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The Genetics of Pediatric Nonalcoholic Fatty Liver Disease (NAFLD)

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in children. Severe fibrosis and cirrhosis are potential consequences of pediatric NAFLD and can occur within a few years of diagnosis. There are racial and ethnic differences in the prevalence of pediatric NAFLD and there is evidence that NAFLD tends to cluster in families. These observations suggest that genetics may be a strong modifying factor in the presentation, severity, and natural history of the disease. The most studied gene in children with NAFLD is *PNPLA3*. At present, data indicate that the I148M allele of *PNPLA3* is associated with higher ALT in children with obesity. There is also evidence that *PNPLA3* is associated with steatosis. Additional studies are warranted to determine the histologic severity of disease associated with this polymorphism. The *TM6SF2* polymorphism may also be associated with hepatic steatosis in children. There is increasing interest in determining at risk populations based on genetics in the hope of finding genotypes that correlate to NAFLD phenotype. Future studies of the genetics of pediatric NAFLD should evaluate multiple genes in a diverse patient population with histologic NAFLD to determine if certain genotypes have higher risk for disease progression. Ultimately, the hope is to be able to tailor therapeutics to genetic predispositions and decrease disease morbidity in children with NAFLD.

Keywords

Nonalcoholic steatohepatitis; children; liver; steatosis; PNPLA3; TM6SF2; obesity; alanine aminotransferase

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in children. The prevalence of fatty liver, after adjusting for age, race, gender, and ethnicity, is estimated at 9.6%.¹ Pediatric NASH (nonalcoholic steatohepatitis) can be distinct from adult NASH and denotes hepatic steatosis with inflammation, with or without ballooning injury to hepatocytes.² This can include zone 3 (venule) centered injury pattern or confluent pattern typically with ballooning or portal predominant (zone 1) centered injury pattern often without ballooning.³ Children with zone 1 steatosis are more likely to present with fibrosis, including advanced fibrosis, compared to children with zone 3 steatosis.⁴ Severe fibrosis and cirrhosis are observed in some children with NAFLD and can occur within a few years of diagnosis in the most severe cases.⁵ Children with NASH are at higher risk of serious co-morbidities such as type 2 diabetes and hypertension.^{6,7} Knowledge of the genetics of pediatric NAFLD may someday improve both diagnosis and treatment. NAFLD is now the leading cause of liver transplantation in young adults, yet a treatment remains to be discovered. Tailoring therapeutics to genetic predispositions is an avenue yet to be explored for this disease.

It is likely that NAFLD has a strong genetic component based on two key observations. The first is the racial and ethnic difference in the prevalence of NAFLD and second is the evidence that NAFLD tends to cluster in families. Hispanic children have the highest prevalence of NAFLD and black children have the lowest. In the Study of Child and Adolescent Liver Epidemiology (SCALE), in which diagnosis was based on liver histopathology, NAFLD was present in 11.8% of Hispanic children, 10.2% of Asian children, 8.6% of white children, and 1.5% of black children.¹ These differences have also been seen in adulthood.⁸

The clustering of NAFLD within families was evaluated by a heritability study by Schwimmer and colleagues. In this study, 33 obese children with biopsy proven NAFLD, 11 obese children without NAFLD, and 152 of their family members (parents, siblings, 2nd or 3rd degree relatives) were studied.⁹ Presence of NAFLD in family members was evaluated by MRI proton density fat fraction (PDFF). In children without NAFLD, 17% of siblings and 37% of parents had NAFLD compared to 59% of siblings and 78% of parents of children with biopsy-proven NAFLD. The heritability estimates (with 0 being no heritability and 1 representing a trait that is completely heritable) were 0.85 for the unadjusted dichotomous variable for NAFLD and 1.0 after adjusting for age, gender, race, and BMI. For the continuous measurement of hepatic steatosis, the adjusted heritability estimate was 0.39 or 39%.

Many aspects of the pathogenesis of NAFLD such as the mechanism for the progression from steatosis to steatohepatitis remain unclear. Additionally, it is not known why NAFLD occurs in some obese individuals and not others. Although less common, NAFLD also exists in 5% of children with a normal BMI.¹ With respect to treatment, there is wide variability in the response of children with NAFLD to lifestyle interventions,¹⁰ and the underlying genetics of NAFLD may play a part in the differential response to dietary and/or exercise interventions. These observations suggest that genetics are a modifying factor, and that there

is an interplay of genetics and environment in the pathogenesis of this disease. Understanding how genes influence the development and progression of pediatric NAFLD will help to address critical gaps in knowledge in the field.

