Understanding the pathophysiological mechanisms in the pediatric non-alcoholic fatty liver disease: The role of genetics

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Abstract
Classically, the non-alcoholic fatty liver disease (NAFLD) physiopathology and progression has been summarized in the two hits hypothesis. The first hit is represented by the action of hyperinsulinemia and insulin resistance, accompanying obesity, that leads to liver steatosis increasing the absolute non esterified fatty acids uptake in the liver and the esterification to form triacylglycerol. The oxidative stress is involved in the second hit leading to the progression to nonalcoholic steatohepatitis (NASH) because of its harmful action on steatosic hepatocytes. However, at the present time, the two hits hypothesis needs to be updated because of the discovery of genetic polymorphisms involved both in the liver fat accumulation and progression to NASH that make more intriguing understanding the NAFLD pathophysiological mechanisms. In this editorial, we want to underline the role of PNPLA3 I148M, GPR120 R270H and TM6SF2 E167K in the pediatric NAFLD development because they add new pieces to the comprehension of the NAFLD pathophysiological puzzle. The PNPLA3 I148M polymorphism encodes for an abnormal protein which predisposes to intrahepatic triglycerides accumulation both for a loss of function of its triglyceride hydrolase activity and for a gain of function of its lipogenic activity. Therefore, it is involved in the first hit, such as TM6SF2 E167K polymorphisms that lead to intrahepatic fat accumulation through a reduced very low density lipoprotein secretion. On the other hand, the GPR120 R270H variant, reducing the anti-inflammatory action of the GPR120 receptor expressed by Kupffer cells, is involved in the second hit leading to the liver injury.

Key words: Pediatric non-alcoholic fatty liver disease; GPR120; PNPLA3; TM6SF2; Alanine transaminase

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the non-alcoholic fatty liver disease (NAFLD) pathophysiological mechanisms. In this editorial, that is not to consider as a comprehensive review, we want to underline the role of three polymorphisms, one older (PNPLA3 I148M) but very important and two recently discovered (GPR120 R270H and TM6SF2 E167K) that add new pieces to the comprehension of the NAFLD pathophysiological puzzle.

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INTRODUCTION

In the last years, the pediatric obesity prevalence has shown a constant increase[1]. The raised pediatric obesity prevalence has determined an increased prevalence of obesity complications and the non-alcoholic fatty liver disease (NAFLD) has become the most common form of liver disease in childhood. In fact, its prevalence has more than doubled over the past years. It currently involves between 3% and 11% of the pediatric population and affects about the 46% of overweight and obese children and adolescents[2]. NAFLD comprehends an extensive range of conditions, including from fatty liver or steatohepatitis with or without fibrosis, to cirrhosis and its complications (e.g., hepatocellular carcinoma and portal hypertension)[3,4]. The NAFLD is defined by hepatic fat infiltration involving > 5% hepatocytes in the absence of excessive alcohol intake or other demonstrable liver diseases[5].

Classically, the NAFLD pathophysiology and progression has been summarized in the two hits hypothesis. Obesity related hyperinsulinemia and insulin resistance represent the first hit. They lead to liver steatosis increasing the absolute non esterified fatty acids uptake in the liver and the esterification to form triacylglycerol[6,5]. The oxidative stress is involved in the second hit leading to the progression to NASH because of its harmful action on steatotic hepatocytes. The reactive oxygen species (ROS), in fact, lead to hepatocellular damage inhibiting the mitochondrial respiratory chain enzymes, and inactivating both the glyceraldehyde-3-phosphate dehydrogenase and the membrane sodium channels. ROS further cause lipid peroxidation, cytokine production, and induce Fas Ligand, contributing to hepatocellular injury and fibrosis[7].

However, at the present time, the two hits hypothesis needs to be updated because of the discovery of 3 genes whose polymorphisms are involved both in the liver fat accumulation and progression to non-alcoholic steatohepatitis (NASH) making intriguing understanding the NAFLD pathophysiological mechanisms. In this editorial, that is not to consider as a comprehensive review, we want to underline the role of three polymorphisms, one older but very important and two recently discovered that added new pieces to the comprehension of the NAFLD pathophysiological puzzle.

