Insights into pediatric non-alcoholic fatty liver disease from genetic variants

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; SNP, single nucleotide polymorphism; PNPLA3, patatin-like phospholipase domain-containing protein 3; GCKR, glucokinase regulator; TM6SF2, Transmembrane 6 superfamily 2; TMC4, transmembrane channel-like protein 4; MBOAT7, membrane-bound O-acyltransferase domain-containing protein 7; LPIN1, lipin 1; UCP2, uncoupling protein 2; ACTR5, actin-related protein 5; CNR2, cannabinoid receptor 2; ETS1, ETS proto-oncogene 1; IL18RAP, Interleukin 18 receptor accessory protein; IRS-1, insulin receptor substrate 1; KLF6, Kruppel-like factor 6; MnSOD, manganese superoxide dismutase; MTP, microsomal triglyceride transfer protein; PPARGC1A, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; SDK1, Sidekick cell adhesion molecule 1; TNFA, tumour necrosis factor alpha; TRAPPC9, trafficking Protein Particle Complex 9; UGT1A1, UDP-glucuronosyltransferase 1A1; LITMUS, Liver Investigation: Testing Marker Utility in Steatohepatitis; RDH16, retinol dehydrogenase 16; HSC, hepatic stellate cell; RBP4, retinol-binding protein 4; RXR, retinoid X receptor; HSD17β13, 17-beta-hydroxysteroid dehydrogenase 13; LYPLAL1, Lysophospholipase Like 1; PPP1R3B, Protein Phosphatase 1 Regulatory Subunit 3B.

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Non-alcoholic fatty liver disease (NAFLD) is a multifactorial condition with a significant genetic contribution to its pathogenesis and progression. Over the past decade, there has been much investigation into genetic variants associated with NAFLD in adults[1] but there have been only a handful of studies focusing on children[2]. In this issue, Hudert et al.[3] combines candidate single nucleotide polymorphism (SNP) testing with proteomics and computational modelling in a cohort of 70 children with biopsy-proven NAFLD to deepen our understanding of these genetic variants. Exploring the role of genetic variants in pediatric NAFLD is particularly important due to its unique histology with periportal/zone 1-predominant localisation of steatosis and inflammation, which is associated with more advanced fibrosis[4].

Hudert et al.[3] selected 14 SNPs previously associated with adult NAFLD, of which 3 were significantly associated with disease in pediatric NAFLD compared to a control group: rs738409C>G in PNPLA3, rs1044498A>C in ENPP1 and rs780094C>T in GCKR, but not rs58542926C>T near TM6SF2 or rs641738C>T near TMC4-MBOAT7.

When SNP-histology associations were examined, rs738409C>G in PNPLA3 and rs13412852C>T near lipin 1 (LPIN1) were significantly associated with the severity of steatosis. Critically, PNPLA3 was associated with periportal/zone 1-predominant steatosis (Figure 1). These histological features are relatively specific to NAFLD in children, particularly those who are pre-pubertal. The same SNP was also associated with an increased risk of progression to fibrosis, along with rs780094C>T in GCKR, rs1260326C>T in GCKR, and rs659366C>T near UCP2.

rs738409C>G in PNPLA3 is recognised as the most important genetic variant in NAFLD. Whilst there have been some conflicting reports, data from several pediatric studies support these findings (Table 1). However, this is the first identification of a genetic variant associated with periportal localisation of disease. What causes the periportal predominant histology of pediatric NAFLD is unclear. Hypotheses have included dietary factors, circulating androgens, and intestinal dysbiosis. It is known that zonal hepatocyte specification causes altered lipid accumulation in NAFLD[5]. It is likely that the Ile148Met variant in PNPLA3 is one factor that primes the periportal area for steatosis and lipotoxicity, which is exacerbated in the context of obesity and insulin resistance, similar to findings in adults[6].

