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The Role of FXR-Signaling Variability in the Development and Course of Non-Alcoholic Fatty Liver Disease in Children

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ABSTRACT

Introduction: Genetic mechanisms among many other factors play a crucial role in the development and progression of nonalcoholic fatty liver disease (NAFLD). The farnesoid X-receptor (FXR) regulates the expression of target genes involved in metabolic and energy homeostasis, so it can be assumed that genetic variations within the *NR1H4* gene, encoding FXR, can affect the development or progression of associated diseases, including NAFLD.

The aim: To study the association of SNP rs11110390 *NR1H4* gene with the probability of development and course of NAFLD in children.

Materials and methods: 76 children aged 9–17 years and overweight were examined. According to controlled attenuated parameter (CAP) measurement (Fibroscan®502touch) children were divided into 2 groups: group 1 consisted of 40 patients with NAFLD, group 2 was composed by 36 patients without hepatic steatosis. According to genetic testing children were divided into 3 subgroups – children with CC-, CT-, TT-genotype SNP rs11110390 *NR1H4* gene.

Results: The frequency of TT-genotype SNP rs11110390 *NR1H4* gene detection in children with NAFLD was 17.5% versus 2.8% in the control group ($p < 0.05$). In children with TT-genotype SNP rs11110390 *NR1H4* gene the liver stiffness ($p < 0.05$) and CAP ($p = 0.1$) were higher than in patients with CC- and CT-genotypes. Patients with the TT-genotype differed from CC-genotype patients with lower levels of IL-10 ($p < 0.05$) and pro-inflammatory cytokine balance ($p < 0.05$). An increase in the concentration of taurine-conjugated bile acid fractions in the hepatic and gallbladder's bile in children with TT-genotype SNP rs11110390 *NR1H4* ($p < 0.05$) was demonstrated.

Conclusions: SNP rs11110390 *NR1H4* is associated with an increased probability of NAFLD development in children. An increase in the steatosis degree and liver stiffness in combination with increased taurine-conjugated bile acids fractions in the hepatic and gallbladder's bile, shift in cytokine balance due to a decrease in IL-10 level in children with TT-genotype SNP rs11110390 *NR1H4* were observed.

KEYWORDS

farnesoid X-receptor; single nucleotide polymorphism; nonalcoholic fatty liver disease; children

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INTRODUCTION

The rapid increase in the prevalence of nonalcoholic fatty liver disease (NAFLD) in recent decades is the result of a combined influence of environment, endogenous factors (such as eating behavior, physical activity, intestinal microbiota composition), and genetic predisposition (1). Among many others genetic mechanisms play an important role in the development and progression of NAFLD (2). The significance of genetic variability underlying the susceptibility to NAFLD has been confirmed in numerous modern genome-wide association studies (3). Studies of candidate genes demonstrated the influence of certain genetic variants on lipid metabolism, inflammation, insulin signaling, fibrogenesis, and oxidative stress activity which determine the severity of NAFLD (4–6). Nowadays, the concept of the role of individual genes/proteins in NAFLD is gradually being replaced by the study of multiple causal interactions using systemic biology approaches. The "NAFLD-Reactome" project confirmed the role of nuclear receptors in the related gene expression regulation in the pathogenesis of NAFLD (7). The *NR1H4* gene encoding farnesoid-X-receptor (FXR) is a member of the nuclear receptors superfamily, so-called ligand-activated transcription factors. It regulates a significant number of target genes involved in a wide range of metabolic processes, such as lipid, carbohydrate, and energy metabolism, bile acid homeostasis, inflammation, cell proliferation, differentiation and death, and regulation of the intestinal microbiota composition and activity (8, 9). Although FXR is most widely presented in tissues involved in the bile acids circulation, including the liver, intestine, and bile duct epithelium, FXR expression is also found in renal tubular cells, adrenal glands, pancreatic tissue, adipocytes, cardiomyocytes, myocytes, endothelial and immune cells (10). The effects of FXR activation are tissue-specific. Both hepatic (hFXR) and intestinal (iFXR) receptors are important mediators of reverse inhibition of bile acid synthesis and induction of bile acids transporters expression, protecting hepatocytes and enterocytes from excessive bile acids toxicity. iFXR activation mainly leads to inhibition of bile acid synthesis, while hFXR activation – to changes in the bile acids pool composition (11). In general, the small number of non-synonymous single nucleotide polymorphisms (SNPs) in the *NR1H4* gene compared to other genes indicates its evolutionary stability and confirms its crucial importance for cell homeostasis and function (12). Considering that FXR is a key metabolic regulator, it is likely that genetic variation within the *NR1H4* gene may have an influence on the development or progression of metabolic-associated diseases (13). Unfortunately, to date, the contribution of *NR1H4* polymorphisms to the NAFLD formation probability and clinical consequences for NAFLD remains unclear.

