Metabolic-Associated Fatty Liver Disease in Childhood and Adolescence

Carolyn Vespoli, MD[a,*], Anoop Mohamed Iqbal, MD[b], Mohammad Nasser Kabbany, MD[a], Kadakkal Radhakrishnan, MD[a]

KEYWORDS

• Fatty liver • Nonalcoholic fatty liver disease • Nonalcoholic steatohepatitis
• Metabolic-associated fatty liver disease • Metabolic syndrome

KEY POINTS

- Metabolic-associated fatty liver disease (MAFLD) has emerged as the most common cause of chronic liver disease among children and adolescents.
- Type I pediatric nonalcoholic steatohepatitis (NASH) shows a similar histopathologic pattern as adult NASH. However, type II pediatric NASH is different from type I NASH as well as adult NASH in that it is defined as the presence of steatosis along with portal inflammation and/or fibrosis in the absence of ballooning degeneration and perisinusoidal fibrosis.
- There are several single-nucleotide gene polymorphisms (*PNPLA3*, *TM6SF2*, *MBOAT7*, and *GPR120*) that are being studied as possible contributors to development of MAFLD and progression to NASH.
- The cornerstone of treatment of pediatric MAFLD is dietary improvement and increased physical activity.
- There are several emerging therapies that target various steps in the pathogenesis and progression of MAFLD in children that show promise for possible pharmacologic treatment options in the future.

INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD), a recently proposed name change from nonalcoholic fatty liver disease (NAFLD) to encompass its close association with

[a] Cleveland Clinic Children's Department of Pediatric Gastroenterology Hepatology and Nutrition, 8950 Euclid Avenue, R Building, Cleveland, OH 44195, USA; [b] Department of Pediatric Endocrinology, Marshfield Clinic Health Systems, Marshfield Children's Hospital, 3rd Floor, 3D, 1000 North Oak Avenue, Marshfield, WI 54449, USA
* Corresponding author.
E-mail address: vespolc2@ccf.org

Endocrinol Metab Clin N Am 52 (2023) 417–430
https://doi.org/10.1016/j.ecl.2023.02.001 **endo.theclinics.com**

metabolic syndrome, has become one of the major causes for chronic liver disease among children and adolescents globally in the past few decades owing to the increasing prevalence of obesity in these age groups.[1] According to the World Health Organization, 39 million children under the age of 5 years were overweight or obese in 2020 and more than 340 million children and adolescents aged 5 to 19 years were overweight or obese in the year 2016.[2] According to the most recent data, the prevalence of childhood obesity-related MAFLD is found to be 52.1%, 39.7%, and 23.0% in Asia, South America, and Europe, respectively.[3] The obesity pandemic is still hitting the global population with the force of an approaching tsunami, which is expected to further worsen the prevalence of MAFLD and liver-related morbidity and mortality in the coming years in young adults. MAFLD is often associated with other diseases, such as hypertension, dyslipidemia, type 2 diabetes mellitus, gallstones, gastroesophageal reflux disease, obstructive sleep apnea, and depression, which substantially increase morbidity among the sufferers.[4] This evidence-based review provides the health care providers with the most up-to-date information on the pathophysiology, clinical evaluation, diagnostic approach, and management of MAFLD among children and adolescents.

EPIDEMIOLOGIC BURDEN

The global level prevalence of MAFLD increased from 19.34 million in 1990 to 29.49 million in 2017 among children and adolescents. The largest increase had been observed in the Middle East and North Africa.[1] MAFLD has emerged as the most common cause of chronic liver disease among children and adolescents. Based on a systematic review, prevalence of MAFLD in children in the general population is likely between 5.5% and 10.3%.[5] This wide range is likely due to regional differences, number of studies, cohort size, and diagnostic modality used. **Fig. 1** shows the estimated prevalence of pediatric MAFLD in every continent based on most recent data. **Fig. 2** shows the estimated prevalence among the obese, pediatric population.

