CONTRIBUTION OF TNFa (G-308A) GENE POLYMORPHISM TO THE DEVELOPMENT OF APLASTIC ANEMIA

Akhmedova Zukhra Bakhtiyorovna, Matkarimova Dilfuza Saburovna, Boboev Kodirzhon Tukhtaboevich

Republican specialized scientific - practical medical center of Hematology Tashkent medical academy

Summary

Purpose of the study. *To analyze the functional significance of polymorphism of the proinflammatory cytokine TNFa (G-308A) gene in the development of aplastic anemia.*

Methods. *Detection of polymorphic loci of the TNFa gene (rs1800629) in 86 patients with AA and 98 healthy ones using polymerase chain reaction in standard mode with visualization of electrophoresis products on a programmable thermal cycler "Rotor Gene Q" (Quagen, Germany).*

In groups of patients with AA and healthy, a molecular genetic analysis was performed with DNA isolation from peripheral blood using a set of reagents "AmpliPrime RIBOT-prep" (Russia) and detection of TNFa genetic polymorphism (rs1800629) using test systems "Litech, NPF LLC" (Russia). The amplification process was reproduced on the GeneAmp PCR-system 2720 thermal cycler (Applied Biosystems, USA). The amplified products were subjected to electrophoresis in 2% agarose gel to study band patterns using ethidium bromide. Statistical processing of the obtained results was carried out using the OpenEpi – 2009 software package (Version 2.3).

Conclusions. *TNFa genetic polymorphism (rs1800629) is not associated with the risk of aplastic anemia.*

Key words: *Tumor necrosis factor-α TNFa (rs1800629), single nucleotide polymorphism (SNP), autoimmune disease, proinflammatory cytokine.*

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Conclusions. *Genetic polymorphism TNFα (rs1800629) is not associated with the risk of developing aplastic anemia.*

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Introduction. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine produced by various immune cells, including antigen-stimulated T cells, lymphocytes and NK cells [2,4,5]. Its effect is to limit concomitant damage to host cells and tissues during an inflammatory reaction and to maintain a balance between inflammatory and anti-inflammatory reactions [1,8,9].

Recent studies have shown that defective functioning of regulatory T cells leads to increased production of interferon gamma (IFN-γ) and tissue necrosis factor (TNF-α), causing damage to stem cells, which leads to bone marrow aplasia [3,6,7,10].

To determine the distribution of SNP loci of the TNFa polymorphic gene (G-308A) and its connection with the formation of AA, we performed a molecular and genetic analysis of this polymorphism.

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Results. The assumptions made were also proved by the results of mathematical analyses of differences between polymorphic loci of the TNFa (G-308A) gene in the studied groups.

Statistically insignificant differences were found between the frequencies of alleles and genotypes of the TNFa (G-308A) polymorphism in the main and control groups, amounting to less than one for the mutant A allele (5.2% vs. 5.6%; $\chi 2<3.84$; P=0.9; RR=1.0; CI: 0.45-2.22; OR=0.9; CI: 0.38 - 2.3), with a risk of developing the disease equal to 1.1 (OR) – for the main genotype (89.5% vs. 88.8%; -2<3.84; P=0.9; RR=1.0; CI: 0.37-2.75; OR=1.1; CI: 0.43-2.75) and less than one for the G/A heterozygote (10.5% vs. 11.2%; $\chi 2<3.84$; P=0.9; RR=0.9; DI: 0.34-2.54; OR=0.9; DI: 0.36-2.35)

According to the polymorphic TNFa gene (G-308A), no statistically significant differences were found in the frequencies of allelic and genotypic variants between groups of patients with mild AA and healthy patients.The proof of this was the absence of significant differences among carriers of the mutant A allele (3.1% vs. 5.6%; $\chi 2=0.3$; P=0.6; RR=1.0; DI: 0.69-1.4; OR=0.5; DI: 0.07-4.22), the main G|G homozygote (93.8% vs. 88.8%; $\chi 2=0.4$; P=0.6; RR=1.1; DI: 0.02-46.7; OR=1.9; DI: 0.24-15.3) and the heterozygote G/A (6.3% vs. 11.2%; $\chi 2=0.4$; P=0.6; RR=0.6; DI: 00.01- 4.6; OR=0.5; DI: 0.07-4.25)

A similar analysis between groups of patients with severe AA and healthy controls confirmed the absence of statistically significant differences among carriers of polymorphic TNFa (G-308A) gene loci: attenuated A allele (5.4% vs. 5.6%; $\chi 2<3.84$; P=0.98; RR=1.0; CI: 0.51-1.94; OR=0.5; DI:0.33-2.87), the main homozygote G/G (89.1% vs. 88.8%; $\chi 2<3.84$; P=0.95; RR=1.0; DI: 0.22-4.53; OR=1.0; CI: 0.34-3.18) and G/A heterozygotes (6.3% vs. 11.2%; $\chi 2<3.84$; P=0.95; RR=1.0; CI: 0.21-4.37; OR=1.0; DI: 0.31-2.96).This means that the polymorphic loci of the TNFa gene (G-308A) do not have an independent role in the formation of severe AA.

