World Journal of Gastroenterology

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DOI:10.3748/wjg.v20.i23.7347

World J Gastroenterol 2014 June 21; 20(23): 7347-7355
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (12): Nonalcoholic fatty liver disease

Pediatric fatty liver disease: Role of ethnicity and genetics

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Author contributions: The authors conceived and wrote the manuscript.
Supported by The American Heart Association (13SDG14640038) and 2012 Yale Center for Clinical Investigation cholar award to Santoro N; This publication was also made possible by CTSA Grant Number UL1 RR024139 from the National Center for Advancing Translational Science, a component of the National Institutes of Health (NIH), and NIH roadmap for Medical Research, Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIH
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Received: October 8, 2013 Revised: January 4, 2014
Accepted: January 20, 2014
Published online: June 21, 2014

Abstract

Non-alcoholic fatty liver disease (NAFLD) comprehends a wide range of conditions, encompassing from fatty liver or steatohepatitis with or without fibrosis, to cirrhosis and its complications. NAFLD has become the most common form of liver disease in childhood as its prevalence has more than doubled over the past 20 years, paralleling the increased prevalence of childhood obesity. It currently affects between 3% and 11% of the pediatric population reaching the rate of 46% among overweight and obese children and adolescents. The prevalence of hepatic steatosis varies among different ethnic groups. The ethnic group with the highest prevalence is the Hispanic one followed by the Caucasian and the African-American. This evidence suggests that there is a strong genetic background in the predisposition to fatty liver. In fact, since 2008 several common gene variants have been implicated in the pathogenesis of fatty liver disease. The most important is probably the patatin like phospholipase containing domain 3 gene (*PNPLA3*) discovered by the Hobbs' group in 2008. This article reviews the current knowledge regarding the role of ethnicity and genetics in pathogenesis of pediatric fatty liver.

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Key words: Non alcoholic fatty liver disease; Ethnicity; Patatin like phospholipase containing domain 3 gene; Obesity; Insulin resistance; Glucokinase regulatory protein; Apolipoprotein C3 gene; Farnesyl-diphosphate farnesyltransferase 1

Core tip: The prevalence of hepatic steatosis varies among different ethnic groups. Ethnicity with the greatest prevalence of non-alcoholic fatty liver disease (NAFLD) is the Hispanic one followed by Caucasian and then African-Americans. NAFLD exhibits tight links with insulin resistance and metabolic syndrome. Several gene variants have been so far identified by Genome Wide Association Studies or by a candidate gene approach as associated with fatty liver disease. The *PNPLA3* rs738409 and the *GCKR* rs1260326 are the strongest variants associated with fatty liver in paediatrics.

Marzuillo P, Miraglia del Giudice E, Santoro N. Pediatric fatty liver disease: Role of ethnicity and genetics. *World J Gastroenterol* 2014; 20(23): 7347-7355 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i23/7347.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i23.7347

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) comprehends

a wide range of conditions, encompassing from fatty liver or steatohepatitis (NASH) with or without fibrosis, to cirrhosis and its complications (*e.g.*, hepatocellular carcinoma and portal hypertension)[1,2]. The NAFLD diagnostic criteria are similar in adults and children: infiltration of more than 5% of hepatocytes, as confirmed by liver histology, in patients with no or low daily alcohol utilization and in absence of either viral, autoimmune or drug-induced liver disease[3-5]. NAFLD has become the most relevant form of liver disease in childhood[6] and its prevalence has highly increased over the past 20 years because of the increased obesity prevalence in children. It currently affects between 3% and 11% of the pediatric population[7,8] reaching the rate of 46% among overweight and obese children and adolescents[6]. Therefore, the screening for NAFLD should be recommended to overweight and obese children[9-11]. The liver histology is the gold standard for NAFLD diagnosis, but to perform biopsies isn't possible in all the cases. Liver enzymes values [aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] are usually slightly elevated in children with steatosis without other causes of fatty liver[12]. Therefore, high serum AST and ALT levels, although they frequently do not well represent the grade of intrahepatic damage, are used as a non-invasive screening for pediatric NAFLD[13] along with liver ultrasound (US), that can detect the disease when steatosis involves > 20% of hepatocytes[14]. Although it does not represent the imaging gold standard, performing liver ultrasound has different advantages as screening : (1) relative cheapness; (2) massive expansion among pediatricians; and (3) practicability in the pediatric population[15]. Furthermore, very recently, in a large prospective pediatric cohort, it has been shown a good correlation between ultrasonographic steatosis score and the grade of fatty liver assessed by hepatic histology[14]. Computed tomography (CT) scan is not recommended in pediatric population because of the unjustifiable radiation related to the process. Magnetic resonance spectroscopy (MRS) and magnetic resonance Imaging (MRI) have been demonstrated to be the best methods to assess and quantify the amount of lipids present into the liver[16].