In this review, the existing data on the genetics of pediatric NAFLD are summarized. The articles cited were identified based on a search of PubMed done in February 2017 using the criteria of “NAFLD and genetic and children” with the results limited to studies in humans.

PNPLA3

PNPLA3 belongs to the patatin-like phospholipase domain-containing family of proteins and it encodes a 481 amino acid protein called adiponutrin, which is involved in lipid metabolism. While the exact role of this protein in the liver is unclear, there is a large body of evidence that *PNPLA3* is associated with NAFLD.

In the landmark study for this field, a genome-wide association study (GWAS) resulted in the discovery of a single nucleotide polymorphism (SNP) in the gene *PNPLA3* that confers susceptibility to NAFLD. The Dallas Heart Study was a multi-ethnic, population based study in adults (n= 1,032 African American, 696 European American, and 383 Hispanic) that evaluated hepatic fat content via proton magnetic resonance spectroscopy and performed a GWAS to search for sequence variations. A single variant in *PNPLA3* (rs739409), a cytosine (C) to guanine (G) substitution of codon 148 resulting in a non-synonymous change to methionine from isoleucine, was highly associated with hepatic fat content independent of BMI, diabetes, or alcohol use.¹¹ The highest frequency of this allele was present in Hispanics (0.49) followed by European Americans (0.23) and African-Americans (0.17), consistent with differing rates of NAFLD by ethnicity and race. Studies in adults have demonstrated an association with *PNPLA3* and histologic severity, with the minor allele associated with increased steatosis, NASH, and fibrosis.¹² These results indicate that the *PNPLA3* gene locus is not only associated with steatosis but likely also with steatohepatitis, or NASH.

Data from pediatric studies have also demonstrated the importance of this gene in children with NAFLD. Several pediatric studies have evaluated the association of *PNPLA3* with ALT, imaging evidence of NAFLD, and histology (Table 1). In a study of 475 overweight or obese children, children who were homozygous for the variant I148M allele had higher serum ALT activity compared to those with homozygous wildtype allele, ALT 21 U/L compared to 32 U/L, respectively.¹³ When stratified by genotype, of those subjects with homozygous minor alleles for *PNPLA3*, 32% had ALT > 30 U/L versus 10% in those with homozygous wildtype alleles. Similarly, in another Italian cohort of 1048 children with obesity, the mean ALT was 25 U/L for those with the homozygous wildtype and 38 U/L in those with the homozygous variant genotype.¹⁴ In a study of 520 Taiwanese children with obesity, ALT value was also associated with the *PNPLA3* genotype in an additive effect. In this study, the mean serum ALT was higher by 4.8 IU/L in carriers and 10.9 IU/L in those with the homozygous variant genotype compared to children with the wildtype genotype. The variant allele was also associated with ultrasound evidence of hepatic steatosis.¹⁵ In a study of over 1000 children from Mexico, the percentage of children with ALT >35 U/L was 8.6% for wildtype, 30.5% for carriers of the variant allele, and 61% for the homozygous variant

PNPLA3.¹⁶ Interestingly, the percentage of children with elevated ALT who had the homozygous variant was 32–33% in the 2 studies of Italian children compared to 61% in the study of children from Mexico. This could be due to the varying cutoffs used to define an elevated ALT as well as differences due to ethnicity. The relationship of an elevated ALT in children carrying the risk allele of *PNPLA3* has subsequently also been observed in children from Finland and Austria.^{17,18}

Two pediatric studies have evaluated the association of *PNPLA3* with liver steatosis, as measured by MRI. Santoro and colleagues evaluated 85 children with obesity and found a positive association of MRI signal fat fraction (SFF) with presence of at least one G allele in Caucasian and African American children.¹⁹ Interestingly, however, this association was not statistically significant in Hispanic children and there was no association with *PNPLA3* and ALT in any racial group. In the second study, the liver fat fraction was evaluated in 188 Hispanic children, and the mean signal fat fraction was 11% for those children with homozygous variant alleles compared to 4.7% with the wildtype alleles.²⁰ One study evaluated *PNPLA3* association with ultrasound evidence of hepatic steatosis. This study was conducted in 1093 overweight or obese children in China and reported that for each variant allele present the odds ratio for hepatic steatosis was 1.57 (95% CI 1.15–2.16).²¹

