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THE LINK BETWEEN MTHFR C677T GENE POLYMORPHISM AND TYPE 2 DIABETES MELLITUS DEVELOPMENT IN UKRAINIAN POPULATION

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WHO postulates that diabetes mellitus is a leading risk factor of the premature death, taking a third place after high arterial blood pressure and smoking. According to the International Diabetes Federation (IDF) about 415 millions of world adult population suffered from this disease, moreover, 318 millions have impaired glucose tolerance which increases diabetes risk emergence in future. Type 2 diabetes mellitus (T2DM) is the most prevalent and it contributes approximately 87-91% of entire diabetes mellitus incidents in countries with high-income level[1-3]. T2DM is considered to be major social and economical problem because of leading many chronic complications further terminating with severe disabling results among abode-bodied population. The most frequent one is diabetic foot syndrome (DFS) characterized by neuropathy and circulation disorders in legs. It results in higher ulceration and infection risks combining with in 25 times increased amputation probability compared to those without diabetes [1-3]. T2DM belongs to multifactorial diseases so genetic predisposition is important for its emergence. Nowadays there is a large amount of researches showing different genes contribution to the T2DM development and methylenetetrahydrofolate reductase (MTHFR) gene is one of them[4-10]. It is coding a protein that participates in homocysteine (Hcy) metabolism – one the central node of T2DM pathogenesis[6]. It is known, that hyperhomocysteinemia (HHcy) causes endoplasmic reticulum stress and increases resistin expression both in vitro and in vivo and suppresses insulin-dependent phosphorylation of tyrosines in β-subunits and insulin receptor substrates[5-9]. MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which serves the methyl donor for Hcy to methionine conversion. Thus, MTHFR limits Hcy concentration and enzyme activity changes could affect plasma level of this metabolite[8, 9, 10]. Nowadays it is known more than 3000 single nucleotide polymorphisms (SNPs) in MTHFR gene according to the National Center for Biotechnology Information (NCBI). The most investigated is C677T SNP of the fourth exon which leads to the alanine to valine substitution in MTHFR catalytic domain. Change in protein primary structure results in its thermolability and loss of effective catalysis. According to the different data 677T-allele decreases MTHFR activity by 35-50% compared to wild type enzyme[6, 8, 9]. The aim of the present study was to investigate the association between MTHFR gene C677T-polymorphic variant and T2DM development and diabetic foot emergence in Ukrainian population. The study was a part of scientific project “Molecular-genetic and morphological features of lower limb tissues regeneration under conditions of chronic hyperglycemia”, no. 0117U003926.

Materials and Methods. Venous blood was selected from 240 patients with diagnosed T2DM (109 females and 131 males; mean age [±SD] 64.6 ± 0.53) and 200 control subjects (88 females and 112 males; mean age [±SD] 76.2 ± 0.74). Diagnosis of T2D was established on the basis of typical symptoms, blood fasting glucose level or glucose tolerance test by perforce and glycated haemoglobin level according to the 1999