We reviewed the literature concerning the role of ethnicity and genetics in the pathogenesis of pediatric fatty liver disease.

PREVALENCE OF NAFLD AMONG ETHNIC GROUPS

Considering the global population in United States, Browning *et al*[17] described that the prevalence of NAFLD is the highest in the American Hispanic population (45%) and the lowest among African Americans (24%), with the Caucasians showing a midway prevalence (33%). The fatty liver prevalence in Europe, Australia, and Middle East encompasses from 20% to 30%[18]. On the basis of recent studies the NAFLD prevalence in Japan and China, such as Latin America, is comparable to the European prevalence (20%-30% in Japan and 15%-30% in China, respectively)[18]. In India, the fatty liver prevalence in urban populations encompasses from 16% to 32%; but in rural India, where there are traditional diets and lifestyles, the prevalence is lower (about 9%)[18]. About the prevalence of NAFLD in Africa there are few data. A Nigerian study estimated the prevalence to be about 9%[18]. This evidence suggests that a sedentary lifestyle and globalization of Western diet could be associated with an increase in the fatty liver prevalence in developing nations.

RISK FACTORS

The principal risk factor for fatty liver in childhood is the obesity. In fact, the pediatric prevalence of NAFLD is particularly high in those countries where childhood obesity is widespread[6,19]. Pediatric NAFLD is also highly correlated with insulin resistance and type 2 diabetes mellitus[20,21]. A high percentage (from 20% to 80%) of children with NAFLD may show associated hypertriglyceridemia and high LDL levels[22]. The prevalence of NAFLD increases in pre-diabetic children, and the subjects affected by NASH have an higher grade of insulin resistance than the individuals with simple fatty liver[8]. The NAFLD can also affect very young children, but its prevalence is higher in adolescents[23]. In fact, sex hormones and insulin resistance in puberty[24,25] and, moreover, the increased propensity for unhealthy food choices and sedentary lifestyle typical of the adolescents[26] can justify the higher rate of NAFLD in adolescents. In all the ethnicity, NAFLD is more prevalent in boys than in girls[22] with a male to female ratio of 2:1. This may be explained by the liver-protective role of estrogens, as well as by the potentially negative role of androgens in aggravating NAFLD[27,28]. Another risk factor that can promote the development of fatty liver is the excessive fructose consumption, in particular the fructose contained in the most common soda[29]. Substantial links have been demonstrated between increased fructose consumption and obesity, dyslipidemia, and insulin resistance (IR). The link between fructose ingestion and NAFLD is mainly explained by an increased hepatic *de novo* lipogenesis[30].

ROLE OF ETHNICITY IN DETERMINING HEPATIC STEATOSIS

The prevalence of hepatic steatosis varies among different ethnic groups. As previously shown, ethnicity with the greater prevalence is the Hispanic one followed by Caucasians and African-Americans[17]. NAFLD exhibits tight links with insulin resistance and metabolic syndrome (MS). It is, therefore, surprising that African-Americans, despite showing a similar or even higher degree of IR than Caucasians and Hispanics, have a lower prevalence of NAFLD[31] and a lower propensity for development of NASH[32]. The dissociation between fatty