Studies of the relationship between *PNPLA3* and liver histology have had inconsistent findings. A study by Rotman and colleagues in 2010 included 223 pediatric patients from the NASH CRN, and reported that there was no association of the *PNPLA3* locus with the histologic severity of NAFLD.²² In a somewhat smaller study, Valenti and colleagues evaluated liver histology in 149 Italian pediatric patients with NAFLD.²³ They reported that the *PNPLA3* variant allele was associated with the severity of steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis. The prevalence of grade 2 and 3 steatosis was greater in children with homozygous variant alleles compared to heterozygotes. Lobular necroinflammation was observed in 3% of the children with wildtype homozygous alleles (2/65), 30% in carriers of the allele (18/61), and 70% in children with homozygous variant alleles (16/23). The prevalence of histologic NASH was 3% (2/65) in those with homozygous wildtype alleles (2/65) versus 74% (46/61) for the heterozygotes, (45/61) and 100% (23/23) in those with homozygous variant alleles. A similar pattern was reported for hepatocellular ballooning. The variant genotype was associated with perivenular fibrosis or higher grade fibrosis in 31% (20/65) of those with homozygous wildtype, 48% (29/61) of those heterozygous, and 74% (17/23) of those homozygous variant. Interestingly, there was no association with peri-portal fibrosis, which is a more common pattern in pediatric NAFLD. Similarly, in another study of 118 Italian children at the same medical center, 100% of children with *PNPLA3* homozygous risk variant had NASH, 73% of heterozygous patients had NASH, and no homozygous wildtype patients had NASH.²⁴

Other Genes

The majority of data that exist for genetic associations with pediatric NAFLD are for the *PNPLA3* polymorphism. After the initial discovery of the association of *PNPLA3* and NAFLD in 2008, many other genes and their relation to pediatric NAFLD have been evaluated. However, for any one genetic polymorphism, most have only been evaluated in

single studies thus far (Table 2). The field of genetics and how it relates to NAFLD is still relatively young, however there is increasing interest in determining at risk populations based on genetics in the hope of finding genotypes that correlate to NAFLD phenotype. The following subsections divide these pediatric studies based on the outcome variable evaluated: ALT, imaging measure of hepatic steatosis, or liver histology.

ALT—The association with ALT was evaluated for two genes: *HIF3A* and *MBOAT7*. Based on prior studies postulating a possible relationship with epigenetic modifications, such as DNA methylation, and obesity, Wang and colleagues evaluated methylation at the first intron of the Hypoxia Inducible Factor 3 Alpha Subunit (*HIF3A*) gene and its relation to obesity and ALT.²⁵ To date, this is the only epigenetic study for pediatric NAFLD. In this study of 110 children with obesity and 110 normal weight and age-matched controls from China, ALT was found to be associated with DNA methylation of *HIF3A* at the CpG11 methylation site after adjusting for BMI ($r=0.226$; $p=0.007$). The other methylation sites evaluated were not significantly associated with ALT once adjusted for BMI.

MBOAT7 (membrane-bound O-acyltransferase domain-containing protein 7) is a gene encoding proteins involved in phospholipid remodeling. This gene has been associated with NAFLD in several adult studies. The first and only pediatric study was conducted in 2016.²⁶ In this study, the association between the minor T allele and ALT was evaluated in 467 children ages 6–9 years. Carriers of this polymorphism had an ALT that was 7% higher than non-carriers (17.8 U/L vs. 19.1 U/L). At 2 year follow-up, the ALT was 10% higher than non-carriers (18.0 U/L vs. 19.7 U/L). In addition, this study also looked at two other genes, *PNPLA3* and *TM6SF2*. Children who were carriers of the minor allele for all three of these genes had an ALT of 32 U/L compared to 19 U/L if they were carriers of *MBOAT* alone, suggesting that multiple “risk” genotypes potentially have an additive effect.