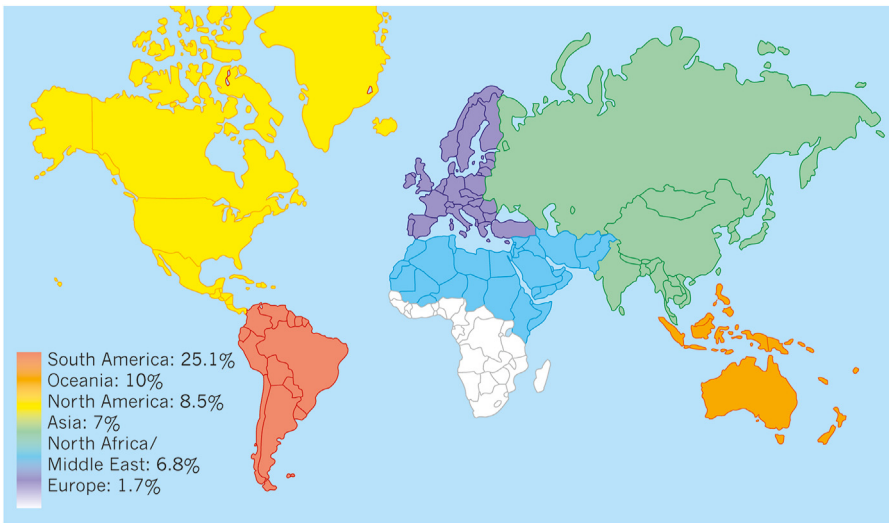

Fig. 1. Estimated MAFLD prevalence within the pediatric population. (Reprinted with permission, Cleveland Clinic Foundation ©2023. All Rights Reserved.)

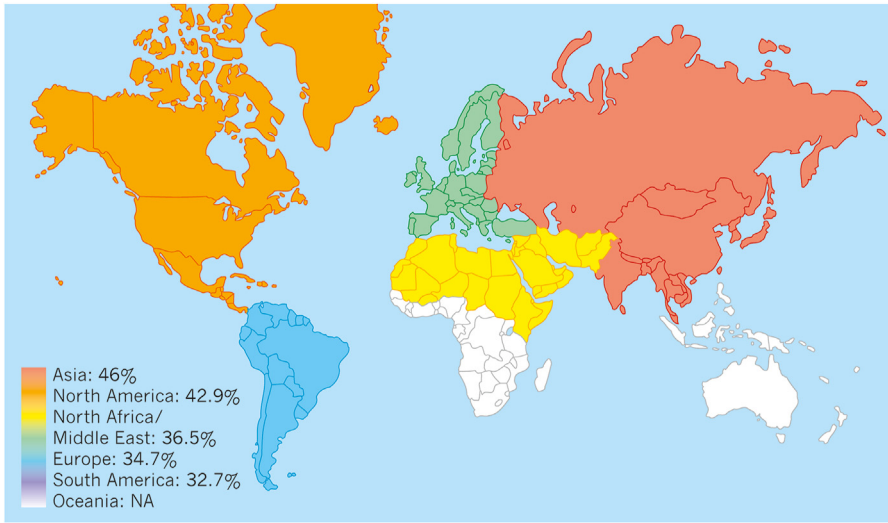

Fig. 2. Estimated MAFLD prevalence within obese pediatric population. (Reprinted with permission, Cleveland Clinic Foundation ©2023. All Rights Reserved.)

MAFLD is considered one of the most common indications for liver transplantation in US adults.[5] Children with MAFLD are at increased risk of developing progressive liver disease.[6] Based on adult data, the annual burden associated with all MAFLD cases in the United States has been estimated at $103 billion, and projections suggest that the expected 10-year burden of MAFLD may increase to $1.005 trillion.[7] Based on a large population-based study, the mean cost per chronic liver disease–related hospitalization was $16,271, whereas the total national estimated cost for chronic liver disease–related hospitalizations from 2012 to 2016 was $81.1 billion.[8]

CAUSE

Similar to adults, MAFLD in children and adolescents includes a spectrum from the relatively benign isolated steatosis, defined as abnormal fat accumulation in greater than 5% of hepatocytes, to inflammation, then the progressive fibrosis, and eventually cirrhosis. Although there is no consensus on the exact cause, recent studies have contributed to a better understanding of this disease process.

Initial theories about the cause of MAFLD were based on the "two-hit" hypothesis. The "first-hit" was the accumulation of hepatic triglycerides in the setting of obesity and insulin resistance, which put the liver at risk for the "second-hit," which was inflammatory cytokines and oxidative stress that sets off a cascade leading to steatohepatitis and fibrosis.[9] This "two-hit" theory was later modified to include the possible role of free fatty acids. In patients with obesity and insulin resistance, there is an increased influx of free fatty acids into the liver. After undergoing β-oxidation or esterification with glycerol to form triglycerides within hepatocytes, free fatty acids are able to cause direct toxicity by increasing oxidative stress and activation of inflammatory cascades.[10]

However, even with the addition of the role free fatty acids play in the pathogenesis of MAFLD, the "two-hit" theory was too simplistic to explain the complex interaction between genetic and environmental factors that leads to the progression of MAFLD.

Thus, the "multiple-hit model" was developed. This model incorporates other factors, including genetic polymorphisms, epigenetics, bile acids, and microbiome.