liver and insulin resistance in African-Americans suggests that this group is protected from hepatic fat accumulation even in presence of IR. Browning *et al*[17] demonstrated that the ethnic differences in the prevalence of hepatic steatosis were not due to differences in the presence of risk factors for NAFLD such as increased body mass index (BMI), reduced insulin sensivity or ethanol ingestion. On the other, although there are no differences in the prevalence of risk factors among ethnicities, there are evident differences in body fat distribution especially concerning the three major fat depots (intraperitoneal, abdominal subcutaneous, and lower extremity)[33]. In fact, African Americans tend to accumulate less intravisceral fat, but more subcutaneous and mainly more gluteal fat than the other ethnic groups. Different is the association between regional adiposity and hepatic fat content. In fact, intra-peritoneal and lower extremities adiposity are strongly correlated with intra-hepatic fat, regardless of ethnicity[33].

While subjects with African ancestries have a low propensity to develop fatty liver, there are other ethnicities/races with a higher propensity to liver fat accumulation such as the subjects with Japanese descents. In fact, in a recent study, Azuma *et al*[34] considered ethnic difference in liver fat content among Japanese American in Hawaii, Japanese in Japan, and non-Hispanic whites in United States. Despite of a very similar BMI, compared with non Hispanic whites, Japanese-Americans had higher liver fat content which tended to become more significant with increasing BMI[34]. On the other hand, compared with Japanese, Japanese-Americans had a lower liver fat content, regardless of BMI[34].

What determines ethnic differences in hepatic steatosis is actually unknown.

In conclusion, insulin resistance and metabolic syndrome play a pivotal role in determining hepatic steatosis but they cannot explain such diversity among ethnic groups. In fact, as previously reported[17], the different ethnic predisposition to accumulate regional fat could partially explain the different ethnic prevalence of hepatic steatosis, since African-Americans have lower intraperitoneal fat accumulation than Hispanics and Caucasians. Therefore, the crucial issue to be resolved is why there is no association, in African-Americans, between the degree of IR and the degree of intra-hepatic fat. Is it possible that the hepatic steatosis does not play such an important role as we believe in developing IR? Why African-Americans despite high degree of IR have lower prevalence of NAFLD than other ethnic groups? Are there gene polymorphisms that could explain these paradoxes?

ROLE OF GENETICS IN DETERMINING HEPATIC STEATOSIS

PNPLA3 rs738409

The most important gene involved in determining hepatic steatosis is the patatin like phospholipase containing domain 3 gene (*PNPLA3*). The *PNPLA3* rs738409 single nucleotide polymorphism (SNP) is a non-synonymous variant, represented by a cytosine to guanosine substitution which encodes an isoleucine to methionine substitution at the amino acid position 148 (I148M) and was showed associated with NAFLD in a multiethnic cohort of adults[35] and children[36,37]. The *PNPLA3* encodes for the adiponutrin an enzyme present in the liver and adipose tissue showing both a lipogenic and lipolytic activity *in vitro*. The prevalence of the *PNPLA3* rs738409 minor allele (G) is 0.460 in Hispanics, 0.305 in Caucasians and 0.186 in African Americans[35].

It has been shown that this variant interacts with environmental factors (*i.e.*, obesity[37,38] and alcohol consumption[39]) that can themselves promote steatosis. In fact, these stressors seem to reveal the association between the rs738409 minor allele (G) and hepatic damage in populations in whom it is otherwise hidden[40]. It is interesting to underline that the same interaction appears with some nutrients. Indeed, the total carbohydrate and high omega (n)-6 to n-3 polyunsaturated fatty acids (PUFA) ratio can enhance the association between steatosis and *PNPLA3* variant[41].

Since the association between the *PNPLA3* rs738409 and fatty liver has been shown[35], a few researches tried to demonstrate this association physiopathology. Probably, this variant may lead to a gain of function of the protein, which could act as a lipogenic factor[42]. In fact, there is evidence that, administrating the mutated *PNPLA3* to knock-out mice for *PNPLA3* through viral vectors[43,44], the knock-out mice obtain a higher susceptibility to fatty liver[42]. Consistently, it was also shown that sterol regulatory element binding transcription factor 1 (*SREBP-1c*), activated by carbohydrate feeding, transcriptionally activates *PNPLA3* and other genes which encodes enzymes implicated in the fatty acid biosynthetic pathway[45]. Other researches demonstrating an interaction between the carbohydrates intake and the *PNPLA3* rs738409 in developing of NAFLD appear to support this mechanism[41]. However, the lack of association of the *PNPLA3* variant with increased plasma triglycerides is in contrast with this hypothesis[35,36].

