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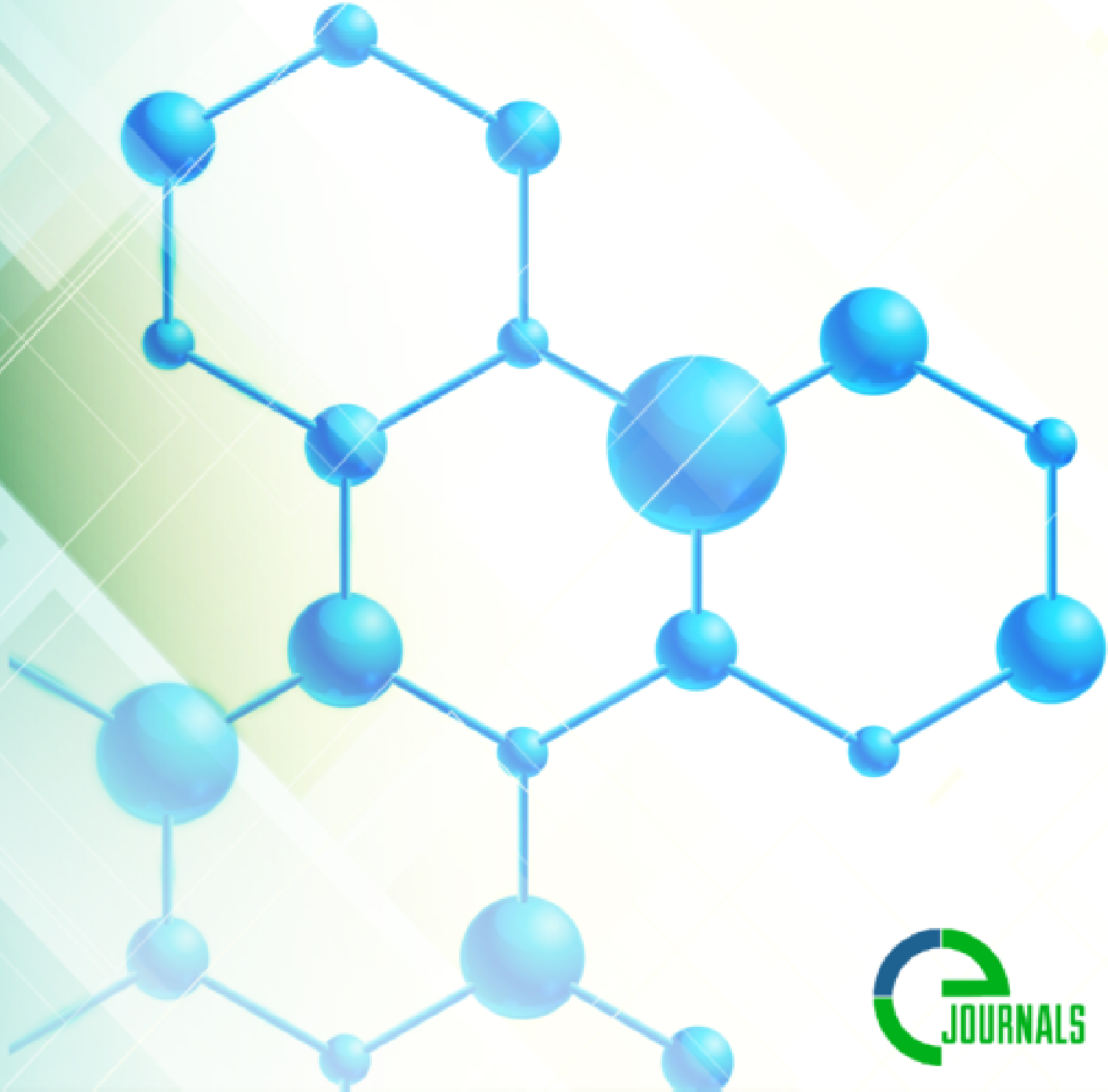

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GENETIC FACTORS OF PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Abstract. The steady increase in the prevalence of non-alcohol fatty liver disease (NAFLD), the appearance of rapidly progressive forms with transformation into liver cirrhosis and hepatocellular carcinoma (HCC) dictate the need for a deeper study of the mechanisms of liver damage. The known facts of the interaction of systemic insulin resistance with the development of fatty liver degeneration cannot fully explain the evolution of the clinical course of this pathology. This review presents the results of a recent study revealing the genetic, molecular aspects of the development and progression of this disease, which, according to forecasts, will soon become the leading cause of end-stage liver disease.