Several polymorphisms have been implicated in the cause of MAFLD. Patatin-like phospholipase domain-containing protein 3 gene (*PNPLA3*) is a gene that encodes for adiponutrin, an enzyme present in liver and adipose tissue.[11] An isoleucine-to-methionine variant of PNPLAs (I148M) has been identified as a major determinant of liver fat content, as it increases lipogenic activity and impairs mobilization of triglycerides from hepatocyte lipid droplets.[12,13] Its role in development of MALFD in children and adolescents is currently being established with 1 study concluding that *PNPLA3* I148M confers susceptibility to hepatic steatosis in obese youths without increasing the level of hepatic and peripheral insulin resistance.[14] Multiple studies suggest that *PNPLA* I148M may represent a general modifier of fibrogenesis in liver disease and, thus, it is important to identify the children with this polymorphism, as they are at a higher risk of progression to nonalcoholic steatohepatitis (NASH).[12]

A cytosine-to-thymine single-nucleotide polymorphism (SNP) in transmembrane 6 superfamily member 2 (*TM6SF2*) gives rise to the E167K variant. This variant decreases very-low-density lipoprotein–mediated lipid secretion and increases susceptibility to liver damage in children.[15] A cytosine-to-thymine SNP in the membrane-bound O-acyltransferase domain-containing protein 7 (*MBOAT7*) is associated with increased risk of fibrosis and, therefore, progression to NASH.

G protein–coupled receptor 120 (GPR120) is a receptor for polyunsaturated fatty acids (PUFAs) that is expressed mainly on adipocytes and Kupffer cells.[16] In adipose tissue, the interaction between GPR120 and PUFAs has an anti-inflammatory effect. A variant of *GPR120*, 270H, has been shown to reduce the signaling effects between GPR120 and PUFAs and thus decrease their anti-inflammatory effects.[17] One study, although underpowered, was able to show that adolescents carrying *GPR120* 270H had significantly higher ALT and ferritin levels than wild-type subjects.[16] Interestingly, this study also showed that subjects with coexisting *GRP120* 270H and *PNPLA3* 148M have significantly higher ALT levels than those with *PNPLA3* 148M alone. However, those subjects with wild-type *PNPLA3* and *GRP120* 270H had normal ALT levels, thus further emphasizing the dynamic pathophysiology described in the "multiple-hit model."[16]

There are several other polymorphisms that have been found to be playing a role in the development of MAFLD and progression to NASH. These polymorphisms are involved in monosaccharide uptake, lipid synthesis, metabolism, lipid excretion, and insulin receptor activity.[13] However, the exact role of these polymorphisms and their degree of correlation with fibrogenesis is not well understood and requires more research, especially in the pediatric population.

PATHOPHYSIOLOGY

The development of steatosis begins with the accumulation of excess carbohydrate precursors. This stimulates the upregulation of de novo lipogenesis. A concurrent process is also occurring whereby increased fat intake from the diet is resulting in increased uptake of fatty acids from chylomicron particles, resulting in lipolysis. These processes together result in the accumulation of hepatic free fatty acids and triglycerides.[18]

These free fatty acids and triglycerides collect in the cytoplasm of hepatocytes and are supposed to be disposed of via mitochondrial β-oxidation. However, if this process becomes overwhelmed, it leads to the inadequate disposal of free fatty acids and increased production of toxic lipids, unesterified cholesterol, and ceramides

that cause hepatotoxicity and increased inflammation.[18] Inflammation leads to the recruitment of multiple cytokines that contribute further to hepatocellular damage. The recruitment of Kupffer cells and activation of hepatic stellate cells result in fibrosis, cirrhosis, and possible hepatocellular carcinoma.[18]

Gut-liver axis is another important process that contributes to this complex system. Bile acids act as the regulators of this system by playing a key role in nutrient absorption and signal transduction to the liver via modulation of the gut microbiome and activation of various receptors. Bile acids act as crucial regulators of liver metabolism. The opposite is also true in that the liver is acting as a key regulator of intestinal homeostasis. Although the exact mechanisms are still an active area of research, an impaired gut-liver axis function has been implicated in the pathogenesis of MAFLD.[19]

This complex web of interconnected processes that leads to the development of MAFLD was discovered through research into adult MAFLD. However, the pathogenesis of pediatric MAFLD has not been given the same consideration. Thus, significant research is required to evaluate possible differences in the development of pediatric MAFLD.

PATHOLOGY AND CLASSIFICATION

Although the exact histologic criteria for adult NASH evolved over time, main features include the presence of macrovesicular steatosis, ballooning degeneration of hepatocytes, and a mix of lobular inflammation with or without a degree of portal inflammation.[20] There are some commonalities in the histopathology of adult and pediatric NASH; however, there are also some notable differences. A 2005 study of more than 100 pediatric patients with NALFD was first able to describe 2 distinct subsets of NASH in children.[21]

Type I NASH shows a similar histopathologic pattern to that of adult NASH. It is defined as the presence of steatosis with ballooning degeneration and/or perisinusoidal fibrosis in the absence of portal features.[21] **Fig. 3** highlights these characteristic features using hematoxylin-eosin (H&E) staining.