Imaging—The *TM6SF2* (Transmembrane 6 superfamily 2 human) gene polymorphism and its association with pediatric NAFLD has been evaluated in two studies. This SNP was initially found to be associated with NAFLD in an adult study. It has been postulated that the variant form of the protein is misfolded, thereby leading to accelerated degradation, which leads to increased intrahepatic fat accumulation and decreased secretion of VLDL from the hepatocyte.²⁷ After *PNPLA3*, the *TM6SF2* SNP is the most studied in NAFLD patients. In a study of 454 children at a pediatric obesity clinic in the United States, Goffredo and colleagues looked at the association between the *TM6SF2* SNP and MRI hepatic fat fraction (HFF).²⁸ The variant allele frequency was 0.061 in Caucasians, 0.033 in African Americans, and 0.089 in Hispanics, which is similar to prior studies. Children carrying the minor allele ($n=92$) had a mean HFF of 11.1%, while children with the wildtype had a mean HFF of 6.7%. When stratified by race and ethnicity, this effect was significant for Caucasian and African American children, but not Hispanic children. Although MRI HFF was not associated with genotype in the Hispanic population, ALT was 26 U/L for wildtype genotype compared to 47 U/L for those carrying the variant allele in Hispanic children. There was no association with ALT in Caucasian or African American children. Additionally, in Caucasians and Hispanics lower low-density lipoprotein, small dense low-density lipoprotein, and very small low-density lipoprotein was observed. In a study of 531

Italian children with obesity and ultrasound evidence of hepatic steatosis, 8.9% were carriers of the variant allele (one child was homozygous), and 89% of children carrying the *TM6SF2* variant allele demonstrated ultrasound evidence of steatosis compared to about 47% with the homozygous wildtype allele.²⁹ There was also an association with ALT, where ALT was 40 in 29% of children who were heterozygous or homozygous for the *TM6SF2* variant allele compared to 16% of children with wild-type allele. In terms of lipids, carriers of the polymorphism had lower total cholesterol (144 mg/dL vs 160 mg/dL), low-density lipoprotein cholesterol (95 mg/dL vs. 85 mg/dL), triglycerides (99 mg/dL vs. 90 mg/dL) and non-high-density lipoprotein cholesterol levels (116 mg/dL vs. 102 mg/dL). [Are these numbers correct as the cited reference (Grandone et al) reports lower LDL, triglycerides, non-high density lipoprotein cholesterol levels in the carrier group. The authors may have reversed the number. Please query them] In addition, the investigators assessed the additive effect of the *PNPLA3* and the *TM6SF2* SNPs. For those patients that were homozygous variant at the *PNPLA3* locus and carriers of the variant *TM6SF2* allele (n=14), the odds ratio of having an elevated ALT was 12.2 (CI 3.8–39.6).

Association with ultrasound evidence of hepatic steatosis in Taiwanese children has been evaluated for the genes *UGT1A1*, *PPARGC1A*, and *HO-1*. *UGT1A1* (uridine-5-diphosphoglucuronosyltransferase 1A1) is responsible for the conjugation of bilirubin in the liver, and defects in this gene are known to cause neonatal hyperbilirubinemia and Gilbert's Syndrome. Two polymorphisms of this gene are common and these polymorphisms were studied in children with obesity from Taiwan. In this population of 234 children, 12% had ultrasound evidence of hepatic steatosis and the *UGT1A1**6 genotype was a protective factor for hepatic steatosis, with an estimated adjusted odds ratio of 0.31 (95% confidence interval: 0.11–0.91). The other polymorphism, *UGT1A1**28, was not associated with hepatic steatosis.³⁰

The *PPARGC1A* (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) gene encodes a protein that regulates oxidative stress, lipogenesis, and gluconeogenesis. The most common polymorphism of this gene has also been associated with diseases in adults including type 2 diabetes, hypertension, obesity, and NAFLD. In the lone pediatric study, this polymorphism was evaluated in 781 Taiwanese children with obesity.³¹ There was a high carrier rate of 68% (532/781). Among the 23% of children with liver ultrasonography consistent with hepatic steatosis, 59% were carriers of the risk allele, which was higher than those with normal liver ultrasound (49% carriers). Carriers of the risk allele also had higher mean ALT, 28.2 U/L, compared to children with the wildtype allele, 22.8 U/L. The association between the *PPARGC1A* polymorphism and ALT level remained significant after controlling for *PNPLA3* genotype.

Heme oxygenase-1 (*HO-1*) plays a role in the oxidative process and its promoter has a GT dinucleotide repeat that modulates transcription in that longer GT repeats decrease gene transcription. In a study of 101 children with obesity from Taiwan, 27% had ultrasound evidence of hepatic steatosis and a higher repeat length was associated with NAFLD where 10/27 with long repeat had NAFLD compared to 1/27 with short repeat with NAFLD. The mean ALT was 30 IU/ml in short repeat and 46 IU/ml in long repeat.³²

Histology—There have been 3 studies thus far evaluating the association between liver histology in children with NAFLD and genetic polymorphisms other than *PNPLA3*. In aggregate, these 3 studies comprise fewer than 300 children.