PATATIN LIKE PHOSPHOLIPASE CONTAINING DOMAIN 3 GENE

The patatin like phospholipase containing domain 3 gene (PNPLA3) is the most important gene involved in hepatic steatosis developing. It encodes for the adiponutrin, an enzyme present in the liver and adipose tissue. Feeding and insulin resistance induce the adiponutrin[8] that shows lipolytic activity on triglycerides[9]. The PNPLA3 rs738409 (PNPLA3 I148M) single nucleotide polymorphism is a non-synonymous variant and it is characterized by a cysteine to guanosine substitution leading to an isoleucine to methionine substitution at the amino acid position 148 (I148M). This aminoacid substitution affects the enzyme function probably reducing the substrates access to the enzyme and then leading to the development of microvesicular steatosis[9]. On the other hand, the adiponutrin could present a gain of lipogenic function, which could further lead to the hepatic fatty acids accumulation[9]. In literature there is strong evidence of association between the PNPLA3 148M allele and NAFLD both in adults[10] and children[11,12].

The PNPLA3 148M allele plays a central role in NAFLD developing interacting with environmental NAFLD risk factors, such as obesity (and in particular visceral fat)[11] and alcohol consumption[13], and then increasing the risk of fatty liver development. In fact, these stressors seem to reveal the association between the PNPLA3 148M allele and hepatic damage in populations in whom it is otherwise hidden[14]. Interestingly, the obesity-driven effect of this polymorphism on liver damage[15] can be reduced by weight loss (expressed as reduction of the waist/height ratio) in obese children and adolescents[15]. Among environmental factors involved in NAFLD development and interacting with PNPLA3 148M allele some nutrients appear. Indeed, the total carbohydrate[16] and high omega (n) 6 to n-3 polyunsaturated fatty acids (PUFA) ratio[17] can enhance the association between steatosis and the PNPLA3 variant.

G-PROTEIN-COUPLED-RECEPTOR 120

G-protein-coupled-receptor 120 (GPR120) is a receptor for PUFAs and it is expressed by adipocytes, Kupffer cells and, at low level, in hepatocytes[18]. PUFAs could play a role in inflammatory response modulation[19]. In fact, it has been shown that, in the adipose tissue, the interaction between PUFAs and macrophagic GPR120 switch off inflammation blocking nuclear factor-kappa-B activity[20]. In particular docosahexaenoic acid (DHA), an n-3 PUFA of the fish oil, has recently shown a potential
role in treatment of liver fat accumulation and of metabolic and hepatic complications of NAFLD[21]. The 270H allele inhibits the GPR120 signalling activity[19] and then it reduce its anti-inflammatory activity[20]. Recently, this variant has been studied in obese children and adolescents and it has been shown that the subject carrying the rare GPR120 270H allele presented higher alanine transaminase (ALT) levels (P = 0.01) and higher ferritin (marker of inflammation) levels (P < 0.003) than wild type subjects[22]. The carriers of the 270H allele showed an odds ratio (OR) to have pathologic ALT of 3.2[22]. Moreover, PNPLA3 148M allele demonstrated an interaction with GPR120 270H allele determining a significant effect on ALT levels (P = 0.00001) suggesting a driving effect of the PNPLA3 148M allele on liver injury in obese children carrying this variant[22]. This is in accord with the findings of Santoro et al[27] demonstrating that the PNPLA3 1148M variant predispose to liver damage in patients with a lower intake of n-3 fatty acids. Therefore, GPR120 R270H variant appear to have an important role in determining liver injury expressed as ALT elevation and the PNPLA3 148M allele on liver injury in obese children carrying this variant[22]. This is in accord with the findings of Santoro et al[27] demonstrating that the PNPLA3 1148M variant predispose to liver damage in patients with a lower intake of n-3 fatty acids. Therefore, GPR120 R270H variant appear to have an important role in determining liver injury expressed as ALT elevation and the PNPLA3 148M allele showed an importance capacity to promote the GPR120 270H allele mediated liver damage. This evidence is in accord with the studies showing that DHA supplementation, activating the GPR120 receptor and then exerting potent anti-inflammatory and insulin-sensitizing activities[20], can reduced liver damage in pediatric NAFLD[23].

