were found regarding diagnostic yield, organ involvement, and disease genes.

In general, PALF cases with a clear biochemical fingerprint (such as tyrosinemia type I, with elevated succinylacetone in urine or dried blood spots) are typically diagnosed based on metabolic investigations, and genetically confirmed in the further workup. The three identified disease groups identified in this study share the feature that no specific laboratory biomarker or metabolic fingerprint by itself can establish the molecular diagnosis or point specifically to a single disease gene. Accordingly, we could not identify a clear biochemical or clinical profile in the cases now diagnosed genetically by WES using already established investigations. More comprehensive metabolomic studies would be needed to extend the search for diagnostic biomarkers. Consequently, with our current knowledge, we advocate performing genetic analyses using WES or whole genome sequencing (WGS) in parallel to metabolic analyses as the timely decision on further appropriate treatment options such as transplantation and/or specific drug therapy or dietary management in case of metabolic diseases will depend on the underlying disorder.

Our study demonstrates that the lack of biomarker specificity also holds true for lactate in serum, as lactate concentration could not differentiate between mitochondrial disorders from other causes of PALF, indicating that lactic acidaemia is also a secondary finding in severe liver dysfunction such as PALF. Extremely high levels of ASAT and ALAT have been associated with NBAS deficiency [6], often presenting as RALF. This reflects the significantly higher ASAT/ALAT levels in this group, however diagnosis cannot be ascertained based on this finding. Age of onset can direct the clinical suspicion towards possible genetic disorders underlying PALF, with mitochondrial disorder most frequently occuring in the neonatal period. Nonetheless, there is a substantial overlap among the different disease groups with respect to age of onset. The most commonly affected extrahepatic organ system was the nervous system,

with hepatic encephalopathy and neurodevelopmental delay reported most often. In analogy to biochemical parameters, also the clinical parameters in our study did not discriminate between cases with and without a genetic diagnosis. This again emphasises the relevance and necessity of genome wide genetic analyses in PALF to determine the underlying cause.

Establishing a (genetic) diagnosis is crucial for clinical management and outcome of children with PALF. While causative treatments are not available for most of the genetic disorders detected in our cohort, there are specific management approaches for several diseases. In patients with TRMU deficiency it has been shown that cysteine supplementation improved survival significantly compared to patients without cysteine supplementation [11]. Forced antipyretic management in ILFS1, ILFS2 and ILFS3 may help to avoid fever triggered RALF [6,7,9,10]. Additionally, decision on transplantation is dependent on the underlying disorder and the expected outcome in relation to both transplant liver survival and extrahepatic features. Our study demonstrates that outcome differs depending on the underlying disease aetiology; native liver survival is significantly higher in individuals with disorders of vesicular trafficking compared to mitochondrial diseases. The role of liver transplantation for individuals with PALF due to genetic diseases is discussed controversially in the literature especially for mitochondrial disorders, due to potentially unfavourable neurological, cardiac or neuromuscular involvement and poor outcome, arguing against liver transplantation [34]. However, there are mitochondrial disorders such as TRMU or hepatic DLD deficiency where extrahepatic involvement is scarce with a favourable neurological prognosis [11,35]. Moreover, multisystemic mitochondrial disorders are not contradicting liver transplant in general, as there are reports of patients e.g. with DGUOK deficiency, with minor neurological involvement who received a liver transplant and had a satisfactory post-transplant course [11,36,37].

In order to help decision making, diagnosis of PALF and the identification of the aetiology needs to be established within a short period of time as the clinical situation typically is critical and decisions are time-sensitive. Turn-around-time in our study was not assessed systematically, and individuals could also be enrolled retrospectively. From a technical point of view, genome sequencing would be faster and more accurate than WES, with reported turn-around-times as low as 24 hours, being a promising option to achieve genetic diagnoses especially in critically ill children [38,39]. Higher investment costs and demanding computational power hampers availability of this technology in most hospitals, but will likely be available in the near future in many countries. Again, rapid establishment of a (genetic) diagnosis has the potential to both save lives and reduce costs [38,39].

Conclusions

In conclusion, this study demonstrates that a relevant number of previously indeterminate PALF cases can be solved by WES. This shifts PALF in the range of other rare disorders for which WES/WGS is now a well-established diagnostic procedure. Major identified groups are mitochondrial disorders, disorders of vesicular trafficking, and cytosolic aminoacyl-tRNA synthetase deficiencies. An ascertained diagnosis helps physicians make treatment decisions and can save lives and costs. For these reasons, we assert that WES, or in the near future WGS, should be within a first line diagnostic approach for every child presenting with PALF.

