Accepted Article

NEXT GENERATION SEQUENCING IN PEDIATRIC HEPATOLOGY AND LIVER TRANSPLANTATION

Emanuele Nicastro and Lorenzo D'Antiga

Pediatric Hepatology, Gastroenterology and Transplantation, Hospital Papa Giovanni XXIII, Bergamo, Italy

Key words: 'next generation sequencing'; 'liver disease'; 'genetic cholestasis'; 'pediatric liver transplantation'; children.

Abstract word count: 250

Manuscript word count: 4398

Number of figures: 3

Number of tables: 2

Abbreviations: A1AT: alpha-1 antitrypsin; A1ATD: A1AT deficiency; AS: Alagille syndrome; BAAT: Bile acid-CoA:amino acid N-acyltransferase; CES: clinical exome sequencing; GGT: gamma-glutamyl transpeptidase; IMD: inherited metabolic disease; LT: liver transplant; MD: monogenic disease; MDS: mitochondrial depletion syndrome; NGS: next generation sequencing; PFIC: progressive familial intrahepatic cholestasis; ROI: region of interest; TGS: targeted gene sequencing; VUS: variant of unknown significance; WES: whole exome sequencing; WGS: whole genome sequencing.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/lt.24964

This article is protected by copyright. All rights reserved.

Accepted Article

Authors do not have any conflict of interest to disclose.

Corresponding Author:

Lorenzo D'Antiga, MD, FEBT

Pediatric Hepatology, Gastroenterology and Transplantation

Hospital Papa Giovanni XXIII, Piazza Oms 1, 24127, Bergamo, Italy

Tel +39 0352673856; Fax +39 0352674959

E-mail: ldantiga@asst-pg23.it

This article is protected by copyright. All rights reserved.

Accepted Article

ABSTRACT

Next Generation Sequencing (NGS) has revolutionized the analysis of human genetic variations, offering a highly cost-effective way to diagnose monogenic diseases (MDs). Since nearly half of children with chronic liver disorders have a genetic cause and approximately 20% of pediatric liver transplants are performed in children with MDs, NGS offers the opportunity to significantly improve the diagnostic yield in this field. Among the NGS strategies, the use of targeted gene panels has proven useful to rapidly and reliably confirm a clinical suspicion, whereas the whole exome sequencing (WES) with variants filtering has been adopted to assist the diagnostic work up in unclear clinical scenarios. WES is powerful but challenging, since it detects a great number of variants of unknown significance, that can be misinterpreted and lead to an incorrect diagnosis. In pediatric hepatology targeted NGS can be very valuable to discriminate neonatal/infantile cholestatic disorders, disclose genetic causes of acute liver failure, diagnose the subtype of inborn errors of metabolism presenting with a similar phenotype (such as glycogen storage disorders, mitochondrial cytopathies or non-alcoholic fatty liver disease).

The inclusion of NGS in diagnostic processes will lead to a paradigm shift in medicine, changing our approach to the patient as well as our understanding of factors affecting genotype-phenotype match. In this review we discuss the opportunities and the challenges offered nowadays by NGS, and we propose a novel algorithm for cholestasis of infancy adopted in our center, including targeted NGS as a pivotal tool for the diagnosis of liver based MDs.

This article is protected by copyright. All rights reserved.

Accepted Article

INTRODUCTION

Over the past decade, next-generation sequencing (NGS) has advanced remarkably, allowing its widespread use in clinical settings (1). Until recently, a genetic test aimed at answering a question arisen from a specific clinical suspicion, pointing towards a selected genetic target (2). This gene-centered approach, although very reliable to detect single mutations, was inefficient and expensive, since it often required several attempts to make a diagnosis.

In recent years, the introduction of NGS has accomplished the simultaneous analysis of a large number of genes, up to whole exome (WES) or even whole genome sequencing (WGS) (3). Nowadays it is possible to sequence the complete human genome within a few hours at relatively low cost, using widely available technologies that are becoming increasingly powerful, and whose limitations are more interpretative than technical (4).

Progress inDNA sequencing and genotyping array technology allow the detection of variants, deletions and duplications at genome level. Among these techniques, targeted gene sequencing (TGS) and WES are particularly useful to retrieve point mutations that are causative of monogenic disorders (MDs) (5).

There is no doubt that genetic testing may contribute to a prompt identification of patients with a MD. Nevertheless, it is illusory to think that the recognition of a genetic defect can fully characterize the clinical features of a mendelian condition (6). Indeed, environmental factors, usually referred to as epigenetic modifiers, deeply affect the phenotypic expression of MDs (7). In this complex setting, a deeper knowledge of genomic variants represents the main stride towards the understanding of the determinants of genotype-phenotype match in MDs (8). Besides, the availability of rapid mutation detection in disease-related locuses can offer the opportunity to identify unexpected clinical pictures for a certain gene variant.

This article is protected by copyright. All rights reserved.

Accepted Article

NGS is a rapidly expanding technology and its applicability is well ahead of the current clinical use. Since approximately 20% of all pediatric liver transplants (LT) are performed in children with MDs affecting the liver (9), the opportunity to simultaneously investigate a panel of genes is potentially increasing the detection rate for an inherited condition. This review aims at describing the value of the NGS as a diagnostic tool in pediatric hepatology and for the decision-making in the setting of pediatric liver transplantation.

NEXT-GENERATION SEQUENCING

Sanger technique has proven enormously successful due to its precision and affordability, but it is unsuitable for large-scale screening projects since it has been designed to sequence only one individually amplified DNA molecule at a time (2,10).

Conversely, massively parallel or "next-generation" sequencing has made it possible to generate large amounts of sequence data analyzed simultaneously, thus providing variant information down to single-base resolution in a rapid, cost-effective and high-throughput fashion on the scale of the whole human genome. Enrichment techniques by either solid or liquid capture hybridization or amplicon methods allow rapid isolation of the candidate regions of interest (ROIs) ranging from hundreds of kilobases in size to the entire protein-coding sequence of an individual (the "exome") (11).

. Briefly, NGS involves three fundamental steps: sample preparation, sequencing, and data analysis (12). Successful sequencing is highly dependent on the efficiency of the enrichment procedure during sample preparation, and depends also on the type of bases of the ROI.

Following sequencing, data analysis involves further steps, including base calling, read alignment, variant calling and a process of prediction of causality for the genetic variants identified, called "annotation". This analysis may include a search for the presence of the variant in public

This article is protected by copyright. All rights reserved.

databases and the degree of evolutionary conservation of the encoded amino acid, and an *in silico* prediction of whether the variant is pathogenic (due to its potential impact on protein function using computer-based algorithms) (13-15).

NGS PROTOCOLS: FROM SCENARIO-SPECIFIC PANELS TO WHOLE GENOME SEQUENCING

Targeted gene sequencing (TGS)

The use of targeted panels has the advantage to focus on a limited number of suspected diseases, has a better coverage of the genes of interest and is time and cost-saving when it comes to reporting (16). Similarly to Sanger sequencing, this technique is suitable as a confirmation of a clinical suspicion based on signs, symptoms and biochemical tests. In pediatric hepatology, TGS is particularly indicated to characterize genetic cholestatic disorders, most commonly presenting early in life with conjugated hyperbilirubinemia, or to identify different disease subtypes causing a similar metabolic derangement, such as glycogen storage disorders, mitochondrial disorders and non-alcoholic fatty liver disease (17-19). In defined conditions or syndromes, clinical features and biochemical markers can guide the investigation towards a specific pathway or group of genes responsible for a given phenotype. In these cases, a TGS analysis has proven efficient and cost-effective (20). Table 1 reports a list of common monogenic disorders of the liver that can be detected by targeted NGS techniques.

