GENETICS, PROTEOMICS AND METABOLOMICS

ASSOCIATION BETWEEN VARIANTS OF *PNPLA3* (rs738409), *UCP2* (rs660339) AND *HFE* (rs1800562, rs1800730, rs1799945) GENES AND CHANGES IN THE FUNCTIONING OF THE LIPID PEROXIDATION – ANTIOXIDANT DEFENSE SYSTEM IN PLASMA IN NON-ALCOHOLIC FATTY LIVER DISEASE PATIENTS

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RESUME

Currently reaching epidemic proportions, non-alcoholic fatty liver disease (NAFLD) particularly affects individuals of employable age. The pathogenesis of NAFLD involves a combination of hereditary factors and external influences that collectively disrupt lipid and carbohydrate metabolic pathways and impair the balance between lipid peroxidation and antioxidant protection mechanisms. To date, there has been limited exploration of the possible relationship between these pathological changes and specific variants of the PNPLA3, UCP2, and HFE genes.

The aim. *To examine the association between some markers of the LPO-AOD system in plasma depending on polymorphic variants of the PNPLA3, UCP2 and HFE genes.*

Materials and methods. *For this study, we collected whole blood samples from 116 patients with NAFLD (65 with steatosis and 51 with steatohepatitis) and 100 healthy volunteers. All participants had peripheral venous blood collected for subsequent molecular genetic and biochemical analysis.*

Results. *Our findings indicate that in steatosis, catalase activity was elevated in carriers of the rs660339 TT genotype, while SOD activity was reduced in those with the rs738409 GG variant.*

For steatohepatitis patients, ceruloplasmin levels were altered in opposite directions based on genotype: the rs1800730 TT variant was associated with lower levels, whereas the rs660339 TT genotype was linked to higher levels.

Conclusions. *Polymorphisms rs738409 of the PNPLA3 gene, rs1800730 of the HFE gene and rs660339 of the UCP2 gene are associated with an imbalance in the LPO-AOD system, which may be caused by an increase of the iron level and a change in the antioxidant activity of the UCP2 protein, as well as an increase in the production of prooxidants.*

Key words: *steatosis, steatohepatitis, PNPLA3, HFE, UCP2, lipid peroxidation, antioxidant defense*

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ВЗАИМОСВЯЗЬ НОСИТЕЛЬСТВА ВАРИАНТОВ ГЕНОВ *PNPLA3* (rs738409), *UCP2* (rs660339) И *HFE* (rs1800562, rs1800730, rs1799945) С НЕКОТОРЫМИ ПОКАЗАТЕЛЯМИ СИСТЕМЫ «ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ – АНТИОКСИДАНТНАЯ ЗАЩИТА» У БОЛЬНЫХ С НЕАЛКОГОЛЬНОЙ ЖИРОВОЙ БОЛЕЗНЬЮ ПЕЧЕНИ

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РЕЗЮМЕ

Неалкогольная жировая болезнь печени (НАЖБП) — это заболевание, принимающее в настоящее время размах эпидемии среди населения трудоспособного возраста. Патогенез НАЖБП обусловлен сочетанным воздействием экзогенных факторов и генетической предрасположенности, что приводит к комплексным нарушениям липидного и углеводного обмена, а также дисфункции системы перекисного окисления липидов и антиоксидантной защиты. Влияние полиморфизмов генов PNPLA3, UCP2 и HFE на указанные процессы остается малоизученным.

