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Gene sequence variants PPARGC1Ars8192678, PPARG2rs1801282, FTORS9939609, LEPRS7799039 and LEPRRS1137101 in non-alcoholic fatty liver disease

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Abstract:
Objective: assessing the association of sequence variants rs8192678, rs1801282, rs9939609, rs7799039 and rs1137101in PPARGC1A, PPARG2, FTO, LEP and LEPR genes, respectively,with non-alcoholic fatty liver disease (NAFLD) inyounger adults (18-44 years old) of the Russian Federation.
Materials and Methods. Our case-control study encompassed 100 patients distributed between two groups: Group 1 (cases) with patients suffering from NAFLD (n=50) andGroup 2 (controls) with individuals without it (n=50). All subjects underwent a conventional sonography of their liver and shear wave elastography (Aixplorer®, France): both ultrasound examinations assessed the severity of liver steatosis and fibrosis.
Results. We discovered two sequence variants associated with an increased risk of NAFLD in women: rs9939609 and rs7799039: A/A rs9939609 genotype (OR 5.33, 95% CI 1.14-24.90,p=0.041) and G/G rs7799039genotype (OR 7.5, 95% CI 1.04-54.12,p=0.026).
Conclusion.The A/A genotype of the rs9939609 gene in younger women of the Russian population yielded the fivefold increase in the likelihood of NAFLD, whereas the G/G genotype of the rs7799039 gene resulted in a 7.5-fold likelihood ofNAFLD occurrence.
Keywords: non-alcoholic fatty liver disease, rs8192678, rs1801282, rs9939609, rs7799039, rs1137101.

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Introduction

In many countries worldwide, non-alcoholic fatty liver disease (NAFLD) is among the most common causes of chronic liver diseases [1, 2]. Genetic and environmental factors play an important role in the development of NAFLD. Sequence variants are useful tools in the course of searching for genetic factors responsible for disease development: that is why they are intensively studied in common chronic non-communicable diseases. Our study focused on sequence variants in the peroxisome proliferator-activated receptor-γ coactivator 1-agene (PPARGC1A; rs8192678), the peroxisome proliferator-activated receptor- γ2 gene (PPARG2; rs1801282), the fat mass and obesity-associated gene (FTO; rs9939609), as well as leptin (LEP; rs7799039) and leptin receptor (LEPR; rs1137101) genes, which are associated with obesity, arterial hypertension, metabolic syndrome and type II diabetes mellitus in various ethnic populations [3-5]. Because replication of a direct association across multiple independently established data sets from different ethnic groups is essential for identifying true population-based susceptibility variants, we examined the role of these five sequence variants among younger adults in terms of their association with NAFLD.

Objective:assessing the association of sequence variants rs8192678, rs1801282, rs9939609,rs7799039 and rs1137101in PPARGC1A, PPARG2,FTO,LEP and LEPRgenes, respectively, with NAFLD in younger adults (18-44 years old) of the Russian Federation.

Materials and Methods

Our case-control study included 100 patients using the continuous sampling method. Inclusion criteria were age from 18 to 44 years, absence of any acute or chronic infection, absence of drug/alcohol abuse, and absence of lactation or pregnancy at the time of the study. Exclusion criteria were other liver diseases, including viral hepatitis; cardiovascular diseases, including stroke and myocardial infarction; diabetes mellitus, cancer in the last five years, and continuous use of antihypertensive, lipid-lowering, hypoglycemic, hormonal and hepatotoxic medicines. All study participants signed written informed consent.

We collected complaints and anamneses of the subjects, followed by their objective examinationand assessment of their anthropometric data. Venous blood samples were obtained from participants after an overnight fast (12 hours) to measure levels of alanine transaminase, asparagine transaminase, fasting blood glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. All biochemical laboratory parameters were measured using a conventional automated analyzer (Sapphire 400, Japan).

All subjects underwent a conventional sonography of their liver and shear wave elastography (SWE) (Aixplorer®, France): both ultrasound examinations assessed the severity of liver steatosis and fibrosis. Based on the examination results and taking into account the inclusion and exclusion criteria, all participants were distributed between two groups: Group 1 (cases) including patients with NAFLD (n=50) andGroup 2 (control subjects) with individuals without NAFLD (n=50). Individuals who met clinical criteria and had typical results of their liver sonography and SWE were recruited as cases. Using a 1:1 matching method, the same number of healthy people without NAFLD, matched by gender, age (less than 5 years difference), professional occupation and regions of residence, were included as controls.