The next two histology-based studies were conducted in children from Italy; genes evaluated included *CB2* and *LPIN1*. Cannabinoid receptor 2 (*CB2*) is found predominantly outside of the central nervous system and data from murine models suggest that it may have a hepatoprotective role. In a study of 118 Italian children with NAFLD, the *CB2* polymorphism was significantly associated with inflammation, but not with steatosis grade or fibrosis stage.²⁴ Grade 2 inflammation was seen 22% of those that were carriers or homozygous for the risk allele, but not in children with homozygous wildtype alleles. There was no association with ALT. Another Italian study looked at the association between *LPIN1* and pediatric NAFLD.³³ Lipin 1 (*LPIN1*) is a phosphatidate phosphatase that is highly expressed in adipose tissue and is involved in the flux of phospholipids between the liver and adipose tissue. In children homozygous for the variant allele, the prevalence of fibrosis was 30% (3/10) compared to 70% (92/132) in those who were carriers or homozygous wildtype. Also, the frequency distribution of the variant allele was lower in those with NAFLD (7%) compared to controls (14%). Controls were defined as children with ALT < 35 IU/mL in boys and ALT < 30 IU/mL in girls.

DISCUSSION

To date, there have been 22 original research articles on the topic of the genetics of pediatric NAFLD. Based on these studies, the best validated finding is an association of *PNPLA3* rs738409 genotype with higher ALT, which has been observed in multiple populations of children. There have been 3,730 children studied from Asia (Taiwan), Europe (Austria, Finland, Italy) and North America (Mexico). Taken in aggregate, mean ALT activity is 9 U/L higher in children with the GG genotype compared to the CC genotype. Although the effect size on ALT seems small in a clinical context, in a population study, it is often the difference between having normal or elevated ALT. The association of *PNPLA3* with imaging evidence of NAFLD is less well developed. Although there have been 1,366 children studied to date, MRI SFF was measured in about 20% with most studies relying upon ultrasound. Because ultrasound performs poorly for the diagnosis or grading of hepatic steatosis in children, the resulting association data are likely to have large errors in their estimates of effect size.³⁴ The data regarding liver histology in children are inconsistent. There have been 490 children evaluated in 3 studies with positive findings between *PNPLA3* and histologic severity in Italian children and negative findings in American children. Thus substantial gaps remain regarding the role of *PNPLA3* in pediatric NAFLD. The second most studied gene in the context of pediatric NAFLD is *TM6SF2*, as it is the only gene with more than one study. Because the c.449 C>T, p.Glu167Lys variant allele has a much lower frequency, large studies are needed. Although the existing studies include approximately 2,000 children, the number of children actually carrying the variant allele was less than 200. The allele frequency of the *TM6SF2* SNP is much lower than that of *PNPLA3*. For example, for the Hispanic population the allele frequency for *PNPLA3* is around 0.49 and for *TM6SF2* is around 0.09. Thus far, these two studies demonstrate a positive association with *TM6SF2* and NAFLD. Additionally, these two studies have also demonstrated an association

with lower lipid levels such as low-density lipoprotein. However, additional large studies are needed to be able to make generalizable conclusions about its association with NAFLD. In particular, data are needed on the effect size of this genetic association, especially with the severity of liver histology. Studies evaluating cardiovascular outcomes are also necessary given the association with lipid levels.

The genetics of pediatric NAFLD is a relatively new field and as such there is growing need for future studies. Major challenges in study design include adequately powered sample sizes, liver histology, and appropriate control groups. Additionally, replication within a diverse pediatric population is required given the key differences in disease histology by age, race and ethnicity, and sex. Studies that included data on clinical outcomes are also a major need.

More recent studies have been evaluating multiple genetic polymorphisms in the same patients and trying to determine the potential “risk” of NAFLD. Studying multiple genes within the same patient population is likely the best approach, as different races and ethnicities may have different “risk” genes. Future studies of the genetics of pediatric NAFLD should look at multiple genes in a diverse patient population with NAFLD diagnosed histologically. With this information, the field can help determine if certain genotypes carry higher risk of NASH and fibrosis, since these are the histologic outcomes that are associated with the largest risk of patient morbidity. These types of studies could help delineate higher risk populations that require closer follow-up and potential therapeutic management. Future clinical trials can then stratify effectiveness of therapy per patient genotype, as one therapy may not be universally efficacious. Only with these types of studies will we be able to take advantage of the growing field of genetics and make progress in understanding how to best care for and develop treatments for children with NAFLD.