Целью исследования. *Изучение взаимосвязи некоторых показателей системы «ПОЛ-АОЗ» в плазме в зависимости от носительства отдельных полиморфных вариантов генов PNPLA3, UCP2 и HFE.*

Материалы и методы. *В исследование включены 216 участников, из которых 116 пациентов с верифицированным диагнозом НАЖБП (65 – со стеатозом, 51 – со стеатогепатитом) и 100 условно здоровых лиц контрольной группы. У всех участников проводился забор периферической венозной крови для последующего молекулярно-генетического и биохимического анализа.*

Результаты. *Выявлена зависимость между генетическими вариантами и активностью ферментов антиоксидантной системы у пациентов с различными формами НАЖБП. В группе со стеатозом было установлено статистически значимое повышение каталазной активности у носителей гомозиготного генотипа TT (rs660339), тогда как у пациентов с вариантом GG (rs738409) наблюдалось снижение активности супероксиддисмутазы (СОД).*

У больных стеатогепатитом зафиксированы разнонаправленные изменения концентрации церулоплазмина. Носители гомозиготы TT (rs1800730) демонстрировали снижение уровня данного показателя, в то время как у пациентов с генотипом TT полиморфизма rs660339 отмечалось его достоверное повышение.

Заключение: *Полиморфизмы rs738409 гена PNPLA3, rs1800730 гена HFE и rs660339 гена UCP2 связаны с дисбалансом в системе «ПОЛ-АОЗ», что может быть вызвано нарушением уровня железа и изменением антиоксидантной активности белка UCP2, а также повышением выработки прооксидантов.*

Ключевые слова: *стеатоз, стеатогепатит, PNPLA3, HFE, UCP2, перекисное окисление липидов, антиоксидантная защита*

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INTRODUCTION

The obesity epidemic, resulting from a sedentary lifestyle and consumption of high-calorie foods, is affecting an increasing number of people of all ages worldwide each year [1]. Abdominal obesity, which leads to insulin resistance and dyslipidemia, is a risk factor for non-alcoholic fatty liver disease (NAFLD). This disease is associated with the accumulation of fat in the liver and gradual damage to hepatocytes, increasing the risk of progression to more severe stages, such as steatohepatitis, fibrosis, and cirrhosis. In some cases, it can even lead to hepatocellular carcinoma [2, 3].

According to various studies, the prevalence of NAFLD varies between 17 % and 35 %, with the condition most commonly affecting middle-aged individuals who are part of the working population.

The development of non-alcoholic fatty liver disease is a complex process influenced by various external factors, such as dietary habits and physical activity, as well as hereditary predisposition [4, 5]. Large-scale studies have shown that certain genetic variations play a key role in the development and progression of this pathology. They affect genes that regulate lipid, carbohydrate, and micronutrient metabolism, particularly iron metabolism [6, 7, 8]. The rs738409 genetic variant in the *PNPLA3* gene has been identified as a significant contributor to NAFLD development. This gene encodes for adiponutrin, a protein that plays a crucial role in hepatic lipid metabolism by participating in the formation of lipid droplets and exhibiting activity as an acetyltransferase and retinol esterase. Individuals with the GG genotype of this variant have an increased risk of developing NAFLD and experiencing a more severe clinical course [9, 10, 11].

The *UCP2* gene and its impact on NAFLD have been relatively understood. The protein encoded by the *UCP2* gene, which belongs to the uncoupling protein family, plays a role in regulating insulin secretion, antioxidant system function, and lipid metabolism. The rs660339 (Val55Ala) polymorphism has been suggested as a potential predictor for NAFLD development. This polymorphism may indirectly influence certain pathogenesis pathways associated with NAFLD [12].

As previously stated, NAFLD progression may be linked to gene variants that are not directly related to lipid and carbohydrate metabolism, such as the *HFE* gene. Its polymorphisms (rs1800562, rs1800730, and rs1799945) have been associated with the inability of the HFE protein to interact with the transferrin receptor. This disrupts the signaling pathway and leads to excessive iron uptake by cells. Researchers have linked this to a more adverse prognosis for NAFLD and an increased risk of hepatocellular carcinoma development [13, 14]. Furthermore, increased iron levels in liver cells can contribute to oxidative stress. The increased lipid peroxidation of hepatocytes, imbalanced antioxidant activity, and development of ferroptosis influence the pathogenesis of NAFLD.

Excess iron in hepatocytes can lead to oxidative stress, which is a significant factor in the development of NAFLD. An imbalance in the function of the various components of the body's antioxidant defense system enhances the harmful effects of intracellular iron on liver cells and can result in ferroptosis.