THE AIM

To study the association of SNP rs11110390 *NR1H4* gene with the probability of development and course of NAFLD in children.

MATERIALS AND METHODS

The study included 76 overweight and obese caucasian children aged 9 to 17 years (average age 12.3 ± 2.5 years). The presence of hepatic steatosis was determined by transient elastometry (FibroScan®502touch, Echosens, France) with the measurement of the controlled attenuation parameter (CAP). According to the presence of NAFLD, children were divided into 2 groups: group 1 (main) consisted of 40 patients with NAFLD, group 2 (control) consisted of 36 patients without hepatic steatosis. To study the association of SNP with laboratory-instrumental data, an intragroup analysis was performed: according to the molecular genetic testing the children with NAFLD were divided into 3 subgroups: children with CC-, CT-, TT-genotypes SNP rs11110390 *NR1H4* gene. The groups did not have significant differences in age and gender. The study has been performed according to the Declaration of Helsinki. The informed consent has been obtained from all patients. All procedures have been approved by the local ethics committee (certificate number 4).

Genetic study to determine the presence of polymorphic variants of the *NR1H4* gene (SNP rs11110390) was conducted in the Scientific Institution of Genetic and Immune Pathology and Pharmacogenetics of "Ukrainian Medical Stomatological Academy". DNA isolation was performed using a set of reagents "DNA-EXTRAN-1" (LLC "NPF SINTOL", Russia) from samples of fasting blood. Determination of the polymorphic variant of the *NR1H4* gene (rs11110390) was performed by allele-specific polymerase chain reaction (PCR) using fluorogenic samples to detect specific products in 25 μl of the reaction mixture, which included: 12.5 μl of 2 × TagMan Master Mix amplification solution; 1.25 μl of TagMan SNP Genotyping Assays containing specific nucleotide sequences for allele C and allele T; and DNA solution in nuclease-free water for PCR (Thermo Fisher Scientific, USA). Polymorphic alleles of the *NR1H4* gene (rs11110390) were amplified and detected in real time by measuring the fluorescent signal from the amplification products in each cycle, the intensity of which was proportional to the concentration of the final PCR product. Amplification was performed under the following conditions: the first cycle took place at a temperature of 95 °C for 10 minutes. The amplicons were accumulated in the next 40 cycles according to the following temperature parameters: 95 °C for 15 seconds, 60 °C for 60 seconds.

Quantitative determination of the concentration of IL-6, IL-10, TNF-α in the serum was performed with enzyme-linked immunosorbent assay (Vector-Best, Russia) using an analyzer "Stat Fax 303 Plus" (USA).

The content of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) in the serum was determined using reagent kits "Cormey" (Poland) using a biochemical analyzer Stat Fax 1904 Plus, Awareness Technology (USA). Low-density lipoprotein (LDL) was calculated by Friedwald's formula. Very low density lipoprotein (VLDL) was determined by empirical calculations of TG levels.

Bile from patients was obtained by duodenal sounding. As a stimulator of bile secretion was used a solution with 33% $MgSO_4$. Determination of the content of taurocholic (TC), taurodeoxycholic (TDC), glycocholic (GC) and

Tab. 1 Distribution of alleles and genotypes of SNP rs11110390 *NR1H4* in the studied groups.

SNP ID	Allele frequency							Genotype frequency						
	Groups	Total		NAFLD		Control		Groups	Total		NAFLD		Control	
	Allele	n	%	n	%	n	%	Genotype	n	%	n	%	n	%
rs11110390	C	97	63.8	47	58.8	50	69.4	CC	29	38.2	14	35.0	15	41.7
								CT	39	51.3	19	47.5	20	55.6
	T	55	36.2	33	41.2*	22	30.6	TT	8	10.5	7	17.5*	1	2.8
Total		152	100	80	100	72	100		76	100	40	100	36	100

Note: * $p < 0.05$ – significance of differences by Fisher's criterion versus the control group.

glycodeoxycholic (GDC) bile acids in bile was performed using the method of thin-layer chromatography (14). The content of bile acids and cholesterol was determined using a densitometer BIAN-170 (λ = 620 nm) after staining the samples on the calibration curves.