Probably more interesting are the data deriving by evidence on the hydrolytic action of the *PNPLA3* given that the *PNPLA3* along with the acylglycerol transacetylase activity also has a triacylglycerol hydrolase function[46]. In fact, it has been recently showed that the rare allele could cause a lack of the *PNPLA3* hydrolytic function[46] reducing the protein capacity in hydrolyzing the n-9 of about 15%[46]. The n-9 are the most common fatty acids deriving from the diet (meat, olive oil, sesame oil, almonds, and avocados) but they can derive also being synthesized from essential polyunsaturated fatty acids such as the n-6[47]. In addition, Perttila *et al*[48] demonstrated that the *PNPLA3* 148M allele significantly slows down the triglycerides hydrolysis and then increases the cellular accumulation of triglycerides in presence of an excess free fatty acids (FFA). This might also explain the association between the PNPLA3 variant and the

Table 1 Gene variants associated with fatty liver disease identified by Genome Wide Association Studies

Ref.	Gene	Polymorphisms	Chromosome	Number of subjects studied
Romeo *et al*[35]	*PNPLA3*	rs738409	22	3383
Speliotes *et al*[71]	*GCKR*	rs1260326	2	7176
Petersen *et al*[60]	*APOC3*	rs2854116	11	258
		rs2854117		
Speliotes *et al*[71]	*NCAN*	rs2228603	19	7176
Speliotes *et al*[71]	*LYPLAL1*	rs12137855	1	7176
Speliotes *et al*[71]	*PPP1R3B*	rs4240624	8	7176
Adams *et al*[73]	*GC*	rs222054	4	928
Adams *et al*[73]	*LCP1*	rs7324845	13	928
Adams *et al*[73]	*SLC38A8*	rs11864146	16	928
Adams *et al*[73]	*LPPR4*	rs12743824	1	928
Kitamoto *et al*[72]	*SAMM50*	rs2143571	22	1326
Kitamoto *et al*[72]	*PARVB*	rs6006473	22	1326
		rs5764455		
		rs6006611		
Chalasani *et al*[66]	*FDFT1*	rs2645424	8	236

dietary lipids in modulating liver injury[49]. More recently, Li *et al*[50], generating mutant *PNPLA3* mice for I148M polymorphism, showed that the *PNPLA3* variant is associated with an increased formation of fatty acids and triacylglycerol and relative depletion of triacylglycerol long-chain polyunsaturated fatty acids. Metabolic studies in the transgenic mice showed that high level expression of *PNPLA3* I148M only in the liver and not in adipose tissue, affected both hepatic triacylglycerol (TAG) synthesis and catabolism. Also, it is interesting to note that *PNPLA3* I148M transgenic mice develop steatosis on a sucrose diet but not on a high-fat diet[51].

Interestingly, Speliotes *et al*[52] showed, among patient selected for NAFLD, that the G allele of the *PNPLA3* rs738409 polymorphism is associated with a favourable metabolic profile including decrease triglyceride levels, BMI, waist circumference and weight. These results argue strongly against rs738409 *PNPLA3* polymorphism increasing risk of NAFLD indirectly through an effect of these components of metabolic syndrome[52]. Despite this evidence, the effect of this polymorphism on liver damage is driven by the amount of visceral fat[37], and it has been demonstrated that weight loss reduce the effect of this polymorphism in obese children[53]. For this reason, the possibility exists that the association between the *PNPLA3* rs738409 SNP and NAFLD is modulated by the degree and the distribution of adiposity; this might explain the differences observed in the rs738409 *PNPLA3* phenotype.