This is the first study to show a correlation between GCKR rs780094C>T and presence of fibrosis in pediatric NAFLD. rs780094C>T in GCKR is well established as a pleomorphic variant associated with insulin resistance and multiple metabolic traits[7]. The group from Berlin went on to perform proteomic analysis using mass spectrometry. rs780094C>T was correlated with decreased hepatic GCKR protein. GCKR regulates the activity of the glucokinase enzyme by forming an inactive complex with the enzyme and transporting it from the cytoplasm to the nucleus; thus, decreased levels of GCKR allow increased glucokinase activity. Pathway enrichment analysis linked the GCKR rs780094 T/T genotype to altered lipid metabolism and mathematical modelling suggested that increased glucokinase activity leads to hepatic fat accumulation, *de novo* fatty acid synthesis and increased glucose uptake. The same variant has been similarly implicated in adult disease, where it has also been associated with increased intrahepatic fat accumulation, risk of NAFLD, and fibrosis[8]. It will be interesting to see whether rs780094C>T achieves genome- or exome-wide significance in larger studies of histologic NAFLD, such as from the European NAFLD Registry / LITMUS consortium[9].

In this study, proteomics revealed that the PNPLA3 SNP resulted in decreased retinol metabolism and decreased hepatic protein levels of retinol dehydrogenase 16 (RDH16), where lower RDH16 levels also correlated with the severity of fibrosis. Precisely how this PNPLA3 variant alters retinol metabolism is unclear. Retinol is a lipid-soluble nutrient that is stored mostly as retinyl esters in lipid droplets of hepatic stellate cells (HSCs). PNPLA3 encodes adiponutrin, an enzyme with retinyl-palmitate lipase function that has been shown to cause retinol release from lipid droplets in response to insulin in HSCs *in vitro* and *ex vivo*[10]. This retinyl-palmitate lipase function is reduced with the PNPLA3 polymorphism, resulting in reduced retinol release from HSCs[10]. Evidence suggesting that wild-type PNPLA3-mediated retinol release reduces secretion of extracellular matrix metalloproteases[11] could explain the relationship of retinol metabolism and liver fibrosis.

Hudert et al. also confirm that hepatic levels of retinol-binding protein 4 (RBP4) are independently associated with fibrosis, while serum RBP4 levels are negatively associated with fibrosis. Studies have linked alterations in liver molecular trafficking and protein catabolism in NAFLD and hepatitis C-associated cirrhosis to accumulation of RBP4[12]. RBP4 was proposed to encourage hepatic steatosis by interfering with the RXR-retinol interaction and to act on adipocytes to interfere with insulin signalling[12]. These hypotheses require further evaluation, but could provide some explanation as to why PNPLA3 Ile148Met is so strongly associated with fibrosis compared to other genetic variants associated with steatosis.

rs72613567T>TA in HSD17β13 is the most recently described genetic variant associated with NAFLD[13]. This variant has a protective function, reducing risk of cirrhosis and severity of histological NAFLD. It also seems to mitigate the harmful effect of rs738409C>G in PNPLA3. Interestingly, recent data have suggested that HSD17β13 acts as a lipid droplet retinol dehydrodgenase[14]. The current study was unable to test for this SNP but the combination of these data may provide a mechanism for interaction between HSD17β13 and PNPLA3 through retinol metabolism.

UCP2 is an inner mitochondrial membrane protein that is expressed in adipose tissue and liver. Akin to GCKR, variants in UCP2 have been associated with many metabolic traits including adiposity[15], BMI, and insulin resistance, in addition to NAFLD[16]. This is the first study to identify a role in pediatric NAFLD. Modelling implicates reduced UCP2 in increased hepatic triacylglycerol storage, as would be expected if uncoupling were reduced. Similar data exists for ENPP1, which is known to modulate insulin receptor signalling and obesity[17], but has relatively little data supporting an effect on NAFLD histology. It remains unclear to what extent these variants have a specific effect on NAFLD or reflect changes in adipose dysfunction and insulin resistance.