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Key Points

- Genetic polymorphisms play a role in the pathogenesis and severity of pediatric NAFLD.
- The *PNPLA3* I148M variant is associated with higher ALT in children with obesity.
- The *TM6SF2* (rs58542926 c.449 C>T, p.Glu167Lys) variant allele is associated with hepatic steatosis in children.
- Future studies of the genetics of pediatric NAFLD should focus on histologic severity and/or clinical outcomes.
- Replication studies will be important due to the heterogeneity in pediatric NAFLD by age, sex, race, and ethnicity.

Table 1

Association of PNPLA3 with Pediatric NAFLD

Study	Year	Population	Location	N	Age (yrs)	NAFLD criteria	Results
ALT							
Romeo et al. ⁹	2010	Obesity clinic	Italy	475	Mean 10	ALT	<ul style="list-style-type: none"> ALT >30 U/L in 32% with GG and 10% with CC Mean ALT 32 U/L with GG and 21 U/L with CC
Giudice et al. ¹⁰	2011	Childhood obesity service	Italy	1048	2–16	ALT	<ul style="list-style-type: none"> ALT > 40 U/L: GG 33%, CG 17%, CC 13%, elevated ALT GG vs CC: OR 2.97 Mean ALT 38 U/L with GG and 25 U/L with CC
Lin et al. ¹¹	2011	Obese children recruited from school	Taiwan	520	6–18	Ultrasound	<ul style="list-style-type: none"> Mean ALT 31 U/L with GG and 22 U/L with CC. The frequency of the GG genotype was significantly higher in the hepatic steatosis group (25% vs 14%)
Larrieta-Carrasco et al. ¹²	2013	Summer camp	Mexico	1037	6–12	ALT	<ul style="list-style-type: none"> GG genotype with higher ALT in normal weight, overweight and obese children compared to CC in each weight category; overall GG with 3.7 OR for elevated ALT Percentage of children with ALT >35 U/L was 9% CC, 31% CG, and 61% GG
Vitasalo et al. ¹³	2015	Children enrolled in school	Finland	481	6–8	ALT	<ul style="list-style-type: none"> G allele carriers with higher ALT at baseline and higher ALT increase at 2yr f/u if overweight
Mangge et al. ¹⁴	2015	Overweight/obese Caucasians	Austria	169	10–20	ALT	<ul style="list-style-type: none"> Mean ALT 37.5 U/L with GG and 23.5 U/L with CC
IMAGING							
Santoro et al. ¹⁵	2010	Pediatric Obesity Clinic	USA	85	8–18	MRI SFF	<ul style="list-style-type: none"> Carriers of G allele with higher SFF (p=0.04) Results not significant in Hispanic population, only Caucasian and Asian No association with ALT
Goran et al. ¹⁶	2010	Hispanic children in General Clinical Research Center	USA	188	8–18	MRI SFF	<ul style="list-style-type: none"> Mean signal fat fraction was 11% for GG genotype and was 5% for CC genotype
Wang et al. ¹⁷	2016	Overweight and obese children from school	China	1093	7–18	Ultrasound	<ul style="list-style-type: none"> For each G allele present the odds ratio for hepatic steatosis was 1.57 (95% CI 1.15–2.16)

Study	Year	Population	Location	N	Age (yrs)	NAFLD criteria	Results
							In inactive children (physical activity < 1 h/d or sedentary behavior 2 h/d), the percentage of children with steatosis increased for each G allele (CC 13%, CG 18%, GG 28%)
HISTOLOGY							
Rotman et al. ¹⁸	2010	NASH-CRN	USA	223	Mean 12.4	Histology	<ul style="list-style-type: none"> No association was found
Valenti et al. ¹⁹	2010	NAFLD Clinic	Italy	149	6–13	Histology	<ul style="list-style-type: none"> PNPLA3 G allele had 1.9 times the odds for presence of fibrosis (95% CI 1.14–3.45 per number of G alleles) Perivenular fibrosis or higher grade fibrosis in 31% (20/65) of CC, 48% (29/61) of CG, and 74% (17/23) of GG
Rossi et al. ²⁰	2012	NAFLD clinic	Italy	118	Mean 10.2	Histology	<ul style="list-style-type: none"> NASH was present in 0% of CC, 73% of CG, and 100% of GG