Type II or pediatric NASH is different from type I or adult NASH in that it is defined as the presence of steatosis along with portal inflammation and/or fibrosis in the absence of ballooning degeneration and perisinusoidal fibrosis, as shown in **Figs. 4** and **5**.[21] This type of NASH is more common overall but especially in male patients as well as in Asians, Native Americans and Hispanics.[20] The presence of portal inflammation, which is unique to type II NASH, has been independently associated with clinically significant fibrosis, suggesting this feature is predictive of a faster disease progression.[22]

Although there is a desire to make types I and II NASH distinct entities for simplicity's sake, there is also significant overlap. One case series demonstrated that 66% of patients with histologically diagnosed NASH fell into this overlapping category.[22] Although the exact percentage of each NASH type, as well as overlap, varies between studies and populations, more research is needed to clarify the natural history of each phenotype.

EVALUATION AND DIAGNOSIS

MAFLD should be suspected in any overweight or obese child with hepatic steatosis on imaging or liver biopsy with or without elevated ALT. However, it should not be assumed as the only cause. Certain conditions and medications may have similar presentation, and missing them may have serious consequences. MAFLD is currently considered a diagnosis of exclusion, and it can coexist with other conditions.[23]

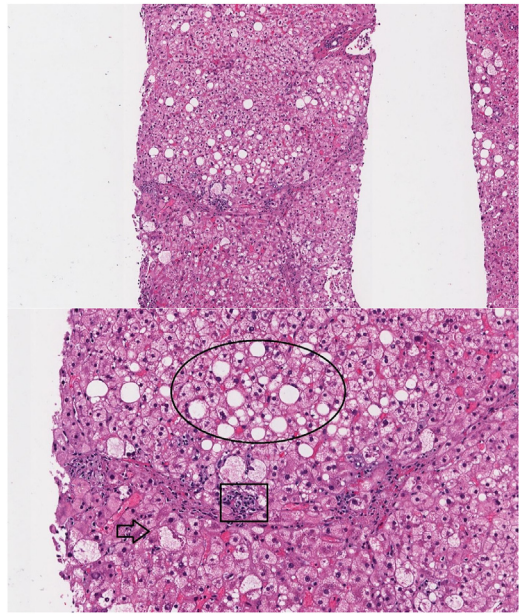

Fig. 3. Adult (type 1) NASH (H&E stain). Steatosis (*circle*), ballooned hepatocyte (*arrow*), inflammation (*square*). (*Courtesy of* Daniela Allende, MD, Cleveland, OH.)

Liver biopsy remains the gold standard to make the diagnosis of MAFLD. It also helps in assessing NASH activity and staging fibrosis. However, in reality, liver biopsy is not performed on every MAFLD patient, and its use is limited for certain indications, ruling out other causes for elevated liver enzymes, assessing NASH activity, and ruling out advanced fibrosis. Liver biopsy has its own limitations, including sampling bias, and the risk of complications, including hemorrhage and, rarely, death.[24]

Emerging noninvasive measures have become appealing in both adult and pediatric MAFLD. Those measures try to evaluate different aspects of the disease, including the presence of steatosis, steatosis grade, NASH activity, and of stage fibrosis. They are generally divided into biomarkers and imaging modalities. Several biomarkers have been developed in adult MAFLD, and few, like FIB-4, are recommended by adult

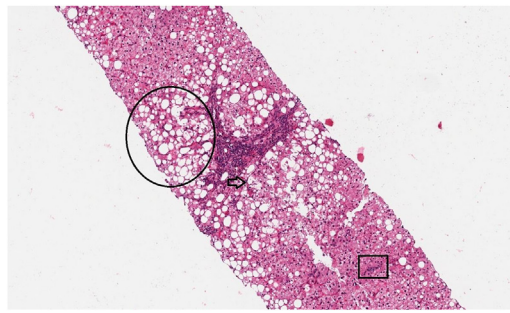

Fig. 4. Pediatric (type 2) NASH (H&E stain). Steatosis (*circle*), ballooned hepatocyte (*arrow*), inflammation (*square*). (*Courtesy of* Daniela Allende, MD, Cleveland, OH.)