Statistical processing of the results was performed using the application package Statistica 6.1 (serial number AGAR909 E415822FA). The conformity of the data distribution to the normal distribution was checked using the Shapiro – Wilk method. The mean (M) and standard deviation (SD) or median (Me), lower (Q1) and upper (Q2) quartiles were used to describe the data. For comparison ANOVA + post hoc test has been applied. Fisher's test was used to compare qualitative features belonging to nominal or ordinal scales. Statistical significance was assessed at a level not lower than 95.0% ($p < 0.05$).

RESULTS

According to the results of molecular genetic examination, the SNP rs11110390 of the *NR1H4* gene in the heterozygous state (CT) was detected in 51.3% of all examined children, while the homozygous (TT) condition was present in 10.5% children (Table 1).

CC-genotype of the *NR1H4* gene was observed in 35% of NAFLD children, CT-genotype – in 47.5% of NAFLD children, and TT-genotype – in 17.5% (Table 1). The frequency of TT-genotype *NR1H4* gene detection in children with NAFLD significantly differed from the control group (17.5% vs 2.8%, $p < 0.05$). The frequency of the minor (T) allele detection among NAFLD children was 41.2% that was significantly higher than in the control group (30.6%) (Table 1).

ASSOCIATION OF SNP RS11110390 *NR1H4* WITH ANTHROPOMETRIC PARAMETERS

The body mass index (BMI) and waist circumference mean values in CT- and TT-genotypes SNP rs11110390 *NR1H4* gene carriers were increased, but the differences were not significant ($p > 0.05$) (Table 2).

ASSOCIATION OF SNP RS11110390 *NR1H4* GENE WITH BIOCHEMICAL PARAMETERS

When comparing the hepatic transaminases activity, no significant differences were found between subgroups (Table 3), but the average levels of γ-glutamyltranspeptidase (GGT) were significantly higher in TT-genotype SNP rs11110390 *NR1H4* carriers (Fig. 1).

Tab. 2 Comparison of the anthropometric parameters depending on the SNP rs11110390 *NR1H4* genotype.

Anthropometric parameters	SNP rs11110390 genotype			p
	CC	CT	TT	
	M ± SD	M ± SD	M ± SD	
BMI, kg/m²	23.8 ± 3.9	24.9 ± 4.3	26.3 ± 3.3	0.2
Waist circumference, cm	81.3 ± 3.2	82.7 ± 2.8	84.7 ± 1.9	0.4

Note: p – significance of differences according to F-criterion between the TT-genotype and CC-genotype patients.

Tab. 3 Comparison of the biochemical parameters depending on the SNP rs11110390 *NR1H4* genotype.

Biochemical parameters	SNP rs11110390 genotype			p
	CC	CT	TT	
	M ± SD	M ± SD	M ± SD	
ALT, U/l	37.2 ± 1.6	36.8 ± 1.4	40.3 ± 1.2	>0.05
AST, U/l	28.7 ± 1.2	29.8 ± 1.1	32.3 ± 0.9	>0.05
GGT, U/l	18.6 ± 0.9	19.2 ± 0.8	27.7 ± 1.1	<0.05

Note: p – significance of differences according to F-criterion between the TT-genotype and CC-genotype patients.

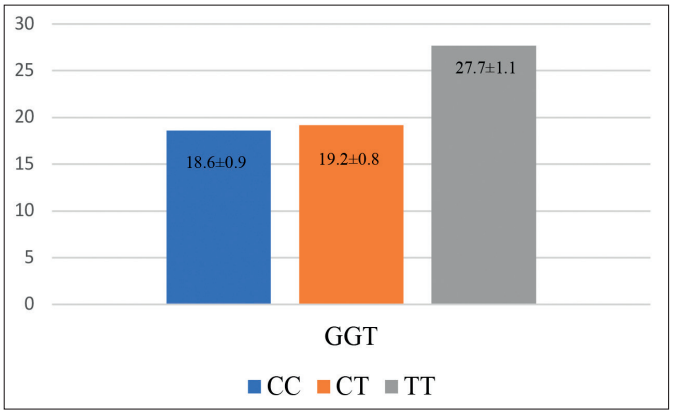

Fig. 1 Mean GGT values depending on the SNP rs11110390 *NR1H4* genotype.

ASSOCIATION OF SNP RS11110390 *NR1H4* GENE WITH LIVER STRUCTURE CHANGES

Children with TT-genotype of SNP rs11110390 *NR1H4* gene were characterized by significantly higher liver stiffness mean values compared to patients with CC- and CT-genotypes ($p < 0.05$) (Fig. 2A). Patients with the TT-genotype also differed from CT- and CC-genotypes carriers with higher CAP levels, but the differences between the groups were a trend ($p = 0.1$) (Fig. 2B).

