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SIGNIFICANCE OF THE ASSOCIATION BETWEEN PNPLA3 GENE POLYMORPHISM AND NON-ALCOHOLIC FATTY LIVER DISEASE IN THE UZBEK POPULATION

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Global statistics indicate a steady increase in the prevalence of non-alcoholic fatty liver disease (NAFLD) across patient populations [1]. However, the absence of standardized diagnostic and screening criteria complicates the development of robust mechanisms for early diagnosis, pharmacotherapy, and prevention of this condition. NAFLD is a multifactorial disorder, the onset and progression of which are influenced by a number of interrelated factors, including genetic predisposition, age, dietary habits, lifestyle, and level of physical activity [1,2]. Genetic factors play a significant role. One of the candidate genes implicated in NAFLD pathogenesis is the PNPLA3 gene.

The PNPLA3 gene is located at locus q13.31 on chromosome 22. It comprises 9 exons and 8 introns, spans 2805 base pairs, and encodes the adiponutrin protein, which contains a patatin-like phospholipase domain. Adiponutrin consists of 481 amino acids. The rs738409 C/G (Ile148Met) polymorphism involves a cytosine-to-guanine substitution at position 10109 of the PNPLA3 nucleotide sequence, resulting in an amino acid substitution of isoleucine with methionine at position 148 of the encoded protein (Figure 1). S. Romeo et al. (2010) [3], through a GWAS study, demonstrated that the Ile148Met variant of PNPLA3 is associated with NAFLD. The exact intracellular function of adiponutrin remains unclear. Its highest concentrations are found in white adipose tissue adipocytes, although it is also expressed in the liver. This enzyme belongs to the calcium-independent phospholipase A2 family and exhibits both triacylglycerol hydrolase and acylglycerol transacylase activity (including the transfer of fatty acid residues to mono- and diacylglycerols) [4,5].

Genetic association studies across various ethnic groups may yield inconsistent results due to the “ethnic-specific genetic associations” phenomenon, highlighting the need for caution when generalizing findings from one population to another. Until now, the association of rs738409 PNPLA3 polymorphism had not

been assessed in Central Asian populations. The objective of this study was to investigate the association of the PNPLA3 rs738409 polymorphism with NAFLD in the Uzbek population.

Materials and Methods: The study included 73 patients diagnosed with NAFLD who were hospitalized at the Republican Specialized Scientific and Practical Medical Center of Therapy and Medical Rehabilitation under the Ministry of Health of the Republic of Uzbekistan. The control group comprised 37 apparently healthy individuals, matched by age, sex, and ethnicity, selected through random sampling.

The diagnosis of NAFLD was based on medical history, laboratory tests, and liver ultrasound. All participants consumed less than 20 g/day of ethanol and tested negative for chronic viral hepatitis B, C, and D; autoimmune and drug-induced hepatitis; Wilson's disease; idiopathic hemochromatosis; and congenital α1-antitrypsin deficiency.

All participants provided written informed consent. The study protocol adhered to the ethical standards of the National Ethics Committee of Uzbekistan, developed in accordance with the Declaration of Helsinki and its amendments (2013).

Genomic DNA was extracted from peripheral blood lymphocytes using the Diatom™ DNA Prep 200 reagent kit (LLC "Laboratory Izogen"), which employs guanidinium thiocyanate-based lysis for cell disruption, solubilization of cellular debris, and nuclease denaturation. DNA was adsorbed onto a NucleoS™ sorbent, washed with ethanol solution to remove salts and proteins, and eluted for direct analysis.

Genotyping of the rs738409 polymorphism was performed by PCR-RFLP [6] using a GeneAmp 9700 thermal cycler (Applied Biosystems). A 333-bp fragment of the gene was amplified using the primers: 5'-TGGGCCTGAAGTCCGAGGGT-3' and 5'-CCGACACCAGTGCCCTGCAG-3'. The 15 µL PCR mix included 5.2 µL ddH2O, 1.5 µL 10× PCR buffer, 1.5 µL 25 mM MgCl2, 1.5 µL each of 2.5 mM dNTPs, 1.5 µL (10 pmol/µL) of each primer, 0.3 µL (1.5 U) of Taq polymerase, and 2 µL of DNA. PCR conditions: 94°C for 5 min; 37 cycles of 94°C for 30 s, 66°C for 30 s, and 72°C for 40 s; final extension at 72°C for 5 min.