Lipid peroxidation processes in patients with NAFLD have been previously studied, and it has been found that patients with steatohepatitis experience higher levels of oxidative stress compared to those with steatosis. Membrane lipid peroxidation can be primarily explained by the excessive fat accumulation in hepatocytes, which leads to mitochondrial dysfunction as the cells attempt to utilize this fat. This process also results in the formation of a significant number of reactive oxygen species, or free radicals [15, 16]. However, recent studies have demonstrated the influence of ferroptosis on the imbalance between prooxidants and antioxidants in cells and plasma in the development of NAFLD. Ferroptosis is a type of cell death that is closely associated with increased intracellular iron concentration and dysfunction of glutathione peroxidase. Increased iron levels in hepatocytes lead to an increase in the number of spontaneous Fenton reactions with intracellular lipids. The ongoing lipid peroxidation processes suppress individual components of the hepatocyte antioxidant system, which causes cell damage and progression of NAFLD [17, 18]. Although this aspect has not been thoroughly investigated, it is known that elevated plasma iron levels and the occurrence of ferroptotic processes in patients with NAFLD are linked to a more unfavorable prognosis [19].

We hypothesize that investigating the associations between *PNPLA3, UCP2*, and *HFE* gene variants and plasma levels of LPO-AOD in patients with steatohepatitis and steatosis associated with NAFLD may be linked to the related disturbances in iron and lipid metabolism. This could indirectly contribute to the development of lipid peroxidation, which could further elucidate the impact of these genetic variants on the pathogenesis of the disease.

THE AIM OF THE STUDY

To investigate the correlation between certain parameters of the LPO-AOD system in plasma and the presence of specific polymorphic variants of the *PNPLA3, UCP2*, and *HFE* genes.

MATERIALS AND METHODS

The study population consisted of 116 patients with a verified diagnosis of non-alcoholic fatty liver disease (43 men and 73 women) who were treated at the internal medicine department of the Krasnoyarsk Science Centre of the Siberian Branch of Russian Academy of Science in 2021–2022. The age range of the participants

was between 21 and 88 years old, with a median age of 62.3 ± 2 years.

The diagnosis of non-alcoholic fatty liver disease and inclusion in the study group were based on the identification of metabolic disorders (BMI > 30 kg/m^2 and changes in carbohydrate and lipid metabolism), the presence of a cytolytic syndrome (increased ALT and AST with a decreased de Ritis coefficient), and characteristic ultrasound changes in liver tissue, including increased echogenicity and the "white liver" phenomenon [20].

The study excluded patients with a history of viral hepatitis, a significant history of alcohol consumption (as confirmed by the CAGE questionnaire), or parasitic infestations. Patients also had to be willing to participate in the study.

Based on their clinical presentation, the gastroenterologist divided the patients with NAFLD into two groups: a group with steatosis (n = 65, mean age 47.4 ± 4 years) and a group with steatohepatitis (n = 51, mean age 47.4 ± 4 years).

Genetic methods were used to assess the presence of *PNPLA3* (rs738409), *UCP2* (rs660339), and *HFE* (rs1800562, rs1799945, rs1800730) variants within the study population. Genomic DNA was extracted from blood leukocytes using the DNA-sorb-B kit (Central Research Institute of Epidemiology, Rospotrebnadzor, Russia). Genotyping was conducted via RT-PCR using hydrolysis probes on a LightCycler 96 thermocycler (Roche, Switzerland) using commercial kits from TestGen LLC (Russia). Following this, lipid peroxidation-antioxidant defense system parameters were measured in blood plasma using a Genesys 10s UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). Malondialdehyde concentration was determined by measuring the colored product formed in the reaction with 2-thiobarbituric acid, employing a wavelength of 532/600 nm [21]. Superoxide dismutase (SOD) activity was assessed through the autoxidation reaction involving adrenaline, by measuring the concentration increase of the product within the sample at 347 nm wavelength [22]. Catalase activity was measured using a reaction with ammonium molybdate, which produces a chromogen with a maximum at 410 nm [23]. Ceruloplasmin concentration was determined by measuring the amount of n-phenylenediamine that reacted with the protein, compared to a blank sample, at a wavelength of 530 nm [24].