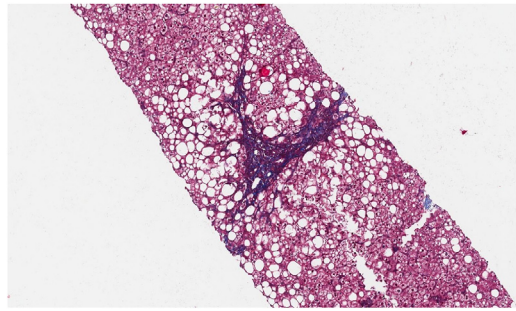

Fig. 5. Pediatric (type 2) NASH (Trichrome stain). (*Courtesy of* Daniela Allende, MD, Cleveland, OH.)

guidelines to screen patients at risk for significant fibrosis.[25,26] Unlike adult MAFLD, there is no reliable biomarker that made it to clinical practice in pediatric MAFLD. On the other hand, imaging modalities show promising results in both age groups.[27]

Conventional ultrasound (US) is a commonly used imaging modality that has been studied extensively. It is cheap, noninvasive, and well tolerated by children. Hepatic steatosis is generally suspected based on increased liver echogenicity compared with adjacent right renal parenchyma. It has shown very good accuracy to detect moderate to severe steatosis when compared with liver biopsy with area under the receiver operating characteristic curve of 0.87.[28] However, low sensitivity and specificity in detecting mild steatosis made it less favorable by the most recent North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition recommendations as a tool to screen for the disease in at-risk children.[23] US is still recommended by the European guidelines as a screening tool.[29] New US-based modalities like quantitative US to detect and grade steatosis are being validated in adults.[30] Magnetic resonance proton density fat fraction (MR-PDFF) is a very accurate imaging tool to quantify liver fat. However, it is not very popular, is only available at tertiary centers, and is commonly used in clinical trials.

Velocity controlled transient elastography (VCTE) is emerging as a popular noninvasive modality to grade steatosis and stage fibrosis. It is a US-based technology that mechanically produces a low-frequency shear wave, which propagates through the liver. The velocity of the shear wave measured by the device correlates positively with liver stiffness, which is measured in kilopascal. The attenuation of that shear wave correlates positively with liver steatosis and is measured using CAP score (controlled attenuation parameter) in decibel per meter. CAP score cutoff for the presence of steatosis ranges between 225 and 241 dB/m based on 2 pediatric studies using biopsy and MR-PDFF as a reference, respectively.[31,32] Although steatosis grade does correlate with CAP score, however, specific cutoff to determine steatosis grade is more problematic owing to significant overlap between cutoff suggested by different studies.

Acoustic radiation force impulse (ARFI) is another US-based technology that is similar to VCTE in using a probe that creates a push pulse wave that propagates through the liver in velocity that correlates with liver stiffness. Stiffness is expressed in meters per second. ARFI differs from VCTE by the ability to choose a region of interest within which the stiffness is measured. This lowers the rate of unsuccessful readings compared with VCTE especially in obese patients and patients with ascites.[27]

MR elastography is a technology that uses MRI and a vibration source that is placed on the patient trunk, which creates a shear wave that propagates throughout the liver. The MRI machine then creates a color-coded map called an elastogram that shows estimated liver stiffness measured in kilopascal. MR elastography has the advantage of assessing the stiffness of the entire liver regardless of the body habitus with very good accuracy. Only a few pediatric studies exist.[33] Small sample size and heterogeneity of chronic liver diseases (besides MAFLD) included make it challenging to define reliable cutoff values to stage fibrosis in pediatric MAFLD. The need for anesthesia remains a limiting factor in young children.

MANAGEMENT AND LIMITATIONS

The goal of treatment of MAFLD is regression in steatosis, inflammation, and ultimately, fibrosis. The cornerstone of treatment of pediatric MAFLD is weight loss through dietary improvement and increased physical activity. The most recent guidelines published in 2017 from the North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition recommends "lifestyle modifications to improve diet and increase physical activity as the first-line treatment for all children with NAFLD."[23] A weight reduction of as little as 1 kg has been shown to improve serum biomarkers of MAFLD in children; thus, many studies have been aimed at determining which diet works most effectively for weight reduction in children.[34]

Multiple studies have shown a decrease in adiposity among overweight and obese adolescents after reduction of sugar-sweetened beverages.[35,36] The addition of high-fructose corn syrup to these beverages acts as a stimulator of glycolysis, lipogenesis, and glucose production in the liver and thus contributes to the pathogenesis of MAFLD.[37] Research also indicates that the Mediterranean diet can reduce liver steatosis and improve insulin sensitivity even without weight loss; however, more research needs to be done in this area.[38] Ultimately, the most effective diet is the one that is able to be sustained over time.