The study of single nucleotide polymorphisms (SNPs) in genes was carried out using the real-time polymerase chain reaction method according to the manufacturer's instructions (Litekh, Russia). For each case-control study, the frequencies of genotypes or alleles were compared between cases and controls in three different modes and their fit to Hardy–Weinberg equilibrium via the Pearson'sχ2 test in the online program known as SNPStats (Catalan Institute of Oncology, Barcelona, Spain) [6]. In the first mode (allele frequency mode), allele frequencies were compared between cases and controls using a 2×2 contingency table. In the second mode (recessive mode), the frequencies of subjects homozygous for allele 1 were compared with the rest using a 2×2 contingency table. In the third mode (dominant mode), the frequencies of subjects homozygous or heterozygotes for allele 1 were compared with the rest using a 2×2 contingency table. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis, and their values were adjusted for age, gender, and body mass index. Data are presented as median and interquartile range for continuous variables using the Mann–Whitney U test, or as absolute and relative frequencies for categorical variables using the Pearson'sχ2 test. Statistical tests wereconducted using the MedCalc statistical software package (version 22.009). Statistical significance was assumed at $p<0.05$.

Results

Study subjects with NAFLD and control group participants (50 individuals in each) were successfully genotyped for further analysis. Male gender accountedfor 26% of controls and 34% of cases. Our study included fewer men than women. At the same time, there were no differences in gender distribution between patients with NAFLD and the control group. There was a difference between cases and controls in terms of mean age (38 [34-40] years in cases vs. 34.5 [28-38] years in controls, $p=0.004$) and body mass index (29 [25-31] kg/m2 in cases and 22.3 [20.4-25.8] kg/m2 in controls, $p<0.001$). We found no significant differences in laboratory metabolic parameters between the groups. Four polymorphisms (rs8192678, rs9939609 rs7799039, rs1137101) corresponded to Hardy–Weinberg equilibrium in the control group ($p>0.05$).

The associations between the studied SNPs and the risks of developing NAFLD using five genetic models (codominant, dominant, recessive, overdominant and log-additive) were assessed using logistic regression analysis. As shown in *Table 1*, a rough analysis did not reveal an association of the studied sequence variants with an increased risk of developing NAFLD. We compared differences in allele frequency distributions between cases and controls using Pearson's χ2 test and found no genes or alleles associated with NAFLD (*Table 2*). However, after adjusting for gender, we revealed two sequence variants associated with an increased risk of developing NAFLD in women: genotype A/A rs9939609 (OR 5.33; 95% CI = 1.14-24.90; $p=0.041$) and genotype G/G rs7799039 (OR 7.5; 95% CI = 1.04-54.12; $p=0.026$) (*Tables 2 and 3*).

By analyzing haplotypes including five sequence variants (rs8192678, rs8192678, rs1801282, rs9939609, rs7799039, rs1137101), we assessed the association between presumed haplotypes and the risk of developing NAFLD.

The block of haplotypes, which included two sequence variants (rs9939609 and rs7799039), is shown in *Figure* below, but we could not establish whether these haplotypes were associated with the risk of developing NAFLD.

Discussion

Genetic factors are important in the development of NAFLD, and recent advances in genotyping techniques allowed identifying genetic variations associated with susceptibility to developing NAFLD. There have been several reports of sequence variants associated with the risk of developing NAFLD, which were responsible for regulating inflammation, lipid metabolism and oxidation [7]. Our study has revealed an association of NAFLD with the AA sequence variant rs9939609 of the FTO gene, which encodes a protein in a cell nucleus comprising 506 amino acids and sharing sequences with iron (II)- and 2-oxoglutarate-dependent oxygenases, making it capable of altering DNA methylation and regulating gene transcription. It is the A allele of rs9939609 that is associated with a higher risk of developing NAFLD among women, which is caused bytheir more emotional attitude towards food due to disruption of the circulating levels of the orexigenic hormone acyl-ghrelin, a key hormone that regulates appetite [8]. The sequence variant rs7799039 is associated with increased serum leptin levels, metabolic syndrome, hypertension, and obesity [9, 10]. Despite the heterogeneous results of the studied associations, our study exhibited a direct association of this variant with the risk of developing NAFLD in women in the Russian population.