Therefore, the *PNPLA3* I148M polymorphism increases the risk of NAFLD without a strong effect on metabolic syndrome components[52] but the abdominal fat, strictly correlated to metabolic syndrome components, can drive the effect of this polymorphism on liver damage. The weight loss, in fact, reduces the effect of this polymorphism[53].

GCKR rs1260326

Another gene that acts together with *PNPLA3* in determining hepatic steatosis is the Glucokinase Regulatory Protein (*GCKR*) gene[54] which encodes for the glucokinase regulatory protein (GCKRP). The GCKRP inhibits the glucokinase (GCK) activity competing with the glucose, substrate of GCK[55-57]. The rs1260326 in the *GCKR* gene is a functionally relevant SNP consisting of a C to T substitution encoding for a proline-to-leucine substitution at position 446 (P446L). It has been demonstrated that the GCKRP L466 variant encodes for a protein that has decreased regulation by physiological concentration of fructose 6 phosphate. This results indirectly in a constant increased GCK activity[58]. The increase in GCK hepatic activity leads to enhancement of the glycolytic flux, and then promotes hepatic glucose metabolism and raises the concentrations of malonyl coenzyme A, a substrate for *de novo* lipogenesis (DNL). DNL may contribute for about 20% in liver fat accumulation[59]. The *GCKR* SNP rs1260326 minor allele (T) frequency was 0.466 in Caucasians, 0.129 in African Americans, and 0.355 in the Hispanics ($P < 0.0001$)[54]. This allele is associated with higher fat content in the liver among all ethnic groups[54]. Therefore, the possibility exists that the lowest prevalence of *PNPLA3* rs738409 minor allele and *GCKR* rs1260326 minor allele (T) in African Americans could explain the lowest prevalence of hepatic steatosis in this ethnic group regardless of the unfavourable metabolic profile.

APOC3 rs2854116 and rs2854117

Apolipoprotein C3 gene (*APOC3*) variants are also involved, probably, in determining NAFLD. Petersen *et al*[60] showed that two SNPs in *APOC3* (rs2854117 and rs2854116 codifying for C-482T and T-455C respectively) are associated with NAFLD and marked insulin resistance in 95 healthy, non-obese, Asian Indian men. The rare-allele carriers and wild-type homozygotes had a NAFLD prevalence of 38% and 0% respectively ($P < 0.001$). The subjects with NAFLD had marked insulin resistance[60]. The mechanism suggested was that the above mentioned *APOC3* variants lead to higher plasma concentrations of apolipoprotein C3. The apo-

Table 2 Proposed mechanism of action of each genetic variant associated with fatty liver disease