**TRANSMEMBRANE 6 SUPERFAMILY MEMBER 2 GENE**

Recently, a new gene variant playing a role in the NAFLD physiopathology has been discovered in the transmembrane 6 superfamily member 2 (TM6SF2) gene[24]. This variant (rs58542926) is characterized by an adenine-to-guanine substitution in the nucleotide 499 which replaces glutamate at residue 167 with lysine (c.499A > G; p.Glu167Lys)[24]. The TM6SF2 minor allele carriage has been causally related to a reduced risk of myocardial infarction[27]. Accordingly, very recent data showed an effect of this polymorphism on reducing carotid atherosclerosis risk in adults[28]. Moreover, Liu et al[29] confirmed, using two histologically characterized cohorts (1074 adults) encompassing steatosis, steatohepatitis, fibrosis and cirrhosis, the TM6SF2 minor allele association with NAFLD and, moreover, with advanced hepatic fibrosis/cirrhosis. The effect of this polymorphism on ALT and cholesterol levels has been confirmed also in obese children and adolescents[29]. Grandone et al[29], in fact, demonstrated, in a cohort of 1010 obese children and adolescents, that the TM6SF2 167K allele is associated with steatosis (P < 0.0001), higher ALT levels (P < 0.001) and lower total cholesterol (< 0.00001), LDL-C (P < 0.0001), triglycerides (P = 0.02) and non-high density lipoprotein cholesterol levels (P < 0.000001)[29]. Interestingly, the subjects homozygous for the PNPLA3 148M allele carrying the rare variant of TM6SF2 showed an OR of 12.2 to present hypertransaminasemia compared to the remaining patients[29]. Therefore, the effect of PNPLA3 and TM6SF2 rare alleles appears additive in determining pediatric NAFLD[29].

**OLD AND NEW CONCEPTS, AN INTEGRATED OVERVIEW**

NAFLD occurs in overweight and obese children deriving from intrahepatic accumulation of triglycerides. The triglycerides accumulated in the liver mostly derive from the adipose tissue lipolysis (60%) and hepatic de novo lipogenesis (26%) whereas only in a little part from the diet as chylomicron remnants (14%)[30]. This fat accumulation, as indicated in the "two hits" hypothesis (Figure 1), is stimulated by obesity-related peripheral insulin resistance and hyperinsulinemia. In fact they stimulate the free-fatty-acids (FFA) uptake in the liver, the esterification of hepatic FFAs to form triglycerides, the FFA synthesis from cytosolic substrates, and the decreased apolipoprotein B-100 synthesis. Then, the export of FFA and triglycerides decreases, while the beta-oxidation of mitochondrial long-chain fatty acids increases[2]. In the last years, the knowledge of NAFLD pathophysiology is constantly increasing[8] with the discovery of new genetic polymorphisms that could promote the NAFLD development and then the progression to the NASH. Each polymorphism plays a role in a different hit and, therefore, in future, the pathophysiology could be described by a "multiple hit hypothesis": PNPLA3 1148M polymorphism plays an important role in the NAFLD development. In fact, it
The hyperinsulinemia and insulin resistance, accompanying obesity, lead to liver steatosis increasing the absolute non esterified fatty acids uptake in the liver and the esterification to form triacylglycerol. The PNPLA3 148M allele encodes for an abnormal protein and predisposes to intrahepatic triglycerides accumulation by both a reduced effect on triglycerides hydrolysis and an enhanced lipogenetic effect. Therefore, it is involved in the first hit. Also the TM6SF2 E167K polymorphism plays a role in the first hit, in fact, it lead to intrahepatic fat accumulation through a reduced VLDL secretion. The oxidative stress is involved in the second hit leading to the progression to NASH because of its harmful action on steatotic hepatocytes. Reactive oxygen species can induce hepatocellular injury and then fibrosis through the inhibition of the mitochondrial respiratory chain enzymes, lipid peroxidation, cytokine production, Fas Ligand induction. The GPR120 270H allele, reducing the anti-inflammatory action of the GPR120 receptor expressed by Kuppfer cells, is involved in the second hit promoting the oxidative stress, mitochondrial dysfunction and pro-inflammatory cytokines release. PNPLA3: Patatin like phospholipase containing domain 3 gene; NASH: Nonalcoholic steatohepatitis; GPR120: G-protein-coupled-receptor 120; TM6SF2: Transmembrane 6 superfamily member 2 gene; NFκB: Nuclear factor-kappa-B; VLDL: Very low density lipoprotein.

**CONCLUSION**

In the last years many polymorphisms playing a role in the NAFLD physiopathology have been identified, therefore our knowledge in this area has been constantly increased. Starting from the genetic association studies we can understand new pathophysiological mechanisms that are firstly implicated in the intrahepatic fat accumulation and then, in the progression to the NASH (Figure 1). This editorial wants to underline how these new genetic findings have improved our comprehension of pediatric NAFLD pathophysiology.

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