Metformin and vitamin E have been studied extensively as possible treatments for pediatric MAFLD owing to their effect on insulin resistance and oxidative stress. The TONIC trial was a 2011 multicenter, phase 3, randomized, placebo-controlled trial that aimed to look at changes in ALT, changes in histologic features of MAFLD, and resolution of NASH after treatment with metformin or vitamin E. This well-powered study concluded that, although treatment with vitamin E showed some histologic improvements, neither metformin nor vitamin E was superior to placebo in attaining sustained reduction in ALT.[39]

Docosahexaenoic acid, fish oil, oral insulin sensitizers, ursodeoxycholic acid, probiotics, and carnitine have all also been studied as possible treatment options for pediatric MAFLD; however, none have been shown to add significantly to the treatment effect of lifestyle modifications alone.[13]

EVOLVING TREATMENTS

In the last several years, different classes of medications have been suggested and are currently being studied as possible treatments for MAFLD in adults. The role of these pharmacotherapies in the treatment of MAFLD in children and adolescents has yet to be determined. However, safety and efficacy have been demonstrated in adults, and studies in the pediatric population will follow soon.[15] **Table 1** outlines the current state of pharmacologic treatment of MAFLD in children and adolescents.

Table 1
Evolving treatment for pediatric metabolic-associated fatty liver disease

Drug	Category	Mechanism of Action/Target	Clinical Trial Phase	Limitations
Elafibranor	Alpha and delta peroxisome proliferator-activated receptor (PPAR) dual agonist (PPAR-α/δ)	• Insulin sensitizer • Improves lipid metabolism	Phase 2 in pediatrics	• Been shown to resolve NASH without worsening fibrosis in adult, but pediatric trials are still ongoing[40] • Adult phase 3 trial terminated due to lack of efficacy
Lanifibranor	Pan-PPAR agonist	• Insulin sensitizer (shown to be more efficacious than dual-PPAR agonists[41]) • Reduces liver fibrosis	Phase 3 in adults	• No pediatric trials
Resmetirom	Thyroid hormone receptor (THR) β-selective agonist	• Targets THR-β receptors in hepatocytes that impact serum cholesterol and triglyceride levels	Phase 3 in adults	• Current adult study shows improvement in ballooning and inflammation within hepatocytes without worsening fibrosis, but no studies in children
Selonsertib	Apoptosis signal-regulating kinase 1 (ASK1) inhibitor	• Inhibits the ASK1 pathway that results in inflammatory and profibrotic changes in the liver • ASK1 is upregulated in patients with NASH and correlates with degree of fibrosis[40]	Phase 3 in adults	• Randomized clinical trials have shown no antifibrotic effect in adult patients with bridging fibrosis or compensated cirrhosis due to NASH[42] • Adult phase 3 trial terminated due to lack of efficacy • No pediatric trials
Obeticholic acid	Farnesoid X receptor (FXR) agonist	• Agonizes the FXR receptor (a bile acid receptor that regulates hepatic and peripheral glucose metabolism), which results in balancing of de novo lipogenesis and fatty acid oxidation as well as anti-inflammatory effects[43]	Phase 3 in adults	• Results in improvement in fibrosis without worsening of NASH in adult patients with stage F2 or F3 fibrosis but there are no pediatric trials[44]

(*continued on next page*)

Table 1
(*continued*)

Drug	Category	Mechanism of Action/Target	Clinical Trial Phase	Limitations
Cenicriviroc	C-C chemokine receptor types 2 and 5 dual antagonist	• Inhibits a pathway that causes fibrogenesis by monocyte and macrophage recruitment to the inflamed tissue and activation on hepatic stellate cells[40] • Downstream target	Phase 3 in adults	• Adult phase 3 trial (AURORA) terminated due to lack of efficacy • No pediatric trials
Aramchol (3β-arachidyl amido cholanoic acid)	Partial inhibitor of hepatic stearoyl-CoA desaturase (SCD1)	• Fatty acid–bile acid conjugate that inhibits SCD1 and results in improved steatohepatitis, fibrosis, and steatosis[45]	Phase 3 in adults	• No pediatric trials
Semaglutide[a]	Glucagon-like peptide-1 analogue	• Increases postprandial insulin level in a glucose-dependent manner, reduces glucagon secretion, delays gastric emptying, and induces weight loss through appetite reduction and decreased energy intake[46]	Phase 3 in pediatrics	• No studies to date exploring direct effect on fatty liver disease (although a phase 3 pediatric study is actively recruiting MAFLD patients currently) • Requires weekly injection
Liraglutide	Glucagon-like peptide-1 analogue	• Increases postprandial insulin level in a glucose-dependent manner, reduces glucagon secretion, delays gastric emptying, and induces weight loss through appetite reduction and decreased energy intake[46]	Phase 3 in pediatrics	• No published studies to date exploring direct effect on fatty liver disease • Requires daily injections

[a] Oral version (Rybelsus) has been proven to have similar efficacy and tolerability in adults but no research is available in pediatrics.

SUMMARY

MAFLD in children and adolescents is a problem that is only growing in scale. Although there are promising therapies on the horizon, patient risk stratification continues to pose a challenge owing to limited data on the disease's natural history in this population. Tackling this growing pandemic in children will certainly have a positive impact on the future of adult MAFLD and its economic burden.