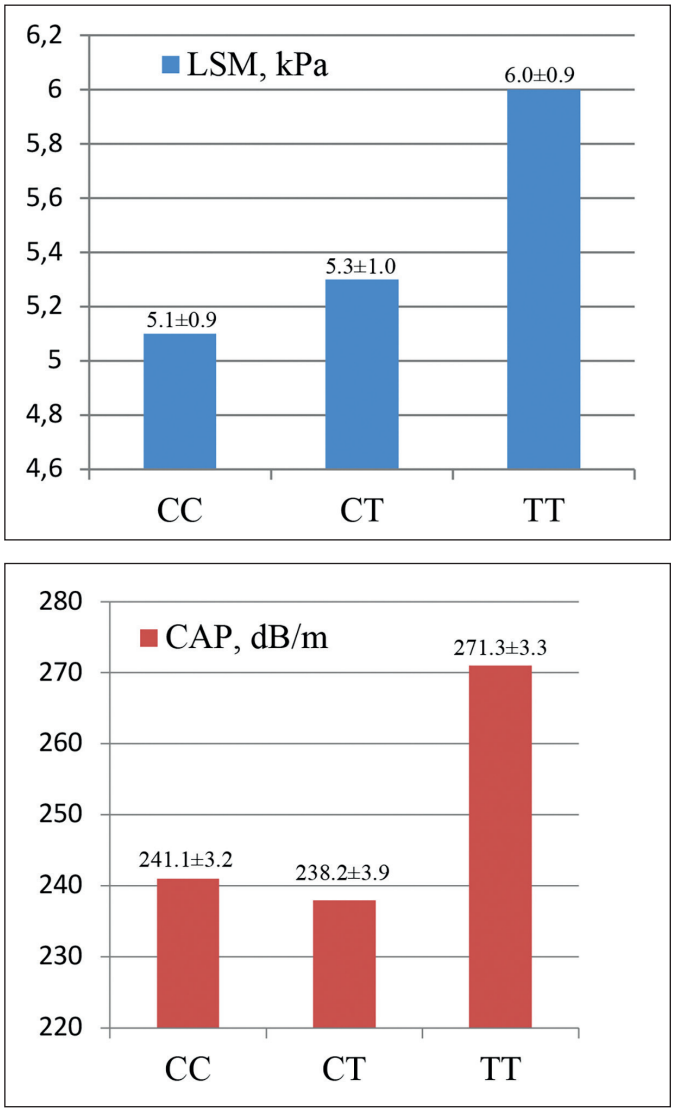

Fig. 2 Liver stiffness (A) and CAP (B) depending on the SNP rs11110390 *NR1H4* genotype.

ASSOCIATION OF SNP RS11110390 *NR1H4* GENE WITH THE CYTOKINE PROFILE

Patients with CT-genotype in contrast to patients with CC-genotype of SNP rs11110390 *NR1H4* gene were characterized by increased levels of proinflammatory cytokines TNF-α and IL-6, but the balance of cytokines was maintained due to a unidirectional increase in the concentration of anti-inflammatory cytokine IL-10 (Table 4). Patients with TT-genotype SNP rs11110390 *NR1H4* differed from patients with CC-genotype with lower IL-10 levels ($p < 0.05$) and a shift in the balance of cytokines towards proinflammatory state (increase in TNF-α / IL-10, $p < 0.05$).

Tab. 4 Cytokine profile depending on the SNP rs11110390 *NR1H4* genotype.

Cytokines	SNP rs11110390 *NR1H4* genotype		
	CC	CT	TT
	Me (Q25, Q75)	Me (Q25, Q75)	Me (Q25, Q75)
IL-6, pg/ml	0.9 (0.4; 1.3)	2.3* (0.3; 5.5)	2.1* (0.2; 4.3)
TNFα, pg/ml	0.7 (0.4; 1.1)	2.1* (0.4; 3.8)	1.1 (0.2; 2.0)
IL-10, pg/ml	2.6 (1.1; 4.1)	4.7* (2.2; 7.3)	1.5* (0.1; 2.9)
TNFα/IL10	0.6 (0.1; 1.1)	0.6 (0.3; 0.8)	2.1* (0.7; 4.9)

Note: * $p < 0.05$ – the significance of differences according to the F-criterion compared to children with CC-genotype.