There are some notable differences between this study's findings and existing literature in adults. rs58542926C>T near TM6SF2 has been associated with steatosis and fibrosis progression in adults[18]. This SNP was not associated with histological severity in this current study, perhaps given its low allele frequency and the relatively small cohort. Similarly, rs641738C>T near TMC4-MBOAT7 was only weakly associated with fibrosis, though some data on this genetic variant in adults is conflicting[19]. No effect was found for rs12137855C>T near LYPLAL1, which had genome-wide significance effect on hepatic fat content in adults and rs4240624 G>A near PPP1R3B was not assessed in this cohort[20]. It will be interesting to see in larger studies whether these reflect a true difference in the genetic aetiology between pediatric and adult disease.

Though this study advances our understanding of genetics and histology, it is limited by a small sample size, which reduces the number of variables that can be controlled for in analyses. This is a common theme pediatric studies as the combination of histology and DNA for genotyping is rarely available. A further consideration for these data is the single (tertiary) centre nature of the cohort, which may bias the spectrum of histology seen. Therefore, the generalisability of these findings may be limited in cohorts of different genetic ancestries.

Summary and future directions

These data complement our understanding of genetic variants in pediatric NAFLD. Firstly, PNPLA3 has zonal-specific effects on histology, suggesting that this variant primes the periportal region for more severe NAFLD in children, but the other factors involved are still unclear. This, and other recent data, provide a possible link between PNPLA3 and HSD17β13 via retinol metabolism. More generally, there are significant differences between genetic variants in children and adults. Whilst these may be influenced by sample size and natural history of the disease, these data support the notion that the pathophysiology of pediatric NAFLD is, at least partially, distinct from that of adults. The results of larger studies, including exome and genome sequencing, will shed further light on these questions.

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Figure legends:

Figure 1. Genetic variants associated with zonal-specific histology in pediatric NAFLD. Unlike adult NASH, pediatric NAFLD shows periportal predominant histology with a lack of ballooning, particularly in pre-pubertal children. Hudert et al[3] found common genetic variants in several genes to correlate with periportal histological features. Other variants were associated with histology without a zonal pattern.

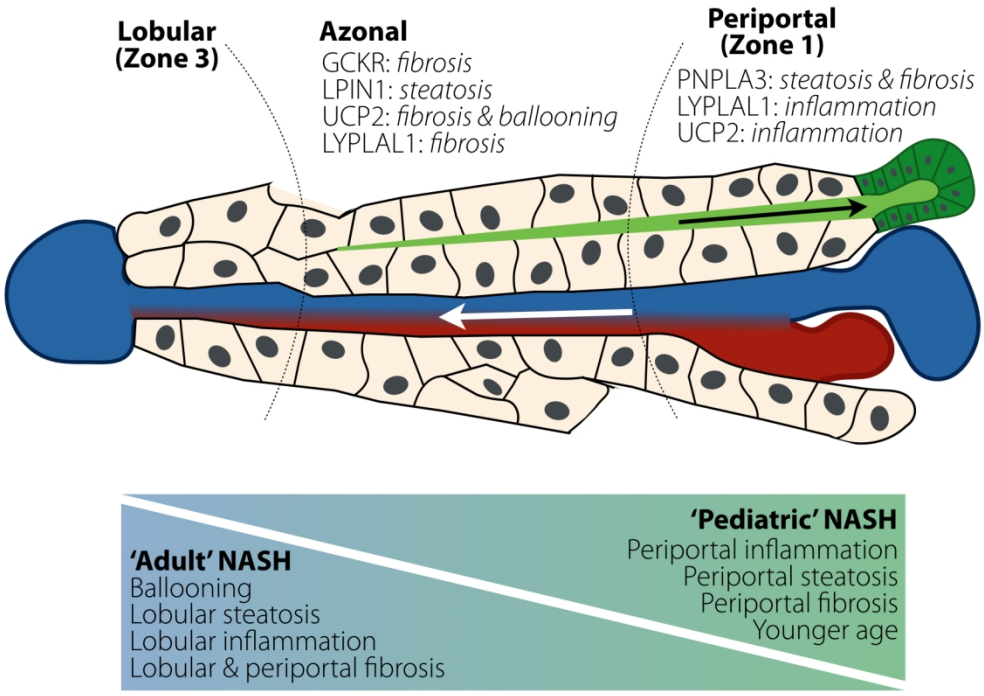
Table legends:

Table 1. Common genetic variants associated with radiological or histological NAFLD in children from candidate gene studies and genome-wide association studies. See Supplementary Material for full references.