CLINICS CARE POINTS

- Increased awareness of metabolic-associated fatty liver disease in children and adolescents can lead to early diagnosis and intervention.
- There are several noninvasive modalities, such as ultrasound and elastography, that can help assist in grading steatosis and staging fibrosis.
- Lifestyle changes and weight loss remain the mainstay of treatment for pediatric metabolic-associated fatty liver disease.
- Emerging therapies that target varied steps in the pathogenesis of metabolic-associated fatty liver disease may play a larger role in treatment in the near future.

DISCLOSURES

The authors have nothing to disclose.

REFERENCES

1. Zhang X, Wu M, Liu Z, et al. Increasing prevalence of NAFLD/NASH among children, adolescents and young adults from 1990 to 2017: A population-based observational study. BMJ Open 2021;11(5):e042843.
2. Obesity and overweight. World Health Organization. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Accessed January 28, 2023.
3. Obita G, Alkhatib A. Disparities in the Prevalence of Childhood Obesity-Related Comorbidities: A Systematic Review. Front Public Health 2022;10. https://doi.org/10.3389/fpubh.2022.923744.
4. Yi M, Peng W, Feng X, et al. Extrahepatic morbidities and mortality of NAFLD: an umbrella review of meta-analyses. Aliment Pharmacol Ther 2022 Oct;56(7):1119–30.
5. Anderson EL, Howe LD, Jones HE, et al. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. PLoS One 2015;10(10).
6. Castillo-Leon E, Cioffi CE, Vos MB. Perspectives on youth-onset nonalcoholic fatty liver disease. Endocrinol Diabetes Metab 2020;3(4). https://doi.org/10.1002/edm2.184.
7. Younossi ZM, Koenig AB, Abdelatif D, et al. Global Epidemiology of Nonalcoholic Fatty Liver Disease-Meta-Analytic Assessment of Prevalence, Incidence, and Outcomes. Hepatology 2016 Jul;64(1):73–84.
8. Hirode G, Saab S, Wong RJ. Trends in the Burden of Chronic Liver Disease among Hospitalized US Adults. JAMA Netw Open 2020;3(4). https://doi.org/10.1001/jamanetworkopen.2020.1997.

9. Fang YL, Chen H, Wang CL, et al. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From “two hit theory” to “multiple hit model. World J Gastroenterol 2018;24(27):2974–83.
10. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. QJM 2009;103(2):71–83.
11. Marzuillo P, Grandone A, Perrone L, et al. Understanding the pathophysiological mechanisms in the pediatric non-alcoholic fatty liver disease: The role of genetics. World J Hepatol 2015;7(11):1439–43.
12. Dongiovanni P, Donati B, Fares R, et al. PNPLA3 I148M polymorphism and progressive liver disease. World J Gastroenterol 2013;19(41):6969–78.
13. Draijer L, Benninga M, Koot B. Pediatric NAFLD: an overview and recent developments in diagnostics and treatment. Expert Rev Gastroenterol Hepatol 2019; 13(5):447–61.
14. Santoro N, Kursawe R, D’Adamo E, et al. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. Hepatology 2010;52(4):1281–90.
15. Nobili V, Alisi A, Valenti L, et al. NAFLD in children: new genes, new diagnostic modalities and new drugs. Nat Rev Gastroenterol Hepatol 2019;16(9):517–30.
16. Marzuillo P, Grandone A, Conte M, et al. Novel association between a nonsynonymous variant (R270H) of the G-protein-coupled receptor 120 and liver injury in children and adolescents with obesity. J Pediatr Gastroenterol Nutr 2014;59(4): 472–5.
17. Oh DY, Talukdar S, Bae EJ, et al. GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-inflammatory and Insulin-Sensitizing Effects. Cell 2010; 142(5):687–98.
18. Phen C, Ramirez CM. Hepatic Steatosis in the Pediatric Population: An Overview of Pathophysiology, Genetics, and Diagnostic Workup. Clin Liver Dis 2021;17(3): 191–5.
19. Xue R, Su L, Lai S, et al. Bile acid receptors and the gut–liver axis in nonalcoholic fatty liver disease. Cells 2021;10(11). https://doi.org/10.3390/cells10112806.
20. Carter-Kent C, Yerian LM, Brunt EM, et al. Nonalcoholic steatohepatitis in children: A multicenter clinicopathological study. Hepatology 2009;50(4):1113–20.
21. Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005;42(3):641–9.
22. Mann JP, Valenti L, Scorletti E, et al. Nonalcoholic Fatty Liver Disease in Children. Semin Liver Dis 2018;38(1):1–13.
23. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). J Pediatr Gastroenterol Nutr 2017;64(2):319–34.
24. Ovchinsky N, Moreira RK, Lefkowitch JH, et al. Liver Biopsy in Modern Clinical Practice: A Pediatric Point-of-View. 2012. Available at: http://www.anatomic.
25. Cusi K, Isaacs S, Barb D, et al. American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD). Endocr Pract 2022;28(5):528–62.
26. Castera L, Friedrich-Rust M, Loomba R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2019; 156(5):1264–81.e4.