ASSOCIATION OF SNP RS11110390 *NR1H4* GENE WITH LIPID PROFILE PARAMETERS

The study of lipid metabolism depending on the presence of SNP rs11110390 *NR1H4* gene showed that in children with TT-genotype it was observed an increase of the atherogenicity index (AI) due to a shift in the balance of HDL / non-HDL to the HDL decrease, but the differences did not have sufficient significance (Table 5).

Tab. 5 Lipid spectrum parameters depending on the SNP rs11110390 *NR1H4* genotype.

Lipid parameters	SNP rs11110390 *NR1H4* genotype			p
	CC	CT	TT	
	M ± SD	M ± SD	M ± SD	
TG, mmol/l	1.2 ± 0.8	0.8 ± 0.4	0.8 ± 0.4	0.2
HDL, mmol/l	1.0 ± 0.4	1.0 ± 0.3	0.8 ± 0.2	0.4
Non-HDL, mmol/l	3.0 ± 0.3	2.5 ± 0.3	3.2 ± 0.2	0.9
VLDL, mmol/l	0.9 ± 0.3	0.4 ± 0.2	0.3 ± 0.2	0.1
AI	3.5 ± 1.4	3.7 ± 1.4	3.9 ± 0.9	0.2

Note: p – significance of differences according to the F-criterion between the parameters of the patients with TT- and CC-genotype.

ASSOCIATION OF SNP RS11110390 *NR1H4* GENE WITH THE BIOCHEMICAL BILE COMPOSITION PARAMETERS

Comparative analysis of the bile acid fractions concentration in patients depending on the SNP rs11110390 *NR1H4* genotype showed a significant increase in the concentration of TC and TDC in gallbladder's bile (bile B), as well as TC in hepatic bile (bile C) (Table 6).

Tab. 6 Biochemical bile composition depending on the SNP rs11110390 *NR1H4* genotype.

Parameters	SNP rs11110390 *NR1H4* genotype			p
	CC	CT	TT	
	M ± SD	M ± SD	M ± SD	
TC bile B, mmol/l	0.4 ± 0.1	0.9 ± 0.1	2.9 ± 0.1	< 0.05
TDC bile B, mmol/l	0.8 ± 0.2	1.4 ± 0.2	2.7 ± 0.3	< 0.05
TC bile C, mmol/l	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	< 0.01

Note: p – significance of differences according to the F-criterion between the parameters of the patients with TT- and CC-genotype.

DISCUSSION

The SNP rs11110390 *NR1H4* gene that we have studied is intronic and localized in the noncoding region of the gene. According to our data, the frequency of the minor T-allele detection among children with NAFLD was 41.2%, that significantly exceeds such parameter of the obese children (36.2%), which coincides with the ALFA-project data that demonstrate the variability of the minor T-allele prevalence in different populations (Fig. 3) with a frequency in the European population of 32.4% (15).

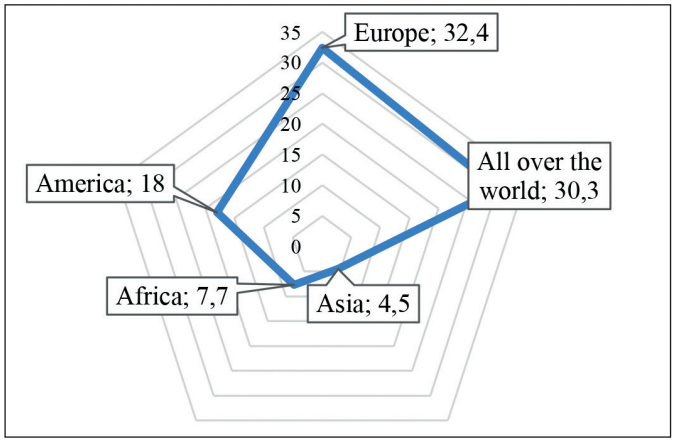

Fig. 3 Frequency of the minor T-allele detection of SNP rs11110390 *NR1H4* gene in different populations (15).

The prevalence of homozygous carriage of SNP rs11110390 *NR1H4* gene among children with NAFLD also differed significantly from the control group and was 17.5%, which may allow the possibility of the association of TT-genotype with NAFLD development. The prevalence of the minor allele and polymorphic genotypes varies considerably between different cohorts of patients. So, Nijmeijer et al. (2011) in the study of adult patients with Crohn's disease and ulcerative colitis demonstrated 32.2% prevalence of the minor T-allele of SNP rs11110390 *NR1H4* gene (16). Lutz et al. (2014), in turn, showed 8.9% prevalence of TT-genotype of SNP rs11110390 *NR1H4* gene among adults with complication by ascites cirrhosis (17).