In the structure of the TNFa polymorphic gene (G-308A), a two-way comparative analysis between groups of patients with superheavy AA and healthy ones allowed us to determine the absence of statistically significant differences in the frequencies of allelic and genotypic variants.Thus, among patients, the frequency of the mutant allele A (6.3% vs. 5.6%; $\chi 2<3.84$; P=0.9; RR=1.0; DI: 0.58-1.75; OR=1.1; CI: 0.3-4.18) and heterozygous genotype G/A (12.5% vs. 11.2%; $\chi 2<3.84$; P=0.9; RR=1.1; CI: 0.14-9.14; OR=1.1; CI:0.29-4.41) turned out to be slightly more than one, and the difference between the studied groups in the frequency of the main homozygote G/G did not even reach one (87.5% vs. 88.8%; -2<3.84; P=0.9; RR=1.0; CI: 0.12-8.1; OR=1.9; DI: 0.23-3.46) and heterozygous.The results obtained serve as evidence of the absence of an independent relationship between polymorphic loci of the TNFa gene (G-308A) and an increased risk of formation of the superheavy form of AA.

Analyzing the significance of differences in the frequencies of polymorphic loci of the TNFa gene (G-308A) in a group of patients with mild AA compared with severe and superheavy forms of AA, there were significant differences in the carriage of the mutant A allele (3.1% vs. 5.4%; $\chi2=0.3$; P=0.6; RR=1.0; DI: 0.47 - 2.03; OR=0.6; DI: 0.06-4.86 and 3.1% vs. 6.3%; $\chi2=0.4$; P=0.6; RR=1.0; DI: 0.3-3.11; OR=0.5; DI: 0.05-4.66), the main genotype is G/G (93.8% vs. 89.1%; $\chi2=0.3$; P=0.6; RR=1.1; DI: 0.03-38.79; OR=1.8; DI: 0.2-16.49 and 93.8% vs. 87.5%; $\chi2=0.4$; P=0.6; RR=1.1; DI: 0.04-32.48; OR=2.1; DI: 0.21-21.68) and heterozygous G/A (6.3% vs. 10.9%; $\chi2=0.3$; P=0.6; RR=0.6; DI: 0.02- 21.2; OR=0.5; DI: 0.06-4.93 and 6.3% vs. 12.5%; $\chi2=0.4$; P=0.6; RR=0.5; DI: 0.02-15.16; OR=0.5; DI: 0.05-4.72) was not detected.

Along with this, comparing the degree of differences in the frequencies of polymorphic loci of the TNFa gene (G-308A) between severe and superheavy forms of AA, there were significant differences in the carriage of the mutant A allele (5.4% vs. 6.3%; $\chi2<3.84$; P=0.9; RR=1.0; CI: 0.16-6.08; OR=0.9; CI: 0.2-3.77), the main genotype G/G (89.1% vs. 87.5%; -2<3.84; P=0.9; RR=1.0; CI: 0.34-3.09; OR=1.2; DI: 0.26-5.38) and heterozygous G/A (10.9% vs. 12.5%; $\chi2<3.84$; P=0.9; RR=0.9; DI: 0.29- 2.63; OR=0.9; DI: 0.19-3.92) has also not been established.

Conclusion. Concluding the discussion on the above analysis, taking into account the literature data, it is important to note that cytokine dysfunction can serve as a key cause of AA development. The mechanisms involved in the pathogenesis of AA include the production of T-helper cytokines, such as TNF-α, which has an inhibitory effect on natural T-killers.

The results of the subsequent mathematical analysis of the differences between the studied loci of TNF-α (G-308A) genetic polymorphism among a sample of patients with AA, both in comparison with healthy ones and between patients with different forms of disease severity, served as evidence of the absence of independent associative links between the studied genetic polymorphism with an increased risk of AA ($\chi2<3.84$; P>0.05) and its severity ($\chi2<3.84$; P>0.05).

Conclusion: The results obtained serve as evidence of the absence of an independent relationship between polymorphic loci of the TNFa gene (G-308A) and an increased risk of AA formation and its severe course in Uzbekistan.

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Akhmedova Zukhra Bakhtiyarovna, basic doctoral student RSSPMCH, number: +99894 726 87 88.

Matkarimova Dilfuza Saburova, Professor of the Department of Hematology, Transfusiology and Laboratory Science of TMA, number: +99897 412 91 18.

Boboev Kodirjon Tukhtabaevich, Head of the Department of Molecular Medicine and Cellular Technologies of the RSSPMCH, number: +99890 319 39 57.