Table 2

Genetic Polymorphisms and their Association with Pediatric NAFLD

Study	Gene	Year	Population	Location	N	Age (yrs)	NAFLD criteria	Results
Lin et al. ²⁶	UGT1A1	2009	Obese elementary school children	Taiwan	234	6–13	Ultrasound	<ul style="list-style-type: none"> • UGT1A1*28 genotypes not significantly associated with hepatic steatosis • UGT1A1*6 genotypes associated with lower odds of hepatic steatosis OR 0.31 (95% confidence interval: 0.11–0.91)
El-Koofy et al. ³⁵	MTP and MnsOD	2011	Pediatric Obesity Clinic	Egypt	33	2–15	Histology	<ul style="list-style-type: none"> • 7 patients with NASH, 8 with steatosis, 18 normal • Of those with both the MTP and MnsOD risk genotypes 6/7 had NASH compared to 2/20 controls
Rossi et al. ²⁰	CB2	2012	NAFLD Clinic	Italy	118	Mean 10.2	Histology	<ul style="list-style-type: none"> • CB2 Q63R variant associated with grade 2 inflammation: 0/13 children with the wildtype genotype, 10/46 heterozygous genotype (22%), and 13/59 with the homozygous variant genotype (22%) • No association with ALT
Valenti et al. ²⁹	LPIN1	2012	NAFLD Clinic	Italy	142	Mean 10.2	Histology	<ul style="list-style-type: none"> • Homozygosity for the LPIN1 T allele was a predictor of the absence of histological fibrosis independent of PNPLA3 genotype: homozygous variant fibrosis rate 30% (3/10) compared to 70% (92/132) in those who were carriers or homozygous wildtype
Lin et al. ²⁷	PPARGC1A	2013	Obese children	Taiwan	781	7–18	Ultrasound	<ul style="list-style-type: none"> • Mean ALT 28.2 U/L in carriers of A allele vs 22.8 U/L in non-carriers • Overall carrier rate 68% with 59.3% in those with hepatic steatosis and 49% in those without hepatic steatosis.
Chang et al. ²⁸	HO-1 promoter	2015	Pediatric Obesity Clinic	Taiwan	101	6–17	Ultrasound	<ul style="list-style-type: none"> • Higher GT repeat length in promoter of HO-1 polymorphism was associated with hepatic steatosis: 10/27 with long repeat with NAFLD and 1/27 with short repeat with NAFLD. • Mean ALT 30 U/ml in short repeat and 46 U/ml in long repeat

Study	Gene	Year	Population	Location	N	Age (yrs)	NAFLD criteria	Results
Wang et al. ²¹	HIF3A methylation	2015	Obese and overweight children and normal weight controls	China	212	7–17	ALT	<ul style="list-style-type: none"> ALT associated with DNA methylation – correlation coefficient 0.263
Goffredo et al. ²⁴	TM6SF2	2016	Pediatric Obesity Clinic	USA	454	Mean 13	MRI SFF	<ul style="list-style-type: none"> Subjects carrying minor allele showed a higher SFF in children of Black or White race, but not Hispanic ethnicity Overall, those carrying the minor allele (n=92) had a mean SFF of 11.1%; children with wildtype SFF 6.7% Mean ALT was 26 U/L for wildtype genotype and 47 U/L for those carrying the variant allele in Hispanics
Grandone et al. ²⁵	TM6SF2	2016	Pediatric Obesity Clinic	Italy	531	4–16	Ultrasound	<ul style="list-style-type: none"> 8.9% were carriers of allele, only 1 patient was homozygous Hepatic steatosis was present in 40/45 children with the variant allele versus 227/486 children homozygous for the wildtype allele ALT 40 U/L in 29% of children with the variant allele and in 16% of children with wildtype allele
Vitasalo et al. ²²	MBOAT7	2016	School children in 1 st grade	Finland	467	6–9	ALT	<ul style="list-style-type: none"> Carriers had an ALT that was 7% higher than non-carriers (17.8 U/L vs. 19.1 U/L) Carriers of MBOAT7, TM6SF2, and PNPLA3 – ALT 32 U/L compared to 19 U/L if carrier of MBOAT7 alone.