All studies were conducted in accordance with the principles of biomedical ethics and approved by the local ethics committee of the Federal Research Center of the Krasnoyarsk Science Centre of the Siberian Branch of the Russian Academy of Science (Protocol No. 4 dated April 12, 2021).

Data analysis was performed using the software STATISTICA and SPSS v.26. Following testing the sample for normality (Shapiro – Wilk test) and rejecting the hypothesis of normality, a nonparametric Mann – Whitney U-test was employed to compare groups. A χ^2 test was utilized to assess whether the observed genotype frequencies conformed to those predicted by the Hardy – Weinberg equilibrium equation. Data are presented as medians with interquartile ranges [Q25-Q75].

RESULTS

In our previous studies, we identified a discrepancy in the lipid peroxidation-antioxidant defense system (LPO-AOD) in plasma among patients with steatosis and steatohepatitis. Additionally, we determined the distribution patterns of specific polymorphic variants of the *PNPLA3, UCP2*, and *HFE* genes linked to the development of particular clinical forms of NAFLD. A test for compliance with the Hardy – Weinberg equilibrium law of genetics revealed no significant differences between the observed values and the expected values (p > 0.05).

It should be noted that in all study groups, there were no individuals with the homozygous TT genotype of the rs1800562 polymorphism, and no statistically significant differences in the frequency of the rs1799945 polymorphism were detected [25, 26]. The influence of genetic factors on the function of the antioxidant system and production of lipid peroxides may occur in two ways: directly, through changes in the functional activity of proteins associated with polymorphic variants in genes encoding antioxidant system proteins, or indirectly, via changes in signaling pathways and cellular metabolism that can lead to an imbalance in oxidative stress.

In this regard, further analysis was conducted to investigate the impact of polymorphic variants rs738409 (*PNPLA3*), rs660339 (*UCP2*) and rs1800730 (*HFE*) on parameters of the LPO-AOD system in patients with steatosis and steatohepatitis (Table 1).

Malondialdehyde (MDA) is a lipid peroxidation product, that is normally formed during prostaglandin synthesis, and functions as a secondary messenger. In pathological lipid peroxidation, MDA serves as a stable reaction product and can be used to assess the severity of the process in different diseases [16].

Superoxide dismutase (SOD) is an antioxidant enzyme that plays a role in the body's defense against oxidative stress by eliminating superoxide anion radicals. Reduced SOD activity may contribute to impaired function of the LPO-AOD system in NAFLD.

Catalase is also a component of the antioxidant system and catalyzes the decomposition of hydrogen peroxide, converting it into water and oxygen. Reduced catalase activity has been suggested as a potential risk factor for insulin resistance development [27].

Ceruloplasmin is a copper-containing plasma protein that is involved in iron transport, and it also exhibits oxidase and glutathione peroxidase activities, making it an antioxidant in plasma [28].

Statistical analysis has revealed significant alterations in antioxidant enzyme activity among individuals with specific genotypes. For example, in patients with steatosis who carry the homozygous GG genotype

(rs738409), superoxide dismutase (SOD) activity was decreased by 1.2-fold, while catalase activity was increased by six-fold. Odds ratio analysis has confirmed a correlation between the CC+CG allele variants and an opposite trend, namely increased SOD activity and reduced catalase levels.

Among patients with steatosis, those carrying the homozygous TT genotype of the rs660339 UCP2 polymorphism demonstrated a threefold reduction in catalase activity. Odds ratio calculations indicate that the presence of this particular genotype significantly increases the probability of enhanced activity of this enzyme.