Keywords: non-alcohol fatty liver disease, genetic factors, pathogenesis, liver fibrosis, liver cirrhosis

Introduction. In the national manual "Gastroenterology" edited by Academician of the Russian Academy of Medical Sciences V.T.Ivashkin, nonalcoholic steatoheplatitis (NASH) is defined as a heterogeneous group of pathological liver changes characterized by inflammatory infiltration against the background of fatty degeneration of hepatocytes in people who do not use algogol in hepatotoxic doses [1]. The recommendations of the American Association for the Study of Liver Diseases (AASLD), the American Gastroenterological Association (American Gastroenterological Association, AGA) and the American College of Gastroenterology (American College of Gastroenterology, ACG) 2012 define NAFLD, which includes two positions:

1) signs of liver steatosis (histological or ultrasound examination);
2) no reason for secondary accumulation of fat in the liver:
- significant alcohol consumption;
- the use of teratogenic drugs;
- the presence of hereditary diseases;
- Hepatitis C of the 3rd genotype [2].

Morphological criteria, according to the opinion of D.Torres et al. recognized by professional associations. (2012), there is a macrovesicular accumulation of fat in more than 5% of hepatocytes [3].

Despite the fact that NASH is a relatively new health problem, clarifying the risk of progression of this pathology and associated conditions is the subject of numerous molecular genetic studies. Some of them have already made it possible to clarify the hereditary determinants of liver steatosis and steatohepatitis, independent of insulin resistance and hyperli pidemia.

For a more accurate understanding of the problem, I would like to concretize the concepts of heredity, genetics and epigenetics [4], on which this review is based.

Table 1
Independent predictors of severe progressive course of NASH

Related factors	Age over 45
	Pathological obesity
	Type 2 diabetes mellitus
	Female gender
	Fibrosis
genetic factors	Oxidation defects
	Changes in the structure of mitochondrial DNA
	Specific loci of antigens of the HLA system
	Tumor necrosis factor α, angiotensin
In the presence of concomitant and genetic risk factors, fibrosis with the formation of cirrhosis of the liver progresses in 20-37% of patients with NASH	

• Heredity is the property (ability) of living organisms to repeat in a number of generations the appearance, type of metabolism, features of development and other signs characteristic of each biological species.

• Genetics (from the Greek. γενητως – originating from someone) – the science of the laws of heredity and variability. In the context of this review, we are talking about the polymorphism of genes responsible for the progression of NAFLD.

• Epigenetics (from Greek. επί – above, above, external) is a science that studies the patterns of epigenetic inheritance – changes in gene expression or cell phenotype caused by mechanisms that do not affect the DNA sequence. When exposed to certain medications, lifestyle changes can affect gene polymorphism and gene aberrations.

Back in 2001 [5] the main independent factors of the progressive course of NAFLD were formulated, which were later supplemented [6] with an emphasis on β-oxidation defects leading to mitochondrial DNA polymorphism (Table 1).

Next, some genetic risk factors for the development and progression of NAFLD will be considered.

Studies proving hereditary mechanisms of NAFLD development

Methods confirming the hereditary nature of the disease include studies of ethnic differences, family clusters and a comparative assessment of clinical observations of twins.

The results of the study of the ethnic difference in the prevalence of NAFLD, NASH and cryptogenic cirrhosis in the outcome of these diseases among Latin Americans, Europeans and African Americans are sufficiently reasoned [7, 8].