In our institution, we have developed a customized TGS panel that – at the time of writing – comprises the locuses of interest of 31 genes related to the 28 most common genetic disorders affecting the liver. The panel has been designed to include the most common monogenic liver diseases irrespective of the clinical phenotype, such as genetic cholestatic disorders, inborn errors of metabolism or defects causing chronic liver disease.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

Except from Alagille syndrome, all diseases included in our panel are inherited in an autosomal recessive manner, and are characterized by homozygous or double heterozygous mutations. For this reason, the availability of the DNA of the parents is essential to confirm that the two variants are located on different alleles (21).

Whole exome sequencing (WES)

Rapid progresses in capture designs have allowed all protein-coding regions to be sequenced without significant increase incosts. For this reason, interest is rapidly shifting towards WES.

The use of WES is recommended to improve the diagnostic yield in cases of an uncertain or compound phenotype, with a likely inheritable background but no clear pointers to the diagnosis.

A tight liaison between the laboratory geneticist and the clinician is necessary to ascertain the correct diagnosis from WES, that involves filtering of gene candidates, on the basis of phenotypic features disclosed by previous clinical investigations, and type of Mendelian inheritance ascertained from parents sequencing (22).

Clinical exome sequencing (CES)

In several cases WES does not allow to point to the genetic diagnosis, since most of generated data includes genes not yet implicated in human disease. In these cases, and especially when the clinical manifestations are not clear, CES represents a good alternative and a good compromise between cost, time and detection rate. This analysis includes all genes associated with known diseases (the “mendeliome”). The clinical exome used in our centre is the TruSight One (Illumina Inc, San Diego, US), that – at the time of writing – includes 4813 disease-genes reported in Human Gene Mutation Database (HGMD) and OMIM (Online Mendelian Inheritance in Man).

This article is protected by copyright. All rights reserved.

Accepted Article

Whole genome sequencing (WGS)

WGS sequences all bases in the genome – coding and non-coding regions – and offers a resolution that is not available with other sequencing methods (23). WGS has the advantage – over WES and TGS – of identifying three categories of variants: structural rearrangements of part of the genome – such as chromosomal translocations or inversions or dosage, otherwise visible only with karyotype or array-CGH; regulatory intronic regions; GC-rich regions, less efficiently amplified by PCR, thus better explored with the most recent PCR-free WGS.

INTERPRETATION OF THE RESULTS AND REPORTING

Each test performed by NGS generates thousands to millions of base pairs of DNA, and detects numerous variants, the number depending on the size of the ROIs (Figure 1). All these data must be filtered and interpreted, with the help of storage facilities, bioinformatic technologies and disease databases.

Classic genetic testing is usually performed to confirm a clinical phenotype linked to a known genetic disease,. However, the novel sequencing technology is often applied as a screening method, especially in unusual and clinically undefined cases. Not rarely in these patients a discrepancy between genetic test and clinical significance often occurs. For this reason, it is often helpful to have a hypothesis of the type of inheritance on the basis of the family history (autosomal dominant or recessive, sporadic condition or X-linked transmission), so that the NGS strategy and reporting can be tailored to the specific case.

Among pathogenic variants, those causing a stop codon or a truncating protein can be considered likely to be causative of a disease, whereas variants related to missense mutations (having variable effects on different proteins) are unpredictable as far as post-transcriptional residual function. Aspects supporting the pathogenicity of a variant causing a missense mutation are: 1) the previous

This article is protected by copyright. All rights reserved.

report in human genome databases; 2) minor allele frequency (MAF) in the general population (the higher the frequency the lower the probability to be causal in rare diseases) (24); 3) evidence of a causative role in published literature; 4) co-segregation with the disease within the same family; 5) de novo variant in a sporadic condition; 6) biostatistical prediction models.

In our center, after filtering, the remaining variants are classified in accordance to the most recent guidelines (25). Only variants associated with the patient phenotype that are present in HGMD Professional 2013.4 (https://portal.biobase international.com/hgmd/pro/start.php) and/or ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) are included in the final report as "pathogenic mutations" (15). Variants found in genes implicated in the patient phenotype and classified as potentially pathogenic by the American College of Medical Genetics (ACMG) that have not been previously described, or those present in HGMD without any functional validation, are reported as VUS (variants of unknown significance) needing further investigations (9,26).

LIMITATIONS OF NGS

Pitfalls of the technique can be technical and interpretative. Indeed, the amount of data (?) is not uniformly distributed on the analyzed region. The coverage, defined as the number of aligned reads that include a given nucleotide in the reconstructed sequence, represents the capability to discriminate between sequencing error and true variant. In general, the larger the number of genes the less accurate the coverage, the bigger the number of variants the more difficult the interpretation (Figure 1) (27).

With broader approaches, such as WES, previous experiences have shown incomplete capture of target regions, with 40% of targeted bases and 20% of known disease-causing sites resulting poorly covered (28). It is therefore clear that a critical phase in the whole exome or genome studies is the enrichment phase, that requires substantial technical improvements (29). Beside

This article is protected by copyright. All rights reserved.

Accepted Article

these technical issues, that are increasingly overcome, the interpretative challenges become more relevant, especially with the increase of the number of genes explored. WGS, more expensive and less feasible in daily practice, produces more than 100 Gb of data per single test, thus a major effort is required to perform bioinformatic analysis and store safely all gathered information (30). Most of the detected variants have not been definitely linked to a disease (VUS, variants of unknown significance) and therefore inevitable incidental findings may lead to divert from the diagnosis in absence of adequate interpretation. If a VUS is found in a gene whose implication is highly suspect for a certain clinical picture, further studies might be required to elucidate its role. These range from extensive studies of biochemical markers of the putative disease, to *in silico* prediction analysis, till relevant mRNA studies in case the mutation is thought to behave as a splice enhancer or silencer.

Finally, although exceedingly effective in identifying single nucleotide variants, substitution and insertion/deletion of few nucleotides, NGS is inherently inaccurate for others genomic variations, such as copy number, structural rearrangements (translocation and inversion) or partial rearrangements of a gene. Furthermore, the most commonly adopted NGS strategies are not able to detect triplet expansion or methylation status of a given genomic region.

THE BURDEN OF MONOGENIC LIVER DISEASES IN CHILDREN

Nearly half of chronic liver disorders presenting in childhood have a genetic cause and approximately 20% of LT in children are performed as a consequence of liver based MDs (9). If we exclude biliary atresia (the most frequent indication for pediatric LT) and autoimmune liver disease, most of the remaining conditions causing progressive serious liver disease in childhood are inheritable. It is therefore clear that, in this setting, a powerful instrument to discover

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

pathogenic variants bears a revolutionary power in the clinical management of a child presenting with liver disease (31).