Gene *Polymorphism*	Chr: bp	Variant(s)	Study	Population	NAFLD assessment modality	Results
ACTR5 *rs6128907* (T>C) *rs6124026* (A>G) *rs6128918* (G>A)	20: 37387862 20: 37399987 20: 37391432	Intronic	Wattacheril et al, 2017	Hispanic boys within NASH Clinical Research Network (CRN) sample Median age: 12.0 n = 208	Biopsy	Three variants (rs6128907[C], rs6124026[G], rs6128918[A]) associated with fibrosis
CNR2 *rs35761398* (CAA/CGG)	1: 23875429	Q63R	Rossi et al, 2012	Italian hospital Mean age: 10.2 n = 118	Biopsy	CB2 Q63R variant associated with severity of inflammation and presence of NASH
ENPP1 rs1044498 (A>C)	6: 131851228	L121G	Hudert et al, 2018	Berlin adolescent NAFLD cohort Age = 10-17 n = 70	Biopsy	ENPP1 rs1044498 [C] variant associated with NAFLD
ETS1 rs3935794 (A>G)	11: 128390677	Intronic	Wattacheril et al, 2017	Hispanic boys within NASH Clinical Research Network (CRN) sample Median age: 12.0 n = 208	Biopsy	ETS1 rs3935794 [G] variant associated with fibrosis
GCKR rs780094 (C>T) rs1260326 (C>T) (in linkage disequilibrium)	2: 27518370 2: 27508073	P446L	Hudert et al, 2018	Berlin adolescent NAFLD cohort Age = 10-17 n = 70	Biopsy	GCKR rs780094 [T] variant associated with NAFLD and decreased levels of GCKR protein GCKR rs780094 [T] and rs1260326 [T] variants associated with fibrosis and decreased levels of GCKR protein
			Lin et al, 2014	Obese Taiwanese children Age = 7-18 n = 797	Ultrasound	GCKR rs780094 [T] variant associated with increased risk of NAFLD
IL18RAP rs11465670 (T>C)	2: 103034440		Wattacheril et al, 2017	Hispanic boys within NASH Clinical Research Network (CRN) sample Median age: 12.0 n = 208	Biopsy	IL18RAP rs14465670 [C] variant associated with fibrosis
IRS-1 rs1801278 (A>G)	2: 226795828	G972A	Dongiovanni et al, 2010	Italian children Mean Age = 11 n = 71	Biopsy	rs1801278 variant associated with increased risk of fibrosis (however, only 2 patients were staged as F>1)
KLF6 rs3750861 (C/T)	10: 3782241	IVS1-27A	Nobili et al 2014	Italian hospital Age = 6-18 n = 152	Biopsy	IVS1-27A variant associated with reduced risk of fibrosis
LPIN1 rs13412852 (C>T)	2: 11774815	Intronic	Valenti et al, 2012	Italian hospital Mean age: 10.2 n = 142	Biopsy	LPIN1 rs13412852 [T] variant associated with reduced NAFLD severity and lower prevalence of fibrosis
			Hudert et al, 2018	Berlin adolescent NAFLD cohort Age = 10-17 n = 70	Biopsy	LPIN1 rs13412852 [T] variant associated with steatosis
MnSOD rs4880 (T>C)	6: 159692840	A16V	El-Koofy et al, 2018	Egyptian paediatric obesity clinic Age = 2-15 n = 76	Biopsy	NASH patients had a higher incidence of the rs4880 (T) variant
MTP promoter rs1800591 (G>T)	4: 99574331		El-Koofy et al, 2018	Egyptian paediatric obesity clinic Age = 2-15 n = 76	Biopsy	NASH patients had a higher incidence of the [G] variant
PNPLA3 rs738409 C>G	22: 43928847	I148M	Hudert et al, 2018	Berlin adolescent NAFLD cohort Age = 10-17 n = 70	Biopsy	PNPLA3 rs738407 [G] variant associated with severity of steatosis, zone 1/periportal disease and fibrosis PNPLA3 rs738407 [G] variant associated with decreased retinol metabolism
			Rotman et al, 2010	NIH centre patients Mean age = 12.4 n = 223	Biopsy	No association between PNPLA3 rs738407 [G] variant with histological parameters PNPLA3 rs738407 [G] variant associated with a younger age of presentation
			Valenti et al, 2010	Italian hospital Age: 6-13 n = 149	Biopsy	PNPLA3 rs738409 [G] variant strongly associated with steatosis severity, hepatocellular ballooning, lobular inflammation and presence of fibrosis No association with PNPLA3 and periportal fibrosis
PPARGC1A rs8192678 (G>A)	4: 23814039	G482S	Lin et al, 2013	Taiwanese obese children Age: 7-18 n = 781	Ultrasound	PPARGC1A rs8192678 [A] variant associated with NAFLD
RAB37 rs12942311 (T>C)	17: 72710796	Intronic	Wattacheril et al, 2017	Hispanic boys within NASH Clinical Research Network (CRN) sample Median age: 12.0 n = 208	Biopsy	RAB37 rs12942311 [C] variant associated with fibrosis
SDK1 rs688020 (T>C)	7: 4228553		Wattacheril et al, 2017	Hispanic boys within NASH Clinical Research Network (CRN) sample	Biopsy	SDK1 rs688020 [C] variant associated with fibrosis