In the study IRAS (Insulin Resistance Atherosclerosis Study), which included 1,142 participants, the highest risk of NAFLD progression was found in Hispanics, while in the group of African-Americans, even in the presence of obesity and diabetes, no necroinflammatory liver reaction and fibrosis were registered [9-11].

Family cluster studies included the assessment of mainly different types of hyperlipidemia. Phenotypic expression has been established not only in probands, but also in their relatives, confirming the hereditary nature of fatty liver degeneration [12, 13].

These studies are well known, and in the applied aspect we have a number of genetic markers of metabolic syndrome, which allow us to timely assess the prognosis of the disease and take preventive measures.

Studies of the twins turned out to be heterogeneous. Most likely, this is due to the different design of the study, the evaluation criteria and the age of the observed patients. In the group of Danish twins, a significant heritability (35-61%) of increased activity of aminotransferases and γ-glutamyl peptidase was established [14].

Up to 50% of inheritance was verified according to the criteria of metabolic syndrome, namely: insulin resistance, hyperlipidemia and high diastolic pressure [15, 16].

At the same time, it was shown that the characteristic ultrasonographic signs of NAFLD in Hungarian twins have a very low percentage of inheritance [17].

Thus, traditional methods of studying the inheritance of NAFLD showed ambiguous results, which dictated the need for gene-molecular studies to more specifically identify independent genetic risk factors.

PNPLA3/148M gene polymorphism is an independent genetic factor in the development and progression of NAFLD and NASH

In 2008, S.Romeo et al. [18], using the genome-wide scans (GWAS) scanning method, first reported the polymorphism of the PNPLA3/148M gene (patatin–like phospholipase domaincontaining 3 PNPLA3 – patatin-like domain containing 3 phospholipase). This gene is responsible for the transition of isoleucine to methionine at the position of protein 148M and has another name "adiponutrin". Adiponutrin encodes a protein consisting of 481 amino acids responsible for the function of the endoplasmic reticulum, the structure and function of mitochondrial membranes and lipid inclusions in hepatocytes and adipocyte membranes. Data have been obtained that the PNPLA3/148M gene interacts with the lipogenesis regulatory gene (SREBP–1c) [19], which contributes to a change in lipid catabolism and a decrease in the synthesis of phospholipids, in particular phosphatidylcholine [20, 21].

To date, we have data that PNPLA3/148M is an independent genetic predictor of the progressive course of NAFLD, almost 3.3 times higher than the control group [22, 23]. The participation of this gene in the formation of liver cirrhosis and transformation into HCC has been proven [24, 25] regardless of external factors (obesity, alcohol consumption), as shown in the figure.

The PNPLA3/148M gene polymorphism is a candidate for consideration of a new gene basis for liver disease with the assumed name PNPLA3-associated steatohepatitis (NASH)

The most dramatic studies of the role of genetic mutations in the development of NAFLD concern the study of this pathology in children. In 2010, a large retrospective cohort study was published [26], in which the natural course of NAFLD was observed in 66 children for 20 years. The follow-up period was 409.6 man-years. 19 (29%) children met the criteria of metabolic syndrome, 13 of them showed progression of liver fibrosis with repeated biopsy with the outcome of cirrhosis in 41% (5 patients) of cases. In 4 out of 5, a recurrence of NAFLD in the transplant was noted, 3 deaths from the progressive stage of liver cirrhosis were recorded while waiting for transplantation. Molecular genetic studies have shown the presence of PNPLA3/148M gene polymorphism in children with an aggressive course of the disease. In addition, the presence of steatohepatitis in childhood and young age is associated with the identification of polymorphisms of the ENPP1/PC-1, Lys121GLN and IRS-1 Gly972Arg genes responsible for the increased risk of fibrogenesis and known microRNA-10b (miRNA-10b) nuclear receptors, dysregulation of PPAR-α, the blockade of which is the direct cause of steatosis [27-29]. The identification of associations of gene aberrations specific to childhood has yet to be clarified, and therefore it is premature to discuss the sequencing of DNA or genomes.