PCR products were digested with BstF5I restriction enzyme (Sibenzyme, Novosibirsk). The reaction mixture included 3.9 µL ddH2O, 1 µL buffer, 5 µL PCR product, and 0.2 µL (2 U) enzyme. Digestion was performed for 16 hours at 65°C. The resulting fragments were separated by 3% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light using a "WiseDoc WGD-30" transilluminator (DAIHAN, Korea). Genotypes were interpreted by band patterns: CC – 200 and 133 bp; CG – 333, 200, and 133 bp; GG – 333 bp.

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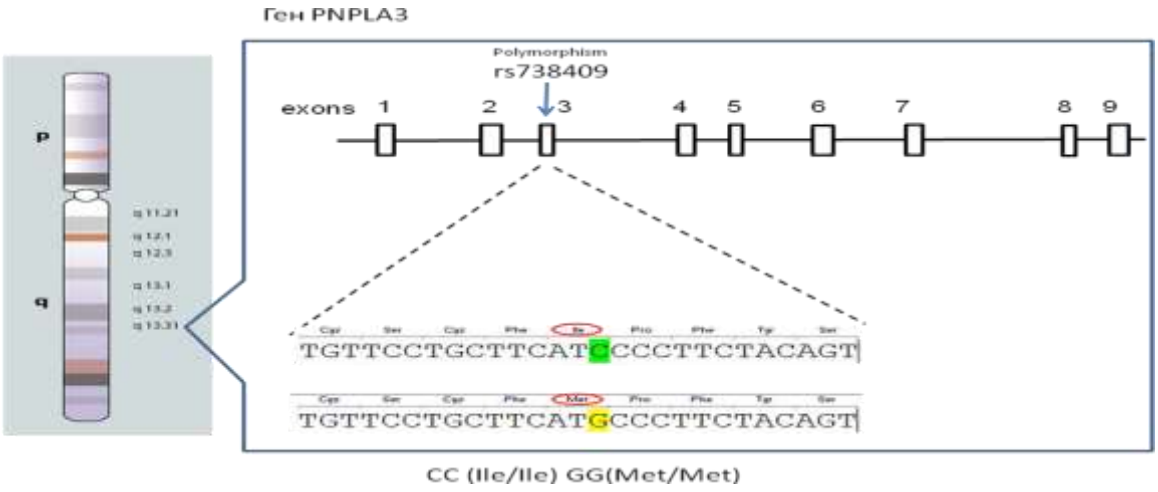
Hardy–Weinberg equilibrium was assessed using Pearson's χ2 test (equilibrium assumed at p > 0.05). Allele and genotype frequencies of PNPLA3 rs738409 were compared between the NAFLD and control groups using Pearson's χ2 test under various inheritance models. A p-value < 0.05 was considered statistically significant. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to determine relative risk. OR = 1 indicated no association; OR > 1 indicated a positive association (increased disease risk); OR < 1 indicated a negative association (reduced risk).

Results: The genotype distribution in the control group was in Hardy–Weinberg equilibrium (χ2 = 0.33; df = 1, p = 0.57). Comparative analysis of allele and genotype frequencies of the PNPLA3 rs738409 polymorphism (Tables 1 and 2) revealed a statistically significant increase in the homozygous GG genotype in patients (45.2%) compared to controls (21.6%) (p = 0.03, additive model; p = 0.02, recessive model). The odds ratio (OR) for NAFLD associated with the GG genotype was 2.99 (CI 95%: 1.21–7.42).

Discussion: Our findings suggest that the PNPLA3 GG (Met/Met) genotype is associated with NAFLD development in the Uzbek population. Molecular genetic prediction using this marker may enable early identification of NAFLD predisposition—even at birth—since an individual's genome remains unchanged throughout life. Furthermore, predisposition may be detected before clinical or biochemical manifestations arise, enabling intervention at the preclinical stage.

Therefore, screening for this polymorphism may be a rational component of NAFLD prevention programs in Uzbekistan.

Figure 1. Location, structure, and rs738409 polymorphism of the PNPLA3 gene



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