Correlation between PNPLA3 and TM6SF gene variations and the occurrence of non-alcoholic fatty liver disease in individuals from Kazakhstan.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) - growing burden on a global scale and considered as the most common liver disease of the 21st century. Genome-wide association studies (GWAS) in the field of liver diseases have made a significant contribution to the understanding of genetic background for NAFLD development. Targeted genes as PNPLA3 and TM6SF2 showed some relationship with the steatosis and hepatocellular carcinoma within NAFLD patients.

The aim of this study is to analyze the frequency of PNPLA3 and TM6SF2 gene polymorphisms and their relationship to changes in instrumental and laboratory markers, the composition of the gut microbiome, the development and progression of NAFLD stage in Kazakhstan.

Methods

- 102 individuals were involved in Medical center hospital of President's affairs administration during outpatient visit for gastroenterologist
- Exclusion criteria: presence of viral hepatitis (A, B, C, D, and E), drug-induced liver injury, autoimmune hepatitis, hepatic storage diseases (glycogen-storage diseases), hereditary liver diseases (alpha-1 antitrypsin deficiency, Wilson's disease), parasitic liver diseases, liver tumors
- The diagnosis was established based on the results of clinical assessment and laboratory-instrumental results
- The microbiome composition of the large intestine was studied by semiconductor sequencing of the bacterial genome using biochips
- The degree of steatosis and liver fibrosis were evaluated by fibroscanning on fibroscan touch 502
- Genotyping of PNPLA3 and TM6SF2 were carried out by PCR

Table 1. Demographics characteristics & main variables

Variables	Mean±SD/(%)	95% CI
Age, years	48.05±8.75	[45.21-50.89]
Male	18 (46.20%)	
Female	21 (53.80%)	
BMI, kg/m^2	30.80±5.28	[29.09-32.51]
BMI <25	5 (12.80%)	
Overweight, 25<BMI<29.9	15 (38.50%)	
Obesity 1, BMI 30-34.9	10 (25.60%)	
Obesity 2, BMI 35-39.9	7 (17.90%)	
Obesity 3, BMI >40	2 (5.10%)	
Diabetes mellitus	4 (10.30%)	
Treatment with statins	19 (48.70%)	

Results

Table 2. Post-hoc analysis of GGT level with Tukey method within PNPLA3 genotypes

Genotype	GGT		p-value
	Mean±SD	95% CI	
C/G	141.97±45.97	89.84-194.10	p1-2=0.043* & p1-3=0.038*
C/C	45.57±29.91	17.90-73.25	p2-1=0.043* & p2-3=0.950
G/G	83.97±12.42	35.17-80.82	p3-1=0.038* & p3-2=0.950

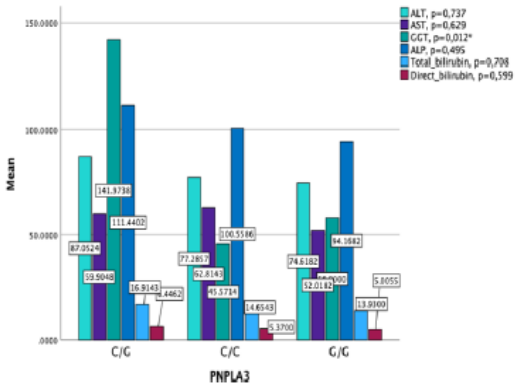

Figure 1. Comparison of liver function parameters depending on PNPLA3 genotypes (Source: Authors' own elaboration)

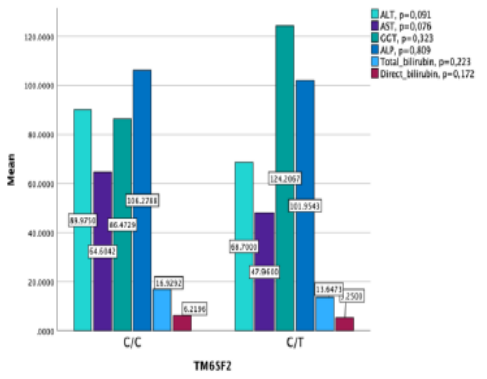

Figure 2. Comparison of liver function parameters depending on TM6SF2 genotypes (Source: Authors' own elaboration)