According to the recent data the association of the wide spectrum of SNP of coding and non-coding regions in the *NR1H4* gene has been confirmed with various dysmetabolic and inflammatory diseases, such as obesity, gallstone disease, intrahepatic cholestasis of pregnancy, Crohn's disease, ulcerative colitis, spontaneous bacterial peritonitis etc. (16–26). Van Mil et al. (2009) identified 4 new SNPs *NR1H4* gene accompanied by functional translation insufficiency and decrease in protein activity in pregnant women with cholestasis, assuming its association with the intrahepatic cholestasis and other cholestatic diseases and dyslipidemia (26). However, a study in an extended cohort (563 pregnant women of European descent with cholestasis) did not confirm the association of these polymorphisms (mostly intronic) with susceptibility to the disease due to their low population frequencies (25). It should be noted that intronic mutations in general account for approximately 10% of all known human pathogenic mutations are accompanied by various rearrangements of mature mRNA due to the influence of SNP on the efficiency of splicing and alternative splicing (27). Introns make up about 24% of the human genome and they have the ability to regulate gene expression through the formation of non-functional or rapidly degrading RNA (negative regulation), intron-mediated enhancement of transcription activity, splicing rate control (28). Therefore, according to the function of introns in the regulation of gene expression, intronic mutations are often associated with severe pathological consequences.

In our study, significant differences in anthropometric parameters (BMI, waist circumference) between patients with different genotypes of SNP rs11110390 *NR1H4* gene were not found in contrast to van den Berg et al. (2009), who demonstrated a strong association between SNP *NR1H4* gene, BMI and waist circumference, assuming the effect of FXR on the adipose tissue distribution and its role in the development of obesity (18). Probably, the differences may be explained by the age difference of the cohorts of patients and the small proportion of morbidly obese patients in our study.

TT-genotype of SNP rs11110390 *NR1H4* gene, according to our data, was associated with impaired bile acid homeostasis: children with TT-genotype showed changes in the biochemical composition of hepatic and gallbladder bile due to increased concentrations of taurine-conjugated fractions associated with biochemical markers of cholestasis (GGT). This is explainable since the leading function of FXR and its target genes is the control of the synthesis, enterohepatic circulation and detoxification of bile acids (29). High level of FXR expression in the gastrointestinal tract allow FXR to play the role of a sensitive sensor of bile acids concentration in the liver and enterocytes, to mediate negative feedback to maintain constant levels of bile acids in hepatocytes and prevent liver damage and cholestasis, to control bile acids reabsorption into the portal bloodstream, to maintain the barrier function of the intestine. FXR-knockout animal models demonstrate the importance of FXR function in maintaining bile acid homeostasis (30).

Disruption of FXR signaling in an experimental model of steatosis in mice on a high-fat diet leads to an increase in the hepatic steatosis degree and the development of endoplasmic reticulum stress (31). Impaired bile acid metabolism in adult NAFLD patients is accompanied by a significant increased risk of liver damage (32). In addition, patients with nonalcoholic steatohepatitis are characterized by a higher level of postprandial bile acid release and a higher likelihood of liver damage by secondary bile acids (33). In our study, patients with TT-genotype of SNP rs11110390 *NR1H4* gene had significantly higher liver stiffness and demonstrated a tendency to increase the steatosis degree in the absence of significant lipid abnormalities, which contradicts the data of Heni et al. (2013) who confirmed the association of SNP rs11110390 *NR1H4* gene with fasting free fatty acids levels (21).

One of the reasons of liver stiffness changes in case of impaired FXR-signaling may be the activation of pro-inflammatory cascades due to increased intestinal permeability. An experimental study of Inagaki et al. (2006) demonstrated that FXR plays a crucial role in maintaining

the integrity of the intestinal epithelial barrier and preventing the development of bacterial overgrowth and bacterial translocation (34). In FXR-knockout mice an increase in intestinal paracellular permeability was observed due to a decrease in the expression of occludin. Furthermore, an increase in aerobic and anaerobic microflora presentation in the ileal mucosa and mesenteric lymph nodes was noticed. Moreover, FXR is able to directly selectively inhibit the NF-kB-associated inflammatory pathway and ensure hepatocyte survival (8). According to our data, changes in the liver stiffness in patients with CT- and TT-genotypes were accompanied by an increase in the concentration of IL-6 and TNF-α, but in heterozygous SNP carriers cytokine balance was maintained due to increased IL-10 production whereas in children with TT-genotype a significant reduction in the IL-10 level was observed, which led to a shift in the balance of pro- and anti-inflammatory cytokines toward inflammation.