Table 1. Association ofrs8192678, rs1801282, rs9939609, rs7799039 and rs1137101 genes with NAFLD в cases vs. controls (n=100, rough analysis)

Model	Genotype	Controls*	Cases*	OR (95%CI)	*p*	AIC	BIC
rs 8192678							
Codominant	G/G	20 (40%)	24 (48%)	1.00	0.71	143.9	151.8
	G/A	26 (52%)	23 (46%)	0.74 (0.33-1.67)			
	A/A	4 (8%)	3 (6%)	0.63 (0.12-3.13)			
Dominant	G/G	20 (40%)	24 (48%)	1.00	0.42	142	147.2
	G/A-A/A	30 (60%)	26 (52%)	0.72 (0.33-1.60)			
Recessive	G/G-G/A	46 (92%)	47 (94%)	1.00	0.69	142.5	147.7
	A/A	4 (8%)	3 (6%)	0.73 (0.16-3.46)			
Overdominant	G/G-A/A	24 (48%)	27 (54%)	1.00	0.55	142.3	147.5
	G/A	26 (52%)	23 (46%)	0.79(0.36-1.72)			
Log-additive	–	–	–	0.76(0.40-1.46)	0.41	142	147.2
rs1801282							
Codominant	C/C	33 (66%)	38 (76%)	1.00	0.39	142.8	150.6
	C/G	12 (24%)	10 (20%)	0.72 (0.28-1.89)			
	G/G	5 (10%)	2 (4%)	0.35 (0.06-1.91)			
Dominant	C/C	33 (66%)	38 (76%)	1.00	0.27	141.4	146.6
	C/G-G/G	17 (34%)	12 (24%)	0.61 (0.26-1.47)			
Recessive	C/C-C/G	45 (90%)	48 (96%)	1.00	0.23	141.2	146.4
	G/G	5 (10%)	2 (4%)	0.38 (0.07-2.03)			
Overdominant	C/C-G/G	38 (76%)	40 (80%)	1.00	0.63	142.4	147.6
	C/G	12 (24%)	10 (20%)	0.79 (0.31-2.05)			
Log-additive	–			0.64 (0.33-1.25)	0.19	140.9	146.1
rs9939609							
Codominant	A/A	19 (38%)	18 (36%)	1.00	0.57	143.5	151.3
	A/T	24 (48%)	21 (42%)	0.92 (0.39-2.21)			
	T/T	7 (14%)	11 (22%)	1.66 (0.53-5.22)			
Dominant	A/A	19 (38%)	18 (36%)	1.00	0.84	142.6	147.8
	A/T-T/T	31 (62%)	32 (64%)	1.09 (0.48-2.45)			
Recessive	A/A-A/T	43 (86%)	39 (78%)	1.00	0.3	141.5	146.7
	T/T	7 (14%)	11 (22%)	1.73 (0.61-4.91)			
Overdominant	A/A-T/T	26 (52%)	29 (58%)	1.00	0.55	142.3	147.5
	A/T	24 (48%)	21 (42%)	0.78 (0.36-1.73)			
Log-additive	–			1.22 (0.70-2.11)	0.49	142.1	147.4
rs7799039							
Codominant	A/A	15 (30%)	10 (20%)	1.00	0.35	142.5	150.3
	G/A	23 (46%)	30 (60%)	1.96 (0.74-5.15)			
	G/G	12 (24%)	10 (20%)	1.25 (0.39-3.99)			
Dominant	A/A	15 (30%)	10 (20%)	1.00	0.25	141.3	146.5
	G/A-G/G	35 (70%)	40 (80%)	1.71 (0.68-4.30)			
Recessive	A/A-G/A	38 (76%)	40 (80%)	1.00	0.63	142.4	147.6
	G/G	12 (24%)	10 (20%)	0.79 (0.31-2.05)			
Overdominant	A/A-G/G	27 (54%)	20 (40%)	1.00	0.16	140.7	145.9
	G/A	23 (46%)	30 (60%)	1.76 (0.80-3.89)			
Log-additive	–			1.14 (0.64-2.02)	0.66	142.4	147.6
rs1137101							
Codominant	G/G	15 (30%)	17 (34%)	1.00	0.59	143.6	151.4
	G/A	24 (48%)	19 (38%)	0.70 (0.28-1.75)			
	A/A	11 (22%)	14 (28%)	1.12 (0.39-3.21)			
Dominant	G/G	15 (30%)	17 (34%)	1.00	0.67	142.4	147.7
	G/A-A/A	35 (70%)	33 (66%)	0.83 (0.36-1.93)			
Recessive	G/G-G/A	39 (78%)	36 (72%)	1.00	0.49	142.1	147.4
	A/A	11 (22%)	14 (28%)	1.38 (0.55-3.43)			
Overdominant	G/G-A/A	26 (52%)	31 (62%)	1.00	0.31	141.6	146.8
	G/A	24 (48%)	19 (38%)	0.66 (0.30-1.47)			
Log-additive	–			1.04 (0.61-1.75)	0.89	142.6	147.8