Although most skilled clinicians would regard NGS as a very inelegant diagnostic tool, the opportunity to confirm the diagnosis with a simple peripheral blood sample, avoiding invasive procedures and complex biochemical tests is undoubtedly appealing. NGS entails a major shift in clinical approach to suspected genetic liver disease: from the classical scenario, in which hypotheses rise from clinical and biochemical data and lead to genetic confirmation, to a new one, in which candidate disease-associated variants are filtered and are matched with the clinical picture. A major advantage of the NGS in pediatric hepatology is that it overcomes possible laboratory pitfalls. In fact, in the subclinical/early phase, clinical and biochemical hallmarks may be absent or insufficient to make the diagnosis until a trigger (diet, oxidative stress, infections) produces a consistent (and sometimes irreversible) metabolic derangement (32). Similarly, at the other end of the spectrum, in a child with end stage liver disease or with acute decompensation, looking for a specific metabolite pattern can be tricky; in fulminant liver failure, genetic testing may represent the only way to achieve a diagnosis of a metabolic condition (33). NGS will never replace biochemical and enzymatic tests in the management of MDs, but it may act as a bundle tool able to provide a fast and reliable diagnosis, especially in unclear phenotypic presentations. Reciprocal confirmation between genetic and biochemical diagnosis is recommended, when feasible. Table 2 reports some clinical hints to the suspicion of MDs in patients presenting with or having an on-going liver disease.

USEFULNESS OF NGS IN PEDIATRIC HEPATOLOGY/LIVER TRANSPLANT CENTERS

Clinical scenario: cholestasis of infancy

This article is protected by copyright. All rights reserved.

Accepted Article

Cholestatic liver disease affects approximately 1/2500 term infants and is most commonly due to biliary atresia (up to 40% of cases) (34). In the past, up to 60% of infants with neonatal cholestasis and a histological pattern of giant cell hepatitis were classified as "idiopathic neonatal hepatitis". This term is now used much less frequently because modern tools allow to recognize specific genetic syndromes; as a consequence, the rate of infants diagnosed with "neonatal idiopathic cholestasis" has dropped to less than 20% of all infants presenting with conjugated jaundice (35). Conversely – in the early NGS era – a genetic cause is being identified in about 40% of the children with intrahepatic (non-biliary atresia) cholestasis; thus this technique represents a very powerful tool to recognize these disorders, expected to further reduce the percentage of "idiopathic" cases. Genetic causes of cholestasis are depicted in Table 1.

Progressive familial intrahepatic cholestasis (PFIC) – a group of disorders caused by a disturbance of the bile acid transport through the canalicular membrane – accounts for more than 10% of the children with neonatal cholestasis. PFICs are almost uniquely associated with a low gamma-glutamyl transpeptidase (GGT) serum level (only the rare inborn errors of bile acid synthesis/conjugation – having the hallmark of low/normal serum primary bile acids – share this feature), and are indistinguishable on clinical ground one from the other (36-40). The most important value of applying NGS in the field of early onset cholestasis is the opportunity to rapidly identify the defect causing *low-GGT PFICs* (*PFIC1, PFIC2, TJP2-related cholestasis* and the recently discovered *Myosin VB-associated cholestasis*) (41,42). Indeed each defect bears the risk of different complications before and after transplantation (such as severe post-LT diarrhea, steatosis and graft loss in *PFIC1*, and the risk of HCC in the native liver or recurrent immune-mediated disease after LT in *PFIC2*) (43-46). Thus, genetic characterization is the crossroad for a decision-making about possible medical, surgical bile acid lowering treatment (e.g. biliary diversion) or towards transplantation.

This article is protected by copyright. All rights reserved.

Accepted Article

The diagnosis of other MDs presenting with infantile cholestasis may be very difficult without genetic testing. Some examples are given by patients with bile acid conjugation defects (such as *familial hypercholanemia* due to Bile acid-CoA:amino acid N-acyltransferase [BAAT] deficiency) having a defect of amidation of primary bile acid that requires mass spectrometry or specific liver immunostaining to be revealed (47). *Citrin deficiency* (neonatal-onset type II citrullinemia), may present clinically with prolonged jaundice and failure to thrive but with otherwise non-specific histology and liver tests, and a normal serum amino acid profile. In all these patients NGS has proven a very effective tool to disclose rare types of genetic cholestasis (48).

Alagille syndrome (AS) is commonly diagnosed on clinical ground, based on historical clinical criteria described by Alagille in 1975 (49). Almost all the genetically-confirmed cases in our experience had previously been suspected (data not published). Nevertheless, due to the variable penetrance of this autosomal dominant condition, AS has a wide spectrum of clinical disease expression and severity; for this reason, many such patients not fulfilling the classical clinical criteria remain undiagnosed, unless JAG1 and NOTCH2 disease-causing variations are regularly looked for (50). Being aware of such condition before LT is offered is important, since it allows to identify and possibly treat coexisting pulmonary artery stenosis or intracranial vascular malformations at risk of potentially devastating complications (51). Remarkably, 5-7% of AS patients have a partial or complete deletion of JAG1, which may not be detected by NGS. Thus, when the clinical suspicion is high and NGS is not diagnostic, a more specific test for structural rearrangements should be performes. The Multiplex Ligation-dependent Probe Amplification (MLPA) is in some way complementary to NGS in these cases: inexpensive and straightforward, it uses pairs of probe oligonucleotides designed so that the amplification product depends on the number of target sites present in the sample DNA. This makes the technique highly reliable in detecting copy number variations of DNA regions and single genes or part of them: in a child with

This article is protected by copyright. All rights reserved.

typical AS phenotype seen at our Unit in which our TGS was negative, MLPA showed an amplification pattern of hemizygous deletion of the whole JAG1, which confirmed the diagnosis.

In some inborn errors of metabolism causing severe liver disease early after birth, NGS overcomes the problem of securing the diagnosis in patients already started on a substrate-free diet. This is especially the case of *galactosemia,* in which the marker metabolite rapidly disappears and a lactose challenge is harmful (52).

Lysosomal storage disorders (such as *Niemann Pick Type B and C, Gaucher disease, Lysosomal acid lipase deficiency*) are hallmarked by splenomegaly, and can present with neonatal cholestasis (53). Although the standard diagnosis is usually made by enzymatic testing, this type of assay is not widely available and need access to a specialized laboratory, with some time lapse before the diagnosis can be made. NGS may not offer faster results, but has the advantage to make the diagnosis and define the genotypic signature of the condition (often corresponding to different predicted outcomes), and can be particularly useful to the pediatric hepatologist as part of a NGS panel for patients presenting early in life with cholestasis (54).

Finally, it is in alpha-1 antitrypsin (A1AT) deficiency (A1ATD) that genetic testing has a limited role. Since the disease can present early in life with clinical and histological features mimicking biliary atresia it should be ruled out before performing a liver biopsy and a direct cholangiography. A borderline or frankly low serum A1AT level is generally a good pointer to the diagnosis.. In unclear cases, A1ATD genetic signature should be therefore obtained either by isoelectrofocusing (giving the detailed A1AT phenotype) or genotyping (55).