27. Chen BR, Pan CQ. Non-invasive assessment of fibrosis and steatosis in pediatric non-alcoholic fatty liver disease. Clin Res Hepatol Gastroenterol 2022;46(1). https://doi.org/10.1016/j.clinre.2021.101755.
28. Shannon A, Alkhouri N, Carter-Kent C, et al. Ultrasonographic quantitative estimation of hepatic steatosis in children With NAFLD. J Pediatr Gastroenterol Nutr 2011;53(2):190–5.
29. Vajro P, Lenta S, Socha P, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: Position paper of the ESPGHAN hepatology committee. J Pediatr Gastroenterol Nutr 2012;54(5):700–13.
30. Lin SC, Heba E, Wolfson T, et al. Noninvasive diagnosis of nonalcoholic fatty liver disease and quantification of liver fat using a new quantitative ultrasound technique. Clin Gastroenterol Hepatol 2015;13(7):1337–45.e6.
31. Shin NY, Kim MJ, Lee MJ, et al. Transient elastography and sonography for prediction of liver fibrosis in infants with biliary atresia. J Ultrasound Med 2014;33(5):853–64.
32. Desai NK, Harney S, Raza R, et al. Comparison of controlled attenuation parameter and liver biopsy to assess hepatic steatosis in pediatric patients. J Pediatr 2016;173:160–4.e1.
33. Xanthakos SA, Podberesky DJ, Serai SD, et al. Use of magnetic resonance elastography to assess hepatic fibrosis in children with chronic liver disease. J Pediatr 2014;164(1):186–8.
34. Friesen CS, Hosey-Cojocari C, Chan SS, et al. Efficacy of Weight Reduction on Pediatric Nonalcoholic Fatty Liver Disease: Opportunities to Improve Treatment Outcomes Through Pharmacotherapy. Front Endocrinol 2021;12. https://doi.org/10.3389/fendo.2021.663351.
35. Ebbeling CB, Feldman HA, Chomitz VR, et al. A Randomized Trial of Sugar-Sweetened Beverages and Adolescent Body Weight. N Engl J Med 2012;367(15):1407–16.
36. de Ruyter JC, Olthof MR, Seidell JC, et al. A Trial of Sugar-free or Sugar-Sweetened Beverages and Body Weight in Children. N Engl J Med 2012;367(15):1397–406.
37. Taskinen MR, Packard CJ, Borén J. Dietary fructose and the metabolic syndrome. Nutrients 2019;11(9). https://doi.org/10.3390/nu11091987.
38. Ryan MC, Itsiopoulos C, Thodis T, et al. The Mediterranean Diet Improves Hepatic Steatosis and Insulin Sensitivity in Individuals with Non-Alcoholic Fatty Liver Disease. J Hepatol 2013;59(1):138–43.
39. Lavine JE, Schwimmer JB, van Natta ML, et al. Effect of Vitamin E or Metformin for Treatment of Nonalcoholic Fatty Liver Disease in Children and Adolescents The TONIC Randomized Controlled Trial. JAMA 2011;305(16):1659–68. Available at: https://jamanetwork.com/.
40. Crudele A, Panera N, Braghini MR, et al. The pharmacological treatment of nonalcoholic fatty liver disease in children. Expert Rev Clin Pharmacol 2020;13(11):1219–27.
41. Francque SM, Bedossa P, Ratziu V, et al. A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. N Engl J Med 2021;385(17):1547–58.
42. Harrison SA, Wong VWS, Okanoue T, et al. Selonsertib for patients with bridging fibrosis or compensated cirrhosis due to NASH: Results from randomized phase III STELLAR trials. J Hepatol 2020;73(1):26–39.
43. Attia SL, Softic S, Mouzaki M. Evolving Role for Pharmacotherapy in NAFLD/NASH. Clin Transl Sci 2021;14(1):11–9.

44. Younossi ZM, Ratziu V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019;394(10215):2184–96.
45. Ratziu V, de Guevara L, Safadi R, et al. Aramchol in patients with nonalcoholic steatohepatitis: a randomized, double-blind, placebo-controlled phase 2b trial. Nat Med 2021;27(10):1825–35.
46. Kelly AS, Auerbach P, Barrientos-Perez M, et al. A randomized, controlled trial of liraglutide for adolescents with obesity. N Engl J Med 2020;382(22):2117–28.