*, adjusted for age, gender and body mass index; NAFLD, non-alcoholic fatty liver disease; AIC, Akaike information criterion; BIC, Bayesian information criterion; OR, odds ratio; CI, confidence interval.

Table 2. Association ofrs9939609 with NAFLD adjusted for gender

Genotype	Gender					
	Male			Female		
	controls	cases	OR (95% CI)	controls	cases	OR (95% CI)
A/A	16	9	1.00	3	9	**5.33(1.14-24.90)**
A/T	16	15	1.67 (0.57-4.90)	8	6	1.33(0.35-5.08)
T/T	5	9	3.20 (0.82-12.53)	2	2	1.78(0.21-14.86)

OR, odds ratio; CI, confidence interval; p-valuefor association is 0.041.

Table 3. Association of rs7799039 with gender in NAFLD

Genotype	Controls		Cases	OR (95%CI)
	Male	Female		
A/A	0	9	8	1.00
	1	6	2	0.38 (0.06-2.41)
G/A	0	18	21	1.00
	1	5	9	1.54 (0.44-5.45)
G/G	0	10	4	1.00
	1	2	6	**7.50 (1.04-54.12)**

OR, odds ratio; CI, confidence interval; p-valuefor association is 0.026.

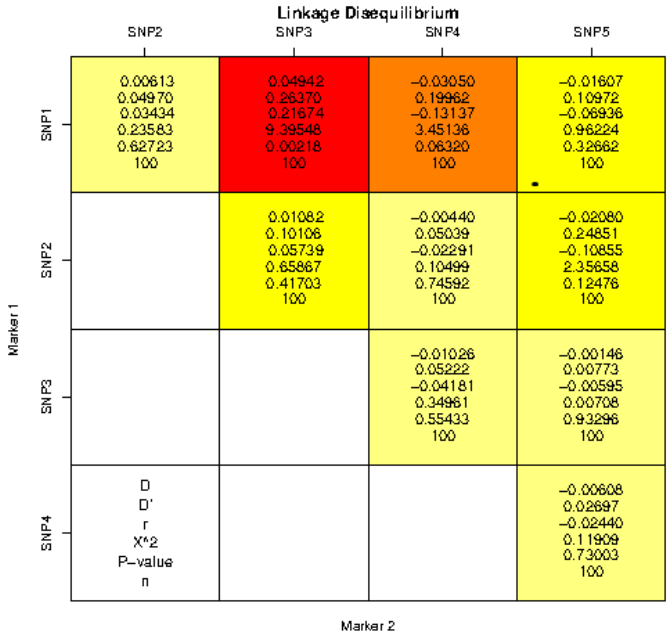

Figure. Multi-SNP analysis. Linkage disequilibrium statistics

Our study had several limitations. First of all, despite our ability to find genetic associations with NAFLD, we did not identify causal mechanisms with all five sequence variants. However, regardless of our small sample size, we were still able to demonstrate several associations with NAFLD in women. Secondly, the diagnosis of NAFLD was based on the results of non-invasive diagnostic techniques, which may have influenced the group allocation of study subjects. Finally, the selection of candidate genes was arbitrary because genome-wide association study of sequence variants in NAFLD was not available.

Conclusion

Hence, we discovered two gene sequence variants (rs9939609 and rs7799039) associated with an increased risk of developing NAFLD in younger women of the Russian population. The A/A genotype of the rs9939609 gene in young women in the Russian population yielded a fivefold increase in NAFLD, while the G/G genotype of the rs7799039 gene resulted in a 7.5-fold likelihood of NAFLD occurrence.

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Conflict of interest. The authors declare no conflicts of interest.

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