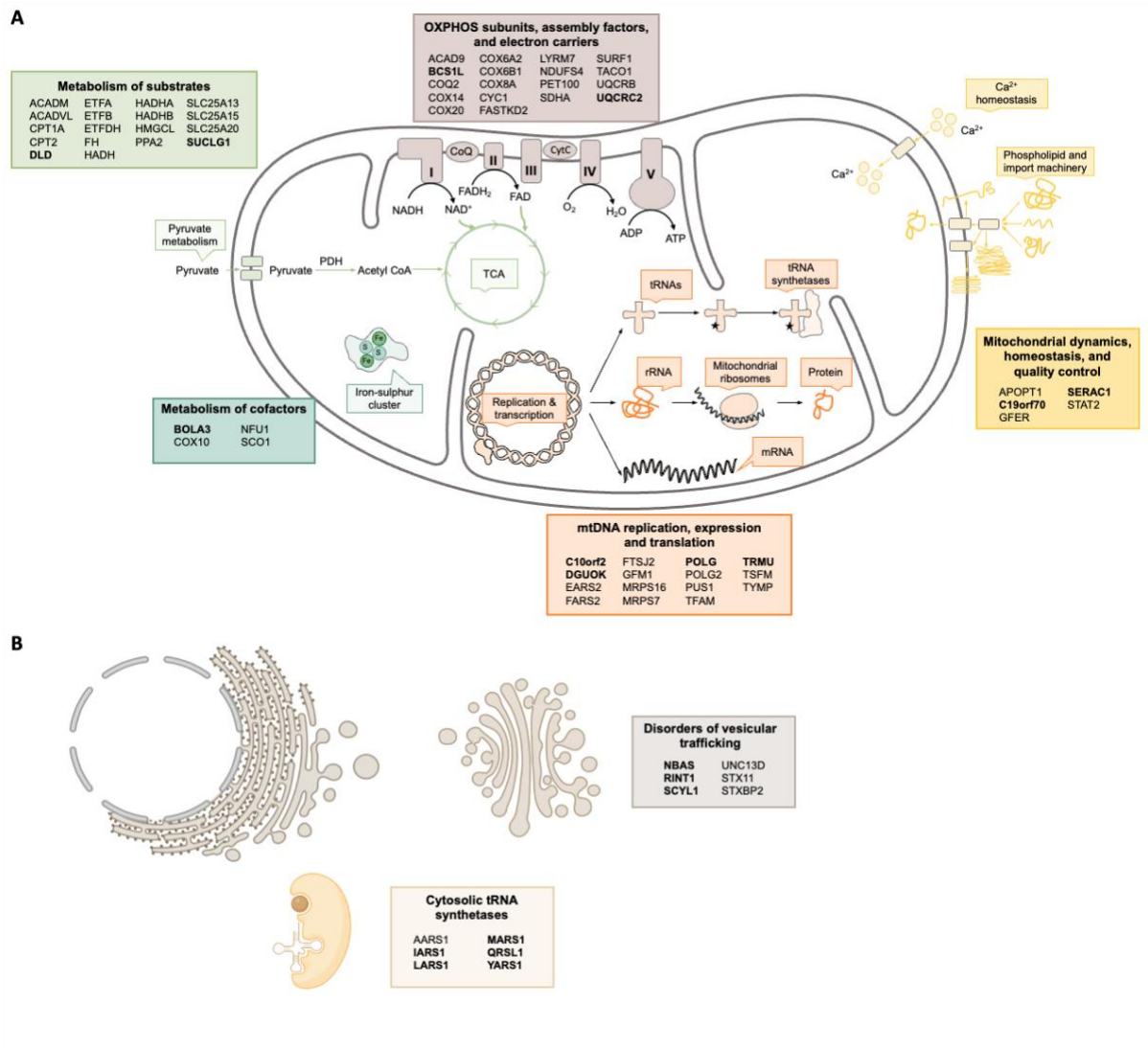


Figure 5 A. Mitochondrial disease genes causing paediatric acute liver failure. B Disorders of vesicular trafficking and aminoacyl-tRNA synthetase deficiency (box) leading to paediatric acute liver failure. Genes that have been identified in cases within this study are shown in bold.

Abbreviations

ALAT	Alanine aminotransferase
ALF	Acute liver failure

ASAT	Aspartate aminotransferase
CNV	Copy number variant
GATK	Genome Analysis Toolkit
HE	Hepatic encephalopathy
HPO	Human phenotype ontology
INR	International normalised ratio
mtDNA	Mitochondrial DNA
PALF	Paediatric acute liver failure
PALFES	Paediatric acute liver failure exome sequencing
PT	Prothrombin time
RALF	Recurrent acute liver failure
SNV	Single-nucleotide variants
TUM	Technical University Munich
WES	Whole exome sequencing
WGS	Whole genome sequencing

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Figure Legends

Table Legends

Supplementary Table 1: List of 229 ALF disease genes.

Supplementary Table 2: List of variants detected in the cohort.

Supplementary Table 3: List of HPO terms describing the clinical phenotype of all 265 patients.