PNPLA3/I148M – the main genetic risk factor for the transformation of NAFLD and NASH into HCC

It is known that the prevalence of HCC in Western countries is 4:100 thousand of the population, while the frequency of occurrence of HCC in patients with diabetes mellitus is 4 times higher than the frequency in the general population. The following factors are considered as the main mechanisms of the latter: accelerated lipolysis, accumulation of lipids in hepatocytes, oxidative stress with the formation of an excess of free radicals, DNA damage and necrosis of hepatocytes. In published in October 2013 The review [30] presents the results of a multicenter study of the prevalence of HCC, ethnic characteristics of the course of the disease, dependence on environmental factors, the presence or absence of infection with hepatitis B or C viruses, survival, the effectiveness of various treatment methods, as well as data from meta–analysis and

case-control observations. The independent influence of the genetic variability of PNPLA3 (rs738409:C>G) on the development of HCC in US residents has been convincingly shown. Currently [25, 31], large-scale studies are planned to evaluate the PNPLA3 genotype GG (rs738409) as a modulator of the risk of developing HCC in patients with NASH without cirrhosis.

Genetic factors affecting the regulation of lipid metabolism

Since 2006, the role of polymorphism of genes responsible for activation of proliferation of the peroxisome receptor α (PPAR), from the family of nuclear hormone receptors, has been actively discussed. Attempts have been made to use eicosanoids, fibrates, and glitazones as inhibitors of these targets. However, contradictory data have been obtained and even information about a higher risk of histological necroinflammation in patients carrying 12Ala PPAR [32].

In 2008, K.Reue identified the candidate Lipin1 (LPIN1) responsible for the metabolism of phospholipids and triglycerides, and confirmed its participation in the normalization of fatty acid metabolism in hepatocytes [15]. Despite some contradictory results, quantitative metabolic phenotypes were confirmed in the case–control study on 17,538 measurements in the Danish population [16]. A meta-analysis, including 8506 observations, showed the protective mechanism of the LPINrs13412852 T allele [17].

The role of phosphatidylcholine in the prevention of NAFLD was proved as early as 1989 [18] and confirmed by later studies [19]. It is known that holodeficiency diets are models of the development of NASH [20, 21]. At the same time, phosphatidylcholine deficiency leads to mutation of the phosphatidylethanolamine-N-methyltransferase (REMT) gene and the development of severe steatosis [22]. And if the above facts were obtained in mice knocked out by the PEMT gene, then the study of J.Song et al. [23] was performed on 28 patients with NAFLD with histological confirmation. Later on 107

In patients compared with 150 healthy individuals [24], it was shown that variants of the PEMT Val175/Met gene are associated with a high risk of developing NASH even in the absence of insulin resistance and obesity.

Genetic factors affecting oxidative stress

From the standpoint of the theory of multiple impacts of the formation of NASH, oxidative stress is key in the induction of inflammation with the subsequent development of fibrosis.

The main gene aberrations associated with oxidative stress were usually recorded in case–control studies or cohort family studies, as well as a number of ethnic observations. The situations with iron overload or damage to the genes responsible for the activity of superoxide dismutase were mainly considered [25-27]. The role of the identified changes has yet to be assessed.

Genetic factors influencing the development of non-inflammatory and systemic inflammatory reactions

The mechanisms of formation of systemic inflammation and the necroinflammatory reaction of the liver during the development of NASH are well studied and described in detail. The subject of the study is the polymorphism of the genes of transmembrane Toll-like receptor 4 (TLR4), which regulates the activation of nuclear factor kB (NF-kB), mitogenactivated protein kinase and phosphatidylinositol-3 kinase (PI3K), thus affecting the activity of inflammation, apoptosis and fibrogenesis [28-30]. Two genes TLR4 D229G and T399I SNPs are considered, which, according to J.Guo et al. [21], reduce the threshold of apoptosis of activated stellate cells and the activity of fibrogenesis.