At our institution, infants with conjugated hyperbilirubinemia undergo a stepwise evaluation in which a NGS expanded panel for genetic liver diseases (*Bergamo Liver Panel*, genes indicated with an asterisk in Table 1) acquires a pivotal role to the diagnosis. Figure 2 illustrates this novel approach, which is currently under evaluation in a prospective study. In this algorithm, that should

This article is protected by copyright. All rights reserved.

Accepted Article

be regarded as a local choice, stool color, liver biopsy pattern and GGT level are the main crossroads, whereas intraoperative cholangiogram and NGS lead to the conclusive diagnosis. Preliminary results show that – in this pediatric LT setting – the detection rate of genetic causes of infantile cholestasis is above 50%, and that NGS is cost-effective when compared with traditional stepwise diagnostic approached based on clinical and biochemical pointers (data not published).

Clinical scenario: acute liver failure in children

The cause of acute liver failure (ALF) in children remains indeterminate in approximately half of patients. An underlying MD causing severe metabolic derangements is identified in 10-28% of children with ALF (56,57), largely depending on the diagnostic capabilities of the center. Genetic causes of ALF and possible clinical and biochemical features are depicted in Figure 3. The earlier the presentation of ALF, the higher the suspicion for an inherited metabolic disease (IMD) should be maintained. The management of a child with ALF is challenging. Children with ALF are often listed for LT before a diagnosis is achieved. Reaching a timely diagnosis, however, is of great importance in this scenario, because IMD may respond to medical treatment, and – conversely – some of them represent relative contraindications to LT (58,59). The importance of a rapid genetic diagnosis is even greater considering that – during acute decompensation – appropriate biochemical and histological evaluations can be unfeasible or ineffective because of the patient instability and the numerous confounders produced by severe organ injury. In this setting, in the next future NGS will probably represent a pillar in the management of ALF, effectively identifying underlying IMDs, provided the turnaround time is fast enough to impact on decision to transplant or not.

The genetic causes of ALF are *galactosemia*, *fructosemia*, *mitochondrial depletion syndromes (MDSs)*, *tyrosinemia type 1*, rarely forms of congenital glycosylation defect (*CDG, especially CDG-*

This article is protected by copyright. All rights reserved.

Accepted Article

1a) and of *fatty acids oxidation defects, often presenting with a Reye-like picture*; in males in the post-neonatal period, *ornithine transcarbamylase deficiency* should be considered. In older children, a relatively common cause is *Wilson disease*.

Galactosemia and *fructosemia* can present as cholestatic liver disease during infancy beside ALF. These diseases respond dramatically to the substrate-free diet (galactose and fructose respectively), but challenging a fragile infant with these sugars to make the definite diagnosis is unadvisable.

Tyrosinemia type 1 is easily diagnosed observing the plasma amino acid profile and urine succinylacetone, and usually genetic testing is performed only as a confirmation.

Mitochondrial disorders (MDSs) are mainly related to mutations in genes involved in the respiratory chain, having heterogeneous phenotypes, most commonly characterized by a defect of cellular energy production. As such, they are systemic diseases in which organ transplantation is generally contraindicated. Those presenting with liver involvement are related to nuclear gene products (inherited with autosomal recessive pattern in 90%, and autosomal dominant and X-linked pattern in 10%), responsible for mitochondrial DNA replication *(POLG1)*, maintenance of deoxyribonucleoside triphosphate (dNTP) pools *(DGUOK)* and membrane mitochondrial integrity *(MPV17)* (60,61). Therefore, mutations in these genes cause mitochondrial DNA depletion, characterized by hepato-cerebral forms inherited with an autosomal recessive pattern. In *Alpers syndrome* (due to mutations in POLG1) sodium valproate administered because of intractable seizures often triggers acute liver decompensation and death. The histological changes on liver biopsy include fatty degeneration, bile duct proliferation, fibrosis, and lobular collapse (62), but NGS warrants a rapid confirmation of the diagnosis, which is therefore relevant for the management of these patients.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

Undoubtedly, the wide clinical and biochemical heterogeneity of MDSs represents a diagnostic challenge. The complex contribution of nuclear and mitochondrial genes on mitochondrial function and integrity, associated with the occurrence of heteroplasmy (random mixture of mitochondria with normal and mutant DNA in different tissues) makes the clinical phenotype unpredictable. Enzymatic assays on muscle biopsy are time-consuming, while a low enzymatic activity on liver biopsy could be secondary. Nevertheless, TGS can be very effective in the suspicion of such defects, since these are caused by mutations in very few genes (63). Remarkably, NGS helps to discriminate cases of acutely ill children in which mitochondrial DNA depletion was secondary to acute liver injury (19). Genetic information, neurologic status and ethical considerations should be part of the evaluation of these children.

A complete list of the genes associated with ALF is in Table 1.

CONCLUSIONS

. NGS allows the detection of a large number of variants in clusters of genes, and is becoming a widely available and standardized technique that, combined with biochemical data and phenotype examination, may rapidly lead to the correct diagnosis of all known monogenic liver diseases. NGS will never replace biochemical testing that is central to establish the clinical phenotype and guide the patient management and follow up, but represents a great tool to make the diagnosis in difficult situations, providing information on genotype-phenotype match, offering diagnostic confirmation to the clinical picture.

Its use may reduce the time to diagnosis, the number of invasive tests or procedures, facilitate decision making, fill the gap of the laboratory diagnostics in certain situations. With the growing adoption of CES, WES and WGS, in the next years it will be important to optimize and standardize a diagnostic procedure that combines clinical phenotype, genetic testing and biochemical tests.

This article is protected by copyright. All rights reserved.

.

Several challenges remain to be faced with this new instrument, such as the choice of NGS techniques in different clinical scenarios, the selection criteria to test a patient, the filtering strategy in WES and WGS, and how to report negative results or VUS. Nevertheless, it is likely that, due to cost-effectiveness, in the future whole genome sequencing will replace targeted sequencing, but only selected "virtual" panels of genes, chosen depending on the patient phenotype, will be analysed; this will offer the possibility to assess the bioinformatically stored data of the entire exome/genome at a later stage.

The inevitable inclusion of NGS in diagnostic algorithms will lead to a new era in medicine, changing our approach to the patient as well as our understanding of the genotype-phenotype match in monogenic disorders.