Gene	Proposed mechanism of action
PNPLA3	The *PNPLA3* encodes for the adiponutrin, an enzyme expressed in the liver and adipose tissue showing both a lipogenic and lipolytic activity. This variant could cause both a gain of function of the enzyme (which could have a lipogenic activity in the liver) and a loss of function (that could predispose to steatosis by decreasing triglyceride hydrolysis in hepatocytes)
GCKR	The gene product is a regulatory protein that inhibits glucokinase in liver and pancreatic islet cells. The polymorphism could lead to increased hepatic glucokinase activity. This enhance the glycolytic flux and then promotes hepatic glucose metabolism and elevates the concentrations of malonyl coenzyme A, a substrate for *de novo* lipogenesis
APOC3	*APOC3* variants could increase the plasma concentrations of apolipoprotein C3. The apolipoprotein C3 could then inhibit the lipoprotein lipase reducing the clearance of triglycerides. Consequence of reduced clearance of triglycerides is the increase of chylomicron-remnant particles that confer a predisposition to both fasting and postprandial hypertriglyceridemia. Higher circulating levels of chylomicron-remnant particles are then especially cleared by the liver through a receptor-mediated process, resulting in NAFLD and hepatic insulin resistance
NCAN	*NCAN* encodes for a chondroitin sulfate proteoglycan thought to be involved in the modulation of cell adhesion and migration. *NCAN* is a risk factor for liver inflammation and fibrosis, suggesting that this locus is responsible for progression from steatosis to steatohepatitis
LYPLAL1	*LYPLAL1* encodes for a lysophospholipase and it is associated with increased hepatic steatosis probably preventing breakdown of triglycerides
PPP1R3B	This gene encodes the catalytic subunit of the serine/theonine phosphatase, protein phosphatase-1. The encoded protein is expressed in liver. It is associated with computer tomography–assessed liver attenuation but not histology-proven NAFLD
GC	*GC* gene is expressed predominately in the hepatocytes where it encodes for VDBP. VDBP is the main vitamin D carrier, which has been implicated in the development of obesity and diabetes. In fact, low vitamin D concentrations could increase adipocyte intracellular calcium, stimulating lipogenesis, whereas vitamin D supplementation improves insulin resistance and down-regulates inflammatory cytokines such as tumor necrosis factor-a and interleukin-6 in cell models. Vitamin D levels are influenced by GC genetic polymorphisms
LCP1	LCP-1 is an actin bundling protein expressed especially in hematopoietic cells and is involved in leukocyte activation and tumor cell proliferation. Its pathogenic role in NAFLD is unknown
SLC38A8 LPPR4	SLC38A8 protein product is a putative sodium-coupled neutral amino acid transporter whose expression is limited to the brain, whereas LPPR4 catalyzes the dephosphorylation of biologically active lipids and is expressed especially in the hypothalamus. While the functional significance of neuronally expressed genes such as SLC38A8 and LPPR4 with NAFLD is not apparent, there is convincing evidence that the nervous system and particularly the hypothalamus play an important role in lipid homeostasis in the liver
SAMM50	*SAMM50* gene encodes for Sam50 a protein that may be involved in mitochondrial dysfunction. The subsequent decreased removal of reactive oxygen species could lead to progression of NAFLD
PARVB	The *PARVB* gene encodes parvin-b, which forms integrin-linked kinase-pinch-parvin complex. Integrins are a large family of heterodimeric cell surface receptors that act as mechanoreceptors by relaying information between cells and from the ECM to the cell interior. Since integrin receptors directly bind to ECM components to control remodeling, they are thought to play a crucial role in the evolution and progression of liver fibrosis. Overexpression of parvin-b leads to a concomitant increase in lipogenic gene expression
FDFT1	The *FDFT1* gene, situated on chromosome 8, is an important modulator of cholesterol biosynthesis[67,68]. It codifies for the Squalene Synthase, an enzyme which converts two molecules of farnesyl pyrophosphate into squalene, a precursor to cholesterol. An hypothesis could be that this SNP is in linkage disequilibrium with a variant in the promoter of squalene synthase gene that through the enhancement of its expression, could lead to an increased activation of the enzyme and to the intra-hepatic accumulation of cholesterol

VDBP: Vitamin D binding protein; NAFLD: Non-alcoholic fatty liver disease; ECM: Extracellular matrix; LCP-1: Lymphocyte cytosolic protein-1; SLC38A8: Solute carrier family 38 member 8; LPPR4: Lipid phosphate phosphatase-related protein type 4 gene; GC: Group-specific component.

lipoprotein C3 could then inhibit the lipoprotein lipase reducing the clearance of triglycerides. Consequence of reduced clearance of triglycerides is the increase of chylomicron-remnant particles that confer a predisposition to both fasting and postprandial hypertriglyceridemia. Higher circulating levels of chylomicron-remnant particles are then especially cleared by the liver through a receptor-mediated process[61-63], resulting in NAFLD and hepatic insulin resistance. Other data available in literature on correlation between *APOC3* and NAFLD are contrasting[64,65]. The differences could be due to the anthropometric profile of subjects included in different researches. Petersen *et al*[60] studied a cohort without any risk factor of metabolic syndrome and with relatively lower body mass index (BMI) of 24.7 ± 3.6 and 24.1 ± 2.9 kg/m^2 in Indian and non-Indian groups, respectively; it is important to underline that a BMI of 24.7 may indicate overweight in Indians but normal body weight in European. In comparison, other works studied larger numbers of subjects with overweight/obesity, dyslipidemia and with the criteria of metabolic syndrome.