The study of polymorphism of tumor necrosis factor α and interleukin-6 genes did not show unambiguous results. The combination of IL28B and PNPLA3 gene polymorphism was recognized as the most significant in assessing the activity of inflammation in NASH in the absence of hepatitis C [22]. The latter was confirmed by observation of 160 patients with confirmed NAFLD biopsy, in whom, when IL28B rs12979860 CC genotype was detected, the severity of necroinflammatory reactions was 4 times higher than the control, regardless of age,

gender, triglyceride levels, hyperuricemia. At the same time, a one–dimensional analysis revealed a direct significant relationship with the severity of fibrosis (stage III-IV).

Applied aspects of genetic research in NAFLD

The genetic basis of the risk of developing NAFLD and NASH with progression to liver cirrhosis and transformation into HCC is beginning to become clear. Variants of polymorphism of genes that are of direct importance in the development of pathology have been identified. However, to date there are no criteria to assess the role of genetic determinants in the response to treatment. Nevertheless, relying on the methodological foundations of epigenetics, it is possible to recommend with a certain degree of confidence the observance of circadian rhythms, the optimal ratio of time for sleep and rest, dietary corrections and exercise regimen.

Markers of predisposition to metabolic diseases have already been validated in practical medicine: ApoE E2/E3/E4, ApoC III (C3238G, S1/S2), ApoC III (T-455C), PON (Gln192Arg), LPL (An291Ser). The inclusion of IL28B CC genotype simultaneously with PNPLA3 in the complex of noninvasive markers of the risk of progression of NASH is actively discussed [22].

The issues of the possibility of drawing up a personalized therapy program based on molecular genetic research are no longer the subject of controversy in a number of nosologies. As for NAFLD, it is possible to correlate the data of clinical and morphological assessment and gene polymorphism only on the basis of comparing the data of fundamental research, understanding the role of oxidative stress in the initiation of genetic aberrations.

In the study H.Dong et al. [23] showed that the administration of a diet rich in choline can affect the reduction of the progression of NAFLD. Scientific publications and recommendations on nutrition indicate the influence of low choline content, in particular phosphatidylcholine, in the diet on methylation processes that change the global gene background, membrane stability and mitochondrial function [24-29].

We must understand that the mechanistic interpolation of experimental studies into the clinic is not entirely correct. Therefore, guidelines on nutrition that recommend choline-rich diets concern the prevention of NAFLD, cardiovascular and cerebrovascular complications. In a series of works by A.Y.Baranovsky and A.Y.Nazarenko, it was shown that the deficiency of unsaturated fatty acids contributes to the progression of NAFLD [30, 31]. Given the importance of phosphatidylcholine in maintaining the structure and function of membranes, in protecting them from reactive oxygen species, the use of drugs with antioxidant properties is pathogenetically understandable. The role of vitamin E as an antioxidant and a means of NAFLD therapy is discussed quite a lot. I would like to mention the publication concerning the positive effect of phosphatidylcholine in the treatment of NASH in patients with hepatitis C associated with steatosis and polymorphism of the IL28CC gene on fibrogenesis and necroinflammatory reaction [32], as well as randomized blind prospective (10 years of follow-up) studies by E.Sas et al., in which it was convincingly shown that long-term use Essentiale forte H in patients with NAFLD, primary and associated with type 2 diabetes, contributes to the stabilization of biochemical parameters, ultrasonographic data, the activity of leptin and a number of proinflammatory cytokines [23, 24].

Conclusion

PNPLA3/I148M is a proven genetic factor in the progressive course of NAFLD. Polymorphism of the main candidate genes is caused by a violation of the oxidation processes leading to damage to mitochondria, prolongation of oxidative stress and activation of inflammatory and fibrogenesis factors. The applied significance of the identified genetic aberrations needs to be investigated and requires a sufficiently long time period to confirm their inheritance. Antioxidant, antifibrotic and antiapoptotic properties of phosphatidylcholine, confirmed in a number of experimental and clinical studies, give grounds to consider it as a drug that affects the epigenetic aspects of the development and progression of NAFLD. Diets enriched with choline and phosphatidylcholine have a protective effect preventing the development of a number of genetic mutations caused by defects in methylation processes, mitochondrial dysfunction and lipid stress.

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