REFERENCES

1. Antonarakis SE, Beckmann JS. Mendelian disorders deserve more attention. Nat Rev Genet. 2006;7:277–282.
2. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 1977;74:5463–5467.
3. Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet. 2010;11:31–46.
4. Stranneheim H, Wedell A. Exome and genome sequencing: a revolution for the discovery and diagnosis of monogenic disorders. J Intern Med. 2016;279:3–15.
5. Kuhlenbäumer G, Hullmann J, Appenzeller S. Novel genomic techniques open new avenues in the analysis of monogenic disorders. Hum Mutat. 2011;32:144–151.
6. Bush WS, Oetjens MT, Crawford DC. Unravelling the human genome-phenome relationship using phenome-wide association studies. Nat Rev Genet. 2016;17:129–145.
7. Mann DA. Epigenetics in liver disease. Hepatology. 2014;60:1418–1425.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

8. Nicastro E, Loudianos G, Zancan L, D'Antiga L, Maggiore G, Marcellini M, et al. Genotype-phenotype correlation in Italian children with Wilson's disease. J. Hepatol. 2009;50:555–561.
9. Sze YK, Dhawan A, Taylor RM, Bansal S, Mieli-Vergani G, Rela M, et al. Pediatric Liver Transplantation for Metabolic Liver Disease: Experience at King's College Hospital. Transplantation. 2009;87:87–93.
10. Collins FS, Morgan M, Patrinos A. The Human Genome Project: lessons from large-scale biology. Science. 2003;300:286–290.
11. Couderc R, Jonard L, Louha M. Next generation sequencing: the technology we need in pediatric laboratories? Clin Biochem. 2011;44:514–515.
12. Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, et al. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. 2013;15:733–747.
13. Ng SB, Nickerson DA, Bamshad MJ, Shendure J. Massively parallel sequencing and rare disease. Hum Mol Genet. 2010;19:R119–24.
14. Brookes AJ, Robinson PN. Human genotype-phenotype databases: aims, challenges and opportunities. Nat Rev Genet. 2015;16:702–715.
15. Christenhusz GM, Devriendt K, Van Esch H, Dierickx K. Focus group discussions on secondary variants and next-generation sequencing technologies. Eur J Med Genet. 2015;58:249–257.
16. Kingsmore SF, Saunders CJ. Deep sequencing of patient genomes for disease diagnosis: when will it become routine? Sci Transl Med. 2011;3:87ps23–87ps23.
17. Wang J, Cui H, Lee N-C, Hwu W-L, Chien Y-H, Craigen WJ, et al. Clinical application of massively parallel sequencing in the molecular diagnosis of glycogen storage diseases of genetically heterogeneous origin. Genet Med. 2013;15:106–114.
18. Togawa T, Sugiura T, Ito K, Endo T, Aoyama K, Ohashi K, et al. Molecular Genetic Dissection and Neonatal/Infantile Intrahepatic Cholestasis Using Targeted Next-Generation Sequencing.

This article is protected by copyright. All rights reserved.

Accepted Article

J Pediatr. 2016;171:171–7.e1–4.

19. McKiernan P, Ball S, Santra S, Foster K, Fratter C, Poulton J, et al. Incidence of Primary Mitochondrial Disease in Children Younger Than 2 Years Presenting With Acute Liver Failure. J Pediatr Gastroenterol Nutr. 2016;63:592–597.
20. Jones MA, Rhodenizer D, da Silva C, Huff IJ, Keong L, Bean LJH, et al. Molecular diagnostic testing for congenital disorders of glycosylation (CDG): detection rate for single gene testing and next generation sequencing panel testing. Mol Genet Metab. 2013;110:78–85.
21. Sakai R, Sifrim A, Vande Moere A, Aerts J. TrioVis: a visualization approach for filtering genomic variants of parent-child trios. Bioinformatics. 2013;29:1801–1802.
22. Rabbani B, Mahdieh N, Hosomichi K, Nakaoka H, Inoue I. Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. J. Hum. Genet. 2012;57:621–632.
23. Jarinova O, Ekker M. Regulatory variations in the era of next-generation sequencing: implications for clinical molecular diagnostics. Hum Mutat. 2012;33:1021–1030.
24. Derkach A, Chiang T, Gong J, Addis L, Dobbins S, Tomlinson I, et al. Association analysis using next-generation sequence data from publicly available control groups: the robust variance score statistic. Bioinformatics. 2014;30:2179–2188.
25. Matthijs G, Souche E, Alders M, Corveleyn A, Eck S, Feenstra I, et al. Guidelines for diagnostic next-generation sequencing. Eur. J. Hum. Genet. 2016;24:2–5.
26. May T, Zusevics KL, Strong KA. On the ethics of clinical whole genome sequencing of children. Pediatrics. 2013;132:207–209.
27. Rizzo JM, Buck MJ. Key principles and clinical applications of “next-generation” DNA sequencing. Cancer Prev Res (Phila). 2012;5:887–900.
28. Sulonen A-M, Ellonen P, Almusa H, Lepistö M, Eldfors S, Hannula S, et al. Comparison of

This article is protected by copyright. All rights reserved.

Accepted Article

solution-based exome capture methods for next generation sequencing. Genome Biol. 2011;12:R94.

29. Niroula A, Vihinen M. Variation Interpretation Predictors: Principles, Types, Performance, and Choice. Hum Mutat. 2016;37:579–597.
30. Belkadi A, Bolze A, Itan Y, Cobat A, Vincent QB, Antipenko A, et al. Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. Proc Natl Acad Sci USA. 2015;112:5473–5478.
31. Fagiuoli S, Daina E, D'Antiga L, Colledan M, Remuzzi G. Monogenic diseases that can be cured by liver transplantation. J. Hepatol. 2013;59:595–612.
32. Blok MJ, van den Bosch BJ, Jongen E, Hendrickx A, de Die-Smulders CE, Hoogendijk JE, et al. The unfolding clinical spectrum of POLG mutations. J Med Genet. 2009;46:776–785.
33. Sallie R. Failure of simple biochemical indexes to reliably differentiate fulminant Wilson's disease from other causes of fulminant liver failure. Hepatology. 1992;16:1206–1211.
34. Fawaz R, Baumann U, Ekong U, Fischler B, Hadzic N, Mack CL, et al. Guideline for the Evaluation of Cholestatic Jaundice in Infants: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr. 2017;64:154–168.
35. Balistreri WF, Bezerra JA. Whatever happened to "neonatal hepatitis"? Clin Liver Dis. 2006;10:27–53– v.
36. Balistreri WF, Bezerra JA, Jansen P, Karpen SJ, Shneider BL, Suchy FJ. Intrahepatic cholestasis: summary of an American Association for the Study of Liver Diseases single-topic conference. Wiley Subscription Services, Inc., A Wiley Company; 2005. p. 222–235.
37. Lu F-T, Wu J-F, Hsu H-Y, Ni Y-H, Chang M-H, Chao C-I, et al. γ-Glutamyl transpeptidase level

This article is protected by copyright. All rights reserved.

Accepted Article

as a screening marker among diverse etiologies of infantile intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 2014;59:695–701.

38. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. Progressive familial intrahepatic cholestasis. Orphanet journal of rare diseases. 2009;4:1.

39. Sambrotta M, Strautnieks S, Papouli E, Rushton P, Clark BE, Parry DA, et al. Mutations in TJP2 cause progressive cholestatic liver disease. Nat. Genet. 2014;46:326–328.

40. Riello L, D'Antiga L, Guido M, Alaggio R, Giordano G, Zancan L. Titration of bile acid supplements in 3beta-hydroxy-Delta 5-C27-steroid dehydrogenase/isomerase deficiency. J Pediatr Gastroenterol Nutr. 2010;50:655–660.

41. Gomez-Ospina N, Potter CJ, Xiao R, Manickam K, Kim M-S, Kim KH, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. Nat Commun. 2016;7:10713.

42. Qiu Y-L, Gong J-Y, Feng J-Y, Wang R-X, Han J, Liu T, et al. Defects in myosin VB are associated with a spectrum of previously undiagnosed low γ-glutamyltransferase cholestasis. Hepatology. 2017;65:1655–1669.