Patients with the TT genotype of the rs1800730 polymorphism in the *HFE* gene showed divergent changes in antioxidant enzyme levels: a 1.5-fold decrease in SOD activity, while catalase activity was 6-fold higher compared to patients with the AA and AT variants. Calculation of the odds ratio revealed no correlation between these changes and the presence of the rs1800730 polymorphism.

The analysis revealed a significant reduction in superoxide dismutase (SOD) activity in patients with steatosis who carry the GG genotype of the rs738409 polymorphism in the *PNPLA3* gene. An increase in catalase levels was observed in carriers of the TT genotype of the rs1800730 polymorphism, GG homozygotes (rs738409), and individuals with the TT variant of the rs660339 polymorphism. No statistically significant changes in plasma malondialdehyde (MDA) and ceruloplasmin concentrations were found depending on the studied genetic variants.

The next phase of the study focused on analyzing various parameters of the lipid peroxidation system, which is the body's natural antioxidant defense, in plasma. This included measuring levels of MDA, SOD, catalase, and ceruloplasmin in the blood samples of patients with steatohepatitis. The relationship between these parameters and the presence of specific genetic polymorphisms in the *HFE, UCP2*, and *PNPLA3* genes was investigated (Table 2).

Individuals carrying of the GG variant of the rs738409 polymorphism in the *PNPLA3* gene and steatohepatitis exhibited a 1.3-fold elevation in ceruloplasmin concentrations. However, the odds ratio (OR) showed no significant association observed between ceruloplasmin levels and the specific variant of the rs738409 polymorphism.

Based on the findings, individuals with the TT genotype (rs660339 in the *UCP2* gene) exhibited abnormalities in the activity of essential antioxidant enzymes. In comparison to the CC and CT genotypes, they demonstrated a four-fold reduction in SOD activity and a two-fold increase in ceruloplasmin concentrations. Furthermore, MDA levels in these patients were reduced by 1.5-fold. Statistical analysis using the OR revealed that the TT genotype was associated with elevated ceruloplasmin levels.

Patients with the homozygous TT (rs1800730) variant showed a two-fold increase in SOD activity compared to patients with the AA and AT genotypes, with a simultaneous 1.5-fold reduction in ceruloplasmin levels in these patients. The odds ratio revealed a significant association between the presence of the TT (rs1800730) genotype and reduced ceruloplasmin concentration.

An analysis of markers related to the lipid peroxidation system, which is an antioxidant defense system,

TABLE 1

MARKERS OF THE LPO-AOD SYSTEM IN PLASMA IN STEATOSIS PATIENTS-CARRIERS OF VARIANTS OF THE *PNPLA3* (rs738409), *UCP2* (rs660339) AND *HFE* (rs1800830) GENES

Gene	Genotypes	MDA µmol/g protein		SOD units/min*ml		Catalase µmol/s* mg protein		Ceruloplasmin mg/l	
		Me	[Q25-Q75]	Me	[Q25-Q75]	Me	[Q25-Q75]	Me	[Q25-Q75]
PNPLA3	**CC+CG**	1	0.5–1.7	186	63–321	0.01	0.002–0.03	233	140–317
(rs738409)	**GG**	0.9	0.6–1.3	155	70–204	0.06	0.01–0.1	229	120–352
OR (CI 95%)		**0.04 (0.01-0.32)**		**0.02 (0.001-0.14)**		**0.1 (0.01-0.86)**		0.8 (0.44–1.46)	
p		0.51		**0.01**		**0.01**		0.77	
UCP2	**CC+CT**	0.8	0.5–1.6	185	66–320	0.01	0.002–0.02	238	139–315
(rs660339)	**TT**	1	0.5–2.4	163	35–394	0.03	0.01–0.1	229	130–340
OR (CI 95%)		0.47 (0.19–1.15)		2.28 (1.07–4.85)		**6.8 (1.84-25.1)**		0.84 (0.42–1.68)	
p		0.73		0.62		**0.003**		0.70	
HFE	**AA+AT**	0.95	0.48–1.73	188.4	63.4–335.2	0.01	0.002–0.03	231	138–310
(rs1800730)	**TT**	0.8	0.6–1.3	123	35–204	0.04	0.01–0.05	212	144–352
OR (CI 95%)		**0.05 (0.01-0.36)**		0.71 (0.04–11.65)		1.79 (0.65–4.92)		1.71 (0.92–3.18)	
p		0.36		**>0.001**		**>0.001**		0.79	