				Median age: 12.0 n = 208		
TM6SF2 rs58542926 (C>T)	19: 19268740	E167K	Goffredo et al, 2016	American paediatric obesity clinic Mean age: 13 n = 454	MRI	TM6SF2 rs58542926 [T] variant associated with high hepatic fat content in Caucasians and African Americans, but not Hispanics
			Dongiovanni et al, 2015	Patients from four centres: Milan, Palermo, Rome, Kuopio n = 1201	Biopsy	TM6SF2 rs58542926 [T] variant associated with increased risk of NASH, advanced fibrosis, increased serum aminotransferase levels, decreased serum lipid levels
TNFA promoter *TNF-α -238/rs361525* (G>A) *TNF-α -308/rs1800629* (G>A)	6: 31575324 6: 31575254		Yang et al, 2012	South Korean hospital n = 111	Biopsy	No association between TNF-α variants and increased risk of NAFLD Two TNF-α variants (G308A, G238A) associated with insulin resistance
TRAPPC9 rs11166927 (C>T) rs11166926 (G>A) rs2242181 (T>C) rs7836476 (T>C)	8: 140796420 8: 140795752 8: 140819819 8: 140819819	?	Wattacheril et al, 2017	Hispanic boys within the NASH Clinical Research Network (CRN) sample Median age: 12.0 n = 208	Biopsy	Four TTRAPC9 variants (rs11166927, rs11166926, rs2242181, rs7836476) associated with increased NAS score
UCP2 rs659366 (C>T)	11: 73983709	G866A	Hudert et al, 2018	Berlin adolescent NAFLD cohort Age = 10-17 n = 70	Biopsy	UCP2 rs659366 [T] variant associated with fibrosis
UGT1A1 rs4148323 (G>A)	2: 233760498	G71R	Lin et al, 2009	Taiwanese obese children Age: 6-13 n = 234	Ultrasound	UGT1A1*6 rs4148323 [A] variant associated with a reduced risk of NAFLD

Table 1. Common genetic variants associated with radiological or histological NAFLD in children from candidate gene studies and genome-wide association studies. See Supplementary Material for full references.



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