43. Knisely AS, Strautnieks SS, Meier Y, Stieger B, Byrne JA, Portmann BC, et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. Hepatology. 2006;44:478–486.

44. Nicastro E, Stephenne X, Smets F, Fusaro F, de Magnée C, Reding R, et al. Recovery of graft steatosis and protein-losing enteropathy after biliary diversion in a PFIC 1 liver transplanted child. Pediatr Transplant. 2012;16:E177–82.

45. Jara P, Hierro L, Martínez-Fernández P, Alvarez-Doforno R, Yánez F, Diaz MC, et al. Recurrence of bile salt export pump deficiency after liver transplantation. N Engl J Med. 2009;361:1359–1367.

This article is protected by copyright. All rights reserved.

Accepted Article

46. Shneider BL. Liver transplantation for progressive familial intrahepatic cholestasis: the evolving role of genotyping. Liver Transpl. 2009;15:565–566.

47. Hadzic N, Bull LN, Clayton PT, Knisely AS. Diagnosis in bile acid-CoA: amino acid N-acyltransferase deficiency. World J. Gastroenterol. 2012;18:3322–3326.

48. Herbst SM, Schirmer S, Posovszky C, Jochum F, Rödl T, Schroeder JA, et al. Taking the next step forward - Diagnosing inherited infantile cholestatic disorders with next generation sequencing. Mol. Cell. Probes. 2015;29:291–298.

49. Alagille D, Odièvre M, Gautier M, Dommergues JP. Hepatic ductular hypoplasia associated with characteristic facies, vertebral malformations, retarded physical, mental, and sexual development, and cardiac murmur. YMPD. 1975;86:63–71.

50. Li L, Dong J, Wang X, Guo H, Wang H, Zhao J, et al. JAG1 Mutation Spectrum and Origin in Chinese Children with Clinical Features of Alagille Syndrome. PLoS ONE. 2015;10:e0130355.

51. Emerick KM, Krantz ID, Kamath BM, Darling C, Burrowes DM, Spinner NB, et al. Intracranial vascular abnormalities in patients with Alagille syndrome. J Pediatr Gastroenterol Nutr. 2005;41:99–107.

52. Mckiernan PJ. Neonatal cholestasis. Semin Neonatol. 2002;7:153–165.

53. Yerushalmi B, Sokol RJ, Narkewicz MR, Smith D, Ashmead JW, Wenger DA. Niemann-pick disease type C in neonatal cholestasis at a North American Center. J Pediatr Gastroenterol Nutr. 2002;35:44–50.

54. Gotti G, Marseglia A, De Giacomo C, Iascone M, Sonzogni A, D'Antiga L. Neonatal Jaundice with Splenomegaly: Not a Common Pick. Fetal Pediatr Pathol. 2016;35:108–111.

55. Snyder MR, Katzmann JA, Butz ML, Wiley C, Yang P, Dawson DB, et al. Diagnosis of alpha-1-antitrypsin deficiency: An algorithm of quantification, genotyping, and phenotyping. Clin Chem. 2006;52:2236–2242.

This article is protected by copyright. All rights reserved.

Accepted Article

56. Squires RH, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. YMPD. 2006;148:652–658.

57. Hegarty R, Hadzic N, Gissen P, Dhawan A. Inherited metabolic disorders presenting as acute liver failure in newborns and young children: King's College Hospital experience. Eur J Pediatr. 2015;174:1387–1392.

58. Gallagher RC, Lam C, Wong D, Cederbaum S, Sokol RJ. Significant hepatic involvement in patients with ornithine transcarbamylase deficiency. J Pediatr. 2014;164:720–725.e6.

59. Staufner C, Haack TB, Köpke MG, Straub BK, Kölker S, Thiel C, et al. Recurrent acute liver failure due to NBAS deficiency: phenotypic spectrum, disease mechanisms, and therapeutic concepts. J Inherit Metab Dis. 2016;39:3–16.

60. Cui H, Li F, Chen D, Wang G, Truong CK, Enns GM, et al. Comprehensive next-generation sequence analyses of the entire mitochondrial genome reveal new insights into the molecular diagnosis of mitochondrial DNA disorders. Genet Med. 2013;15:388–394.

61. Dames S, Chou L-S, Xiao Y, Wayman T, Stocks J, Singleton M, et al. The development of next-generation sequencing assays for the mitochondrial genome and 108 nuclear genes associated with mitochondrial disorders. J Mol Diagn. 2013;15:526–534.

62. Spinazzola A, Invernizzi F, Carrara F, Lamantea E, Donati A, Dirocco M, et al. Clinical and molecular features of mitochondrial DNA depletion syndromes. J Inherit Metab Dis. 2009;32:143–158.

63. Menezes MJ, Riley LG, Christodoulou J. Mitochondrial respiratory chain disorders in childhood: insights into diagnosis and management in the new era of genomic medicine. Biochim. Biophys. Acta. 2014;1840:1368–1379.

This article is protected by copyright. All rights reserved.

Accepted Article

FIGURE LEGEND

Figure 1

Total number of variants (SNPs and indels) upon step-wise expansion of analysed genomic portions, expressed as base pairs and sequencing strategy (from targeted genes to whole genome). In the bottom part of the figure it is shown the correlation among the platform throughput, the technical accuracy and the interpretative complexity. While the technical accuracy decreases, the amount of data and its complexity raise exponentially. SNV: single nucleotide variant.

Figure 2

Algorithm adopted in Bergamo for the diagnostic work up of cholestatic infants. A1ATD: alpha-1 antitrypsin deficiency. CF: cystic fibrosis. GGT: gamma-glutamyl transpeptidase. NICCD: neonatal idiopathic cholestasis due to citrin deficiency. PFIC: progressive familial intrahepatic cholestasis. NP-C: Niemann-Pick type C disease. ARC: arthrogryposis renal dysfunction and cholestasis syndrome. TALDO: transaldolase deficiency. *In infants with acholic stools A1AT is tested in serum before Kasai portoenterostomy, whereas its genetic testing is included in the NGS panel we use in children with cholic stools.

Figure 3

Genetic causes of acute liver failure and possible clues for their identification. ALF: acute liver failure; Tyr: tyrosinaemia; Galactos: galactosaemia; FAO: fatty acid oxidation defects; HFI: hereditary fructose intolerance.

This article is protected by copyright. All rights reserved.