TABLE 2

MARKERS OF THE LPO-AOD SYSTEM IN PLASMA IN STEATOHEPATITS PATIENTS-CARRIERS OF VARIANTS OF THE *PNPLA3* (rs738409), *UCP2* (rs660339) AND *HFE* (rs1800830) GENES

Gene	Genotypes	MDA µmol/g protein		SOD units/min*ml		Catalase µmol/s* mg protein		Ceruloplasmin mg/l	
		Me	[Q25-Q75]	Me	[Q25-Q75]	Me	[Q25-Q75]	Me	[Q25-Q75]
PNPLA3	**CC+CG**	1.1	0.7–2.2	200	24–371	0.04	0.02–0.06	210	99–309
(rs738409)	**GG**	1	0.8–1.2	266	245–288	0.07	0.01–0.1	269	207–330
OR (CI 95%)		0.33 (0.03–3.51)		1.17 (0.28–4.87)		**6.5 (1.13-37.4)**		1.29 (0.33–4.97)	
p		0.05		0.14		0.87		**0.03**	
UCP2	**CC+CT**	1.1	0.8–2.36	245	138–386	0.04	0.01–0.05	175	94–277
(rs660339)	**TT**	0.7	0.1–1.2	60	54–160	0.05	0.04–0.07	321	290–330
OR (CI 95%)		1.13 (0.21–6.05)		0.5 (0.07–3.85)		0.9 (0.1–7.78)		**15 (2.02-111.18)**	
p		**0.04**		**0.005**		0.07		**0.001**	
HFE	**AA+AT**	1.1	0.7–2.3	181	11.1–344	0.04	0.02–0.6	249	114–318
(rs1800730)	**TT**	0.8	0.3–1.5	349	203–393	0.03	0.01–0.04	156	88–223
OR (CI 95%)		**36 (3.67-352.66)**		2.8 (0.62–12.6)		0.33 (0.03–3.68)		**0.08 (0.01-0.8)**	
p		0.154		**0.02**		0.074		**0.04**	

in patients with steatohepatitis has revealed a statistically significant association with changes in ceruloplasmin levels in plasma, depending on the presence of certain genetic variations. Patients with the TT genotype of the rs1800730 polymorphism in the *HFE* gene exhibited a decrease in ceruloplasmin, while those with the TT genotype of the rs660339 polymorphism in the *UCP2* gene exhibited an increase. There were no significant associations between the carriage of the polymorphic variants of *PNPLA3, UCP2*, and *HFE* genes and other parameters studied (MDA, SOD, and catalase).

DISCUSSION

The imbalance of the LPO-AOD system in NAFLD is due to several factors. Primarily, it is caused by the excessive accumulation of lipids within cells, leading to mitochondrial dysfunction and an increase in cellular ROS levels. Additionally, excessive fat can cause chronic inflammation, which exacerbates the imbalance of the antioxidant defense system and leads to pro-oxidation. Iron overload in hepatocytes may also contribute to the development of a prooxidant condition. The development of this cascade may be influenced, among other factors, by genetic factors.

According to the data obtained, individuals with the GG genotype of the rs738409 polymorphism and steatosis were found to have reduced SOD activity. However, among patients with steatohepatitis, this genetic variant was not linked to any significant alterations in the functioning of the LPO-AOD system. Moreover, the TT genotype of the rs660339 polymorphism among patients with steatosis was associated with elevated catalase activity. Among patients with both steatosis and the TT genotype, ceruloplasmin levels were increased.

A clear correlation has been established: carriers of the TT variant of the rs1800730 polymorphism and steatohepatitis show decreased ceruloplasmin concentrations. In contrast, no correlation has been found between the studied genetic marker and the LPO-AOD system parameters for cases of steatosis.