Thus, our study demonstrated that the variability of the *NR1H4* gene (SNP rs11110390) is associated with an increased likelihood of developing NAFLD in children. Changes in the bile acid fractions distribution in patients with TT-genotype are characterized by a significant increase in TC and TDC in the gallbladder's and hepatic bile, accompanied by a significant increase in GGT level, increased liver stiffness and steatosis degree. Cytokine profile in children with CT-genotype of SNP rs11110390 *NR1H4* gene is characterized by higher levels of pro-inflammatory cytokines – IL-6, TNF-α, but maintaining the balance due to compensatory increase of IL-10, while children with TT-genotype are characterized by shift the balance towards pro-inflammatory cytokines due to reducing the IL-10 level. Taking into account the prevalence of TT-genotype of SNP rs11110390 *NR1H4* gene in the pediatric population (10.5%), features of the course of NAFLD in SNP carriers, obese NAFLD children with TT-genotype should be considered at risk of the disease progression, which requires timely correction.

CONCLUSION

TT-genotype of SNP rs11110390 *NR1H4* gene carriage are associated with an increased likelihood of NAFLD development in obese children. In children with TT-genotype of SNP rs11110390 *NR1H4* gene an increase in the liver stiffness and steatosis degree is observed in combination with an increase in taurine-conjugated fractions of bile acids in hepatic and gallbladder's bile connected with biochemical signs of cholestasis. In TT-genotype carriers of SNP rs11110390 *NR1H4* gene a shift of cytokine balance towards inflammation is observed due to reduction of IL-10 levels.

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CONFLICTS OF INTEREST

Authors have no conflict of interest to declare.

REFERENCES

1. Stepanov YM, Abaturov OE, Zavhorodnia NY, Skirda IY. Non-alcoholic fatty liver disease in children: current view on diagnostics and treatment (Part I). Gastroenterologia 2015; 2: 99–107.
2. Mantovani A, Zusi C. The dawn of a new era for nonalcoholic fatty liver disease? Hepatobiliary Surg Nutr 2019; 8(Suppl 6): 629–31.
3. van der Sijde MR, Ng A, Fu J. Systems genetics: From GWAS to disease pathways. Biochim Biophys Acta 2014; 1842(Suppl 10): 1903–9.
4. Goldner D, Lavine JE. Nonalcoholic Fatty Liver Disease in Children: Unique Considerations and Challenges. Gastroenterology 2020; 158(Suppl 7): 1967–83.
5. Ismaiel A, Dumitrascu DL. Genetic predisposition in metabolic-dysfunction-associated fatty liver disease and cardiovascular outcomes-Systematic review. Eur J Clin Invest 2020; 50: e13331.
6. Zusi C, Mantovani A, Olivieri F, et al. Contribution of a genetic risk score to clinical prediction of hepatic steatosis in obese children and adolescents. Dig Liver Dis 2019; 51: 1586–92.
7. Sookoian S, Pirola CJ, Valenti L, Davidson NO. Genetic Pathways in Nonalcoholic Fatty Liver Disease: Insights From Systems Biology. Hepatology 2020; 72(Suppl 1): 330–46.
8. Chen, X, Wang L, Shan Q, et al. A novel heterozygous NR1H4 termination codon mutation in idiopathic infantile cholestasis. World J Pediatr 2012; 8(Suppl 1): 67–71.
9. Gomez-Ospina N, Potter C, Xiao R, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. Nat Commun 2016; 7: 10713.
10. Pavlovic N, Stanimirov B, Mikov M. Bile Acids as Novel Pharmacological Agents: The Interplay Between Gene Polymorphisms, Epigenetic Factors and Drug Response. Curr Pharm Des 2017; 23(Suppl 1): 187–215.
11. Ticho AL, Malhotra P, Dudeja PK, Gill RK, Alrefai WA. Intestinal Absorption of Bile Acids in Health and Disease. Compr Physiol 2019; 10(Suppl 1): 21–56.
12. Koutsounas I, Theocharis S, Delladetsima I, Patsouris E, Giaginis C. Farnesoid X receptor in human metabolism and disease: the interplay between gene polymorphisms, clinical phenotypes and disease susceptibility. Expert Opin Drug Metab Toxicol 2015; 11(Suppl 4): 523–32.
13. Hiebl V, Ladurner A, Latkolik S, Dirsch VM. Natural products as modulators of the nuclear receptors and metabolic sensors LXR, FXR and RXR. Biotechnol Adv 2018; 36(Suppl 6): 1657–98.
14. Veselsky SP, Lyashchenko PS, Lukyanenko IA. Method for determination of bile acids in biological fluids. Laboratory Work 1979; 3: 176–81.
15. National Center for Biotechnology Information U.S. National Library of Medicine. ALFA: Allele Frequency Aggregator. (Accessed March 10, 2020, at www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/.)
16. Nijmeijer RM, Gadaleta RM, van Mil SW, et al. Farnesoid X receptor (FXR) activation and FXR genetic variation in inflammatory bowel disease. PLoS One 2011; 6(Suppl 8): e23745.
17. Lutz P, Berger C, Langhans B, et al. A farnesoid X receptor polymorphism predisposes to spontaneous bacterial peritonitis. Dig Liver Dis 2014; 46: 1047–50.
18. van den Berg SW, Dollé ME, Imholz S, et al. Genetic variations in regulatory pathways of fatty acid and glucose metabolism are associated with obesity phenotypes: a population-based cohort study. Int J Obes (Lond) 2009; 33: 1143–52.
19. Hu M, Lui SS, Tam LS, Li EK, Tomlinson B. The farnesoid X receptor -1G>T polymorphism influences the lipid response to rosuvastatin. J Lipid Res 2012; 53: 1384–9.
20. Liu M, Wu XJ, Zhao GL, et al. Effects of Polymorphisms in NR1H4, NR1I2, SLCO1B1, and ABCG2 on the Pharmacokinetics of Rosuvastatin in Healthy Chinese Volunteers. J Cardiovasc Pharmacol 2016; 68: 383–90.
21. Heni M, Wagner R, Ketterer C, et al. Genetic variation in NR1H4 encoding the bile acid receptor FXR determines fasting glucose and free fatty acid levels in humans. J Clin Endocrinol Metab 2013; 98: E1224–9.
22. Attinkara R, Mwinyi J, Truninger K, et al. Association of genetic variation in the NR1H4 gene, encoding the nuclear bile acid receptor FXR, with inflammatory bowel disease. BMC Res Notes 2012; 5: 461.
23. Kovacs P, Kress R, Rocha J, et al. Variation of the gene encoding the nuclear bile salt receptor FXR and gallstone susceptibility in mice and humans. J Hepatol 2008; 48: 116–24.