Accepted Article

Table 1. Most common liver based monogenic disorders

Disease name	Defective protein	OMIM	Gene	Locus	Pointers to the diagnosis
NEONATAL/INFANTILE CHOLESTASIS					
Progressive familial intrahepatic cholestasis type 1 (PFIC 1)	FIC1	211600	ATP8B1*	18q21.31	-Low GGT -Short stature -Diarrhoea
Progressive familial intrahepatic cholestasis type 2 (PFIC 2)	Bile Salt Export Pump (BSEP)	601847	ABCB11*	2q31.1	-Low GGT
Progressive familial intrahepatic cholestasis type 3 (PFIC 3)	Multidrug resistance protein 3	602347	ABCB4*	7q21.12	-High GGT -Early portal hypertension
Progressive, familial intrahepatic cholestasis type 4 (PFIC 4)	Tight Junction Protein 2	615878	TJP2*	9q21.11	-Low GGT
Progressive, familial intrahepatic cholestasis type 5 (PFIC 5)	Farnesoid X-activated receptor	617049	NR1H4	12q23.1	-Low GGT
Myosin VB-	Myosin VB	606540	MYO5B	18q21.1	-Low GGT

This article is protected by copyright. All rights reserved.

associated cholestasis					
Alagille syndrome	Jagged-1, Notch2	118450 610205	JAG1* NOTCH2*	20p12.2 1p12	-High GGT -Peculiar face -Bile duct paucity
Alpha-1 antitrypsin deficiency	Alpha-1-antitrypsin	613490	SERPINA1*	14q32.13	-Low serum A1AT - Steatosis
Bile acid synthesis defects: Δ4-3-oxosteroid-5β-reductase deficiency	Δ4-3-oxosteroid-5β-reductase	235555	AKR1D1*	7q33	-Low GGT -Normal serum primary bile acids -No itching
Bile acid synthesis defects: 3β-hydroxy- Δ5-C27-steroid dehydrogenase deficiency (3βHSD)	3β-hydroxy- Δ5-C27-steroid dehydrogenase	607765	HSD3B7*	16p11.2	-Low GGT -Normal serum primary bile acids -No itching
Bile acid synthesis defects: Oxysterol 7α-hydroxylase deficiency	Oxysterol 7α-hydroxylase	613812	CYP7B1*	8q12.3	-Low GGT -Normal serum primary bile acids -No itching
Familial hypercholanemia due to amidation defects (BAAT)	-Bile acid-CoA:amino acid N-acyltransferase	602938	BAAT*	9q31.1	-Low GGT -High serum primary bile acids (unconjugated) -Itching
Arthrogryposis-renal	VPS33B protein VIPAR protein	208085 613404	VPS33B*, VIPAR*	15q26.1 14q24.3	-Low GGT -Arthrogryposis

This article is protected by copyright. All rights reserved.

dysfunction-cholestasis (ARC) syndrome					
Transaldolase deficiency	TALDO	606003	TALDO1*	11p15.5	-Low GGT -Hepatosplenomegaly
Neonatal sclerosing cholangitis (NISCH)	Claudin-1	607626	CLDN1	3q28	-Neonatal sclerosing cholangitis -Ichthyosis
Neonatal sclerosing cholangitis	Doublecortin Domain-containing Protein 2	605755	DCDC2	6p22.3	-Neonatal sclerosing cholangitis
Citrin deficiency (NICCD)	CMC2	605814	SLC25A13*	7q21.3	-Mild cholestasis, fatty changes -Elevated citrullin -Resolves by 6-12 months with MCT-based formula
Niemann Pick A,B	SMPD1	257200 607616	SMPD1*		-Early hepatosplenomegaly -Progressive developmental delay -Pulmonary involvement
Niemann Pick C	NPC1 NPC2	257220 607625	NPC1* NPC2*	18q11.2 14q24.3	-Early hepatosplenomegaly -Progressive developmental delay
Gaucher disease	Glucosylceramidase	230800	GBA*	1q22	-Early hepatosplenomegaly -Pancytopenia

This article is protected by copyright. All rights reserved.

Accepted Article

CHRONIC LIVER DISEASE AND/OR PORTAL HYPERTENSION					
Congenital hepatic fibrosis	Fibrocystin	263200	ARPKD*	6p12.3-p12.2	-Hepatosplenomegaly -Polycystic kidney -Normal liver function
LAL-deficiency (mild form)	Lysosomal acid lipase	278000	LIPA*	10q23.31	-Microvescicular steatosis -Raised cholesterol level
Congenital defects of glycosylation 1b	Phosphomannose isomerase	602579	MPI*	15q24.1	-Chronic diarrhea -Low antithrombin III -Non cirrhotic portal hypertension -Normal mental status
Wilson disease	Copper-transporting ATPase 2	277900	ATP7B*	13q14.3	-Low ceruloplasmin -Fatty liver -Neurologic symptoms later in life
ACUTE LIVER FAILURE					
Galactosemia	GAL-1-PUT	230400	GALT*	9p13.3	-Triggered by galactose in diet -Hypoglycaemia -E.Coli sepsis -Renal tubular dysfunction
Tyrosinemia	Fumarylacetoacetase	276700	FAH*	15q25.1	-Triggered by tyrosine in diet

This article is protected by copyright. All rights reserved.

					-Renal tubular dysfunction -Rickets -Early cirrhosis and HCC
Hereditary fructose intolerance	Fructose-bisphosphate aldolase B	229600	ALDOB*	9q31.1	-Triggered by fructose/sucrose in diet -Hypoglycaemia -Vomiting, shock
MCAD deficiency	Medium-chain specific acyl-CoA dehydrogenase	201450	ACADM*	1p31.1	-Reye-like presentation -Fatty liver
Mitochondrial DNA Depletion sdr (POLG)	DNA polymerase subunit gamma-1	203700	POLG*	15q26.1	-Intractable epilepsy -Triggered by valproate -Severe encephalopathy, lactic acidosis
Mitochondrial DNA Depletion sdr (DGUOK)	Deoxyguanosine kinase	251880	DGUOK*	2p13.1	-Hypoglycaemia -Neurological involvement -Lactic acidosis
Mitochondrial DNA Depletion sdr (MPV17)	Mpv17	256810	MPV17*	2p23.3	-Hypoglycaemia -Neurological involvement
Ornithine transcarbamylase (OTC) deficiency	Ornithine carbamoyltransferase	311250	OTC*	Xp11.4	-Male subjects -Hyperammonemia -Encephalopathy
Recurrent acute liver failure	Neuroblastoma-amplified sequence	616483	NBAS	2p24.3	-Triggered by fever -Recurrent ALF

This article is protected by copyright. All rights reserved.

(RALF)	episodes

*genes included in the targeted NGS panel at our Institution.

Accepted Article

This article is protected by copyright. All rights reserved.

Table 2: Pointers to the diagnosis of genetic liver disease

Medical history (consanguinity, previous affected siblings, dietary triggers, provocative symptoms, hypoglycaemia, acidosis, neurological symptoms, rhabdomyolysis)	Tyrosinaemia, Galactosaemia, Fructosaemia, Urea cycle defects, Fatty acid oxidation defects, Mitochondrial cytopathies
Hydrops fetalis	Lysosomal storage disorders, Wolman disease, Congenital defects of glycosilation
Neonatal cholestasis	A1ATD, PFIC, Galactosaemia, Tyrosinaemia, Niemann Pick C, Inborn errors of bile acid, Cystic fibrosis, Ciliopathies
Cholestasis with low GGT	PFIC 1, 2 and 4, Bile acid defects, ARC, TALDO
Fatty liver early in life	Hereditary fructose intolerance, fatty acid oxidation defects, LAL deficiency, mitochondrial cytopathies
Isolated hepatomegaly	Glycogen storage disease, LAL deficiency, Fatty acid oxidation defects, Mucopolysaccharidosis
Hepatosplenomegaly	All lysosomal storage disorders, congenital hepatic fibrosis
Neonatal cholestasis with splenomegaly	Niemann Pick C, Gaucher disease
Chronic liver disease	A1ATD, Wilson disease, Glycogen Storage Disease 3 and 4
Liver failure	Tyrosinaemia, Galactosaemia, Fructosaemia, Urea cycle defects, Fatty acid ox defects, Wilson disease, mitochondrial cytopathies

A1ATD: alpha-1 antitrypsin deficiency. CF: cystic fibrosis. GGT: gamma-glutamyl transpeptidase. NICCD: neonatal idiopathic cholestasis due to citrin deficiency. PFIC: progressive familial intrahepatic cholestasis. NP-C: Niemann-Pick type C disease. ARC: arthrogryposis renal dysfunction and cholestasis syndrome. TALDO: transaldolase deficiency. LAL: lysosomal acid lipase

This article is protected by copyright. All rights reserved.