Adiponutrin, a product of the *PNPLA3* gene, influences various aspects of lipid metabolism within the liver. When its function is impaired, it leads to an excessive lipid accumulation by hepatocytes, resulting in the development of fatty liver disease and eventually steatohepatitis. Fatty cell infiltration caused by adiponutrin dysfunction likely leads to an increase in β-oxidation. Increased oxidation, in turn, increases the production of ROS, particularly the superoxide anion radical. The excessive superoxide anion radicals can inhibit SOD activity, which may be a contributing factor to the decreased activity seen in patients with steatosis and those carrying the GG genotype of the rs738409 polymorphism.

The effect of the rs738409 polymorphism on oxidative stress development in patients with steatosis has not been previously investigated. For the first time we have demonstrated a decrease in SOD activity in this patient group associated with carriage of a particular variant of the *PNPLA3* gene. This finding may explain the decreased SOD activity

observed in patients with NAFLD, which was also reported by Javed A. et al. [16].

The impact of the rs660339 polymorphism in the *UCP2* gene on the development of steatohepatitis in NAFLD may be related to the fact that the substitution of alanine to valine at position 55 disrupts the functional activity of the UCP2 protein. This may lead to altered uncoupling activity, which indirectly affects the regulation of ROS, insulin secretion, and lipid metabolism. The relationship between the rs660339 variant in the *UCP2* gene and alterations in the LPO-AOD system in plasma among patients with steatosis and steatohepatitis has not yet been investigated. It is hypothesized that these changes may result from the active role of *UCP2* in controlling ROS production and the disruption caused by the Ala55Val substitution. This impairment may be offset by increased catalase activity in patients with steatosis who carry the TT variant (rs660339), as shown in our study. Additionally, in patients with steatohepatitis and the TT genotype, we observed elevated ceruloplasmin levels, which may be associated with severe oxidative stress and the antioxidant function of ceruloplasmin as a ferroxidase and glutathione peroxidase.

The role of the rs1800730 polymorphism in the development and progression of NAFLD has received the least attention in research. However, the contribution of polymorphic variants of the *HFE* gene and elevated iron levels to the development of oxidative stress in other pathologies, such as pulmonary fibrosis, has been established, as well as an effect on ceruloplasmin activity reduction, as reported in the studies of Sangiuolo F., Laine F., and Stevens R. [29, 30, 31].

Our study emphasizes the significance of alterations in the α1 domain structure linked to this polymorphic site and their influence on the development of a particular clinical form of NAFLD. The reduced plasma ceruloplasmin levels observed in patients with steatohepatitis who carry the TT genotype might be linked to impaired iron metabolism and a probable substrate inhibition of the expression of this protein. Although connection between the rs1800730 polymorphism and alterations in antioxidant defense indicators in patients with steatohepatitis has not been investigated, Xia Z. et al. have demonstrated that reduced ceruloplasmin levels in patients with NAFLD correlate with a worse prognosis, further supporting our study and expanding our comprehension of the potential mechanism behind this phenomenon [32].

Thus, variants in the *PNPLA3, UCP2,* and *HFE* genes are linked to opposing alterations in the function of the LPO-AOD system, specifically its antioxidant component, depending on the clinical form.

CONCLUSION

In patients with steatosis, there is a characteristic relationship between polymorphisms and changes in enzymatic activity: rs660339 (TT) is associated with alterations in catalase activity, whereas rs738409 (GG) is linked to suppression of SOD activity. Conversely, in steatohepatitis, the polymorphisms have the opposite effect on ceruloplasmin levels: rs1800730 (TT) correlates with a reduction, while rs660339 (TT) correlates with an increase.

The rs1800730 polymorphism within the *HFE* gene and the rs660339 polymorphism in the *UCP2* gene have been associated with an imbalance in the LPO-AOD system, which may contribute to dysregulation of iron levels and alterations in the antioxidant activity of the UCP2 protein.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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