FDFT1 rs2645424

A SNP in the farnesyl-diphosphate farnesyltransferase 1 (*FDFT1*) gene has been associated with the degree of liver injury: the rs2645424. This variant, in fact, has been showed to be associated with NAFLD activity score by a GWAS study. This study examined 236 non-Hispanic white women with fatty liver[66]. The *FDFT1* gene, situated on chromosome 8, is an important modulator of cholesterol biosynthesis[67,68]. It codifies for the Squalene Synthase, an enzyme which converts two molecules of farnesyl pyrophosphate into squalene, a precursor to cholesterol. The rs2645424 is an intronic variant and therefore it is difficult to explain how it may affect the enzyme activity; an hypothesis could be that this SNP is in linkage disequilibrium with a variant in the promoter of squalene synthase gene that through

the enhancement of its expression, could lead to an increased activation of the enzyme and to the intra-hepatic accumulation of cholesterol. Evidence in animals has, in fact, shown that transient over-expression of the *FDFT1* gene in the liver of both wild-type and LDL Receptor knockout mice led to higher *de novo* cholesterol biosynthesis, over-secretion of cholesterol-rich LDL, increased cholesterol concentrations and a 37% enhancement in liver weight compared with controls related to hepatocyte proliferation[69]. This hypothesis would also be in agreement with recent researches demonstrating the role of intra-hepatic cholesterol accumulation in the pathogenesis of NASH[70].

NOVEL GENE VARIANTS ASSOCIATED WITH HEPATIC FAT CONTENT

More recently, more gene variants have been associated with fatty liver disease (Table 1), the mechanisms of action are summarized in Table 2.

Speliotes *et al*[71], in addition to *GCKR*, identified variants in novel loci *NCAN* and *LYPLAL1* associated with both increasing computer tomography (CT) hepatic steatosis and histological NAFLD and identified variants in another locus, *protein phosphatase 1 regulatory subunit 3B* (*PPP1R3B*), associated with CT steatosis but not histologic NAFLD[71]. Recently Kitamoto *et al*[72] found that *PNPLA3, sorting and assembly machinery component* (*SAMM50*), *parvin beta* (*PARVB*) genetic regions was significantly associated with NAFLD in the Japanese population. Adams *et al*[73] showed that SNPs in two genes expressed in liver were associated with NAFLD adolescents: *group-specific component (GC)* and *lymphocyte cytosolic protein-1 (LCP1)*. SNPs in two genes expressed in neurons were also associated with NAFLD: *lipid phosphate phosphatise-related protein type 4 (LPPR4)* and *solute carrier family 38 member 8 (SLC38A8)*[73].

FUTURE DIRECTIONS

Although the last few years have shed light in the pathogenesis of fatty liver, more researches are needed to be done and novel approaches to this problem are needed to be thought especially in the pediatric population. In fact, a limitation of this review is that several of the quoted studies have been performed in adults, but unfortunately accurate studies in pediatrics are quite limited also because the state of art techniques to assess hepatic fat content (MRI, MRS and liver biopsy) are difficult to perform in the pediatric population and very expensive.

One promising line of research that will allow to uncover novel mechanisms in the pathogenesis of fatty liver is the study of gut microbiome. Several lines of evidence, in fact, suggest a strong interaction between gut flora and liver. In fact, the liver receives 70% of its blood flow from the intestine through the portal vein and act as the first line of defence against gut-derived antigens, therefore it is one of the most exposed organs to gut-derived toxic factors[74]. The joint between gut microbiota and the development of fatty liver has been demonstrated both in murine model and in humans. In mice, Bäckhed *et al*[75] observed that the transplantation of normal caecal microbiota to germ-free mice induced, 15 d later, a 60% increase in body fat along with a more than two-fold increase in hepatic triglyceride content. For these reasons several researchers are now investigating the role of the gut flora in determining and influencing non-alcoholic fatty liver disease.

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P- Reviewers: Chatterjee S, Makisalo H
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ISSN 1007-9327

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