24. Alashkar F, Weber SN, Vance C, et al. Persisting hyperbilirubinemia in patients with paroxysmal nocturnal hemoglobinuria (PNH) chronically treated with eculizumab: The role of hepatocanalicular transporter variants. Eur J Haematol 2017; 99: 350–6.
25. Dixon PH, Wadsworth CA, Chambers J, et al. A comprehensive analysis of common genetic variation around six candidate loci for intrahepatic cholestasis of pregnancy. Am J Gastroenterol 2014; 109(Suppl 1): 76–84.
26. Van Mil SW, Milona A, Dixon PH, et al. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. Gastroenterology 2007; 133(Suppl 2): 507–16.
27. Patrushev LI, Kovalenko F. Functions of non-coding sequences of the mammalian genome. Adv Biol Chem 2014; 54: 39–102.
28. Ramírez-Bello J, Jiménez-Morales M. Functional implications of single nucleotide polymorphisms (SNPs) in protein-coding and non-coding RNA genes in multifactorial diseases. Gac Med Mex 2017; 153(Suppl 2): 238–50.
29. Chiang JYL, Ferrell JM. Bile acid receptors FXR and TGR5 signaling in fatty liver diseases and therapy. Am J Physiol Gastrointest Liver Physiol 2020; 318(Suppl 3): G554–G573.
30. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 2000; 102(Suppl 6): 731–44.
31. Alvarez-Sola G, Uriarte I, Latasa MU, et al. Fibroblast growth factor 15/19 (FGF15/19) protects from diet-induced hepatic steatosis: development of an FGF19-based chimeric molecule to promote fatty liver regeneration. Gut 2017; 66: 1818–28.
32. Mouzaki M, Wang AY, Bandsma R, et al. Bile Acids and Dysbiosis in Non-Alcoholic Fatty Liver Disease. PLoS One 2016; 11(Suppl 5): e0151829.
33. Ferslew BC, Xie G, Johnston CK, et al. Altered Bile Acid Metabolome in Patients with Nonalcoholic Steatohepatitis. Dig Dis Sci 2015; 60(Suppl 11): 3318–28.
34. Inagaki T, Moschetta A, Lee YK, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A 2006; 103(Suppl 10): 3920–5.