Accepted Article

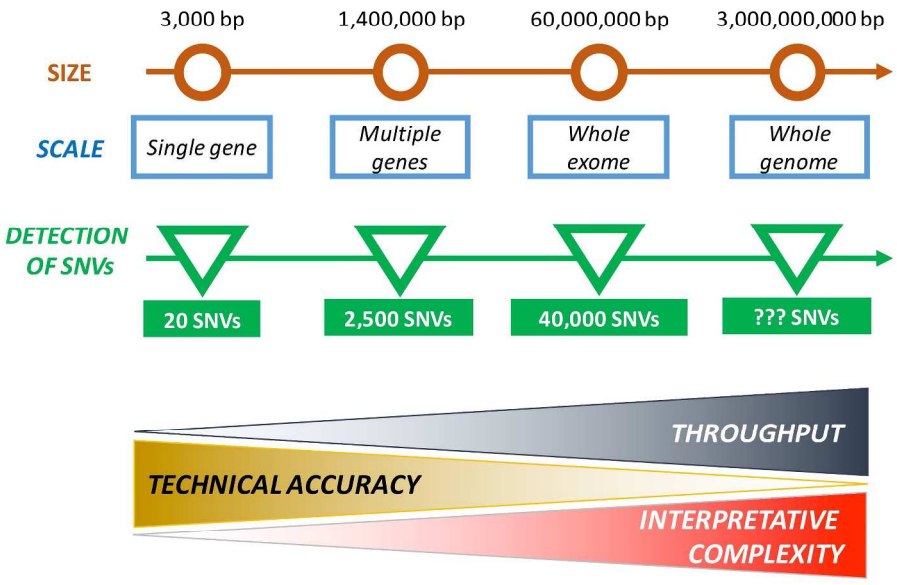

Figure 1

600x424mm (300 x 300 DPI)

Accepted

This article is protected by copyright. All rights reserved.

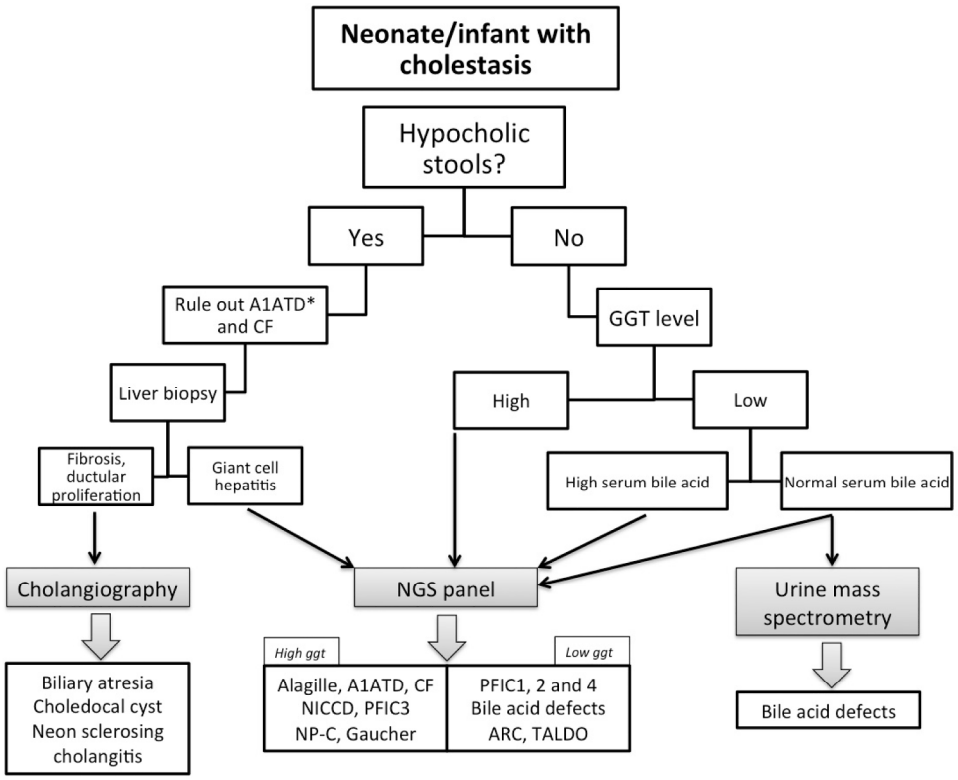

Figure 2

600x469mm (300 x 300 DPI)

Accepted

This article is protected by copyright. All rights reserved.

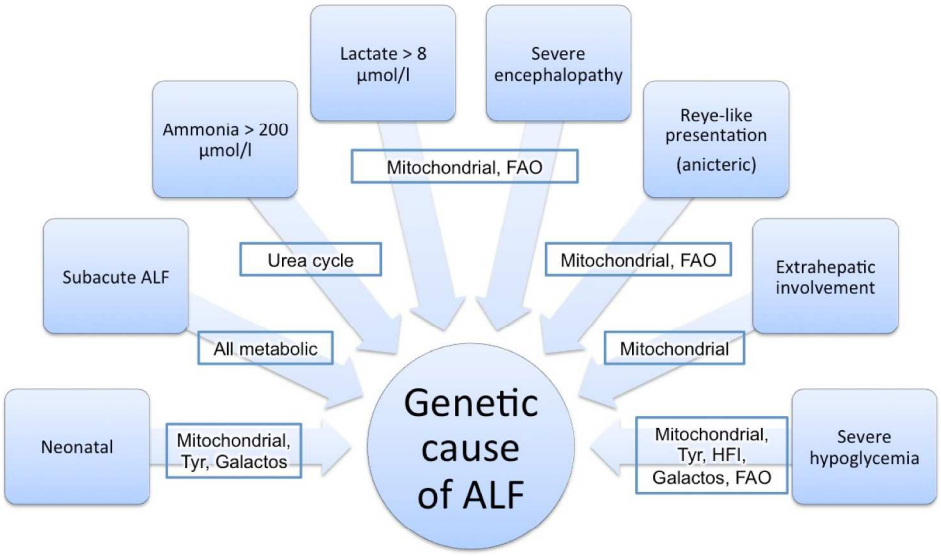

Figure 3

600x424mm (300 x 300 DPI)

Accepted

This article is protected by copyright. All rights reserved.