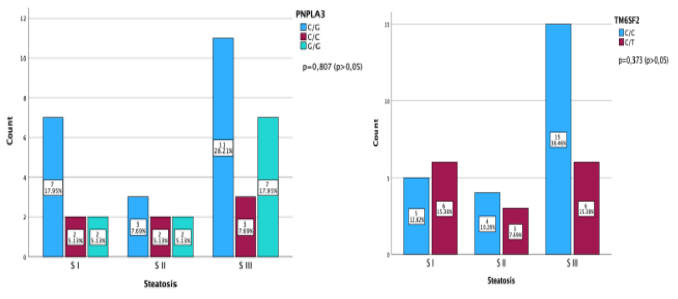

Figure 3. PNPLA3 & TM6SF2 genotypes & steatosis stages

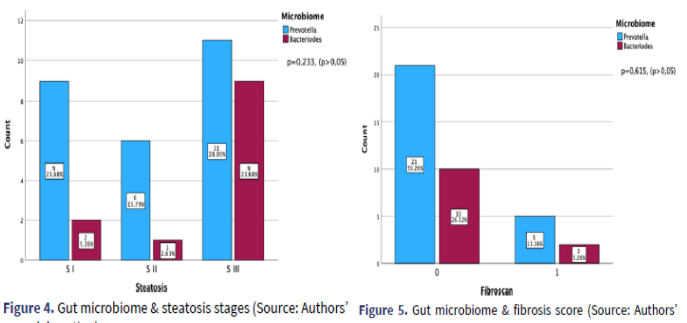

Figure 4. Gut microbiome & steatosis stages (Source: Authors' own elaboration)

Figure 5. Gut microbiome & fibrosis score (Source: Authors' own elaboration)

Table 3. Analysis of total cholesterol depending on PNPLA3 genotypes

Genotype	Total chholesterol		p-value: 0.036*
	Mean±SD	95% CI	
C/G	5.76±1.00	5.30-6.21	p1-2=0.810 & p1-3=0.640
C/C	6.00±0.79	5.26-6.73	p2-1=0.810 & p2-3=0.620
G/G	4.98±0.71	4.49-5.46	p3-1=0.640 & p3-2=0.620

Table 4. LDL analysis depending on PNPLA3 genotypes

Genotype	LDL		p-value: 0.006*
	Mean±SD	95% CI	
C/G	3.90±1.03	3.42-4.37	p1-2=0.980 & p1-3=0.007*
C/C	3.96±0.58	3.42-4.50	p2-1=0.980 & p2-3=0.031*
G/G	2.82±0.71	2.34-3.30	p3-1=0.007* & p3-2=0.031*

Table 5. Analysis of fibrosis on results of liver fibroscan (fibrosis score)

Nucleotide	Percentage (%)		Exact Fischer test (p-value)
	Fibrosis 0: n=34	Fibrosis 1: n=5	
TM6SF2 C/C	21 (61.70%)	3 (60.00%)	1.00 (p>0.05)
TM6SF2 C/T	13 (38.30%)	2 (40.00%)	
PNPLA3 C/G	19 (55.80%)	2(40.00%)	0.767 (p>0.05)
PNPLA3 C/C	6 (17.70%)	1(20.00%)	
PNPLA3 G/G	9 (26.50%)	2(40.00%)	
TM6SF2 C/C	21 (61.70%)	3 (60.00%)	

Table 6. Analysis of liver parameters between enterotypes

P	Enterotypes				p-value
	Prevotella: n=26		Bacteriodes: n=12		
	Mean±SD	95% CI	Mean±SD	95% CI	
AST	51.40±3.75	43.67-59.13	73.7±9.31	53.27-94.28	0.042*
ALT	74.69±4.25	65.93-83.44	97.99±21.38	50.92-145.05	0.144
GGT	90.34±15.69	58.02-122.65	98.48±27.40	38.16-158.80	0.785
ALP	102.37±8.84	84.16-120.58	111.83±7.51	95.29-128.38	0.505
TB	14.38±1.74	10.78-17.99	17.708±3.59	9.87-25.69	0.343
DB	5.16±0.60	3.92-6.41	7.19±1.58	3.71-10.67	0.152

Conclusion

- Polymorphisms in PNPLA3 and TM6SF2 genes were diagnosed in patients with NAFLD, namely the NASH stage
- Previous study results suggests homozygous G/G genotype being the highest risk group for NAFLD development
- In our study G/G genotype was the lowest risk, instead C/G genotype possesses the highest risk and GGT being the statistically significant difference between them in PNPLA3 gene
- TM6SF2 and gut microbiome analysis did not reveal any statistically significant differences
- Considering the relatively small sample size of our study, further studies are required with a larger sample to obtain more sensitive results.

Reference

- European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388-402. https://doi.org/10.1016/ j.jhep.2015.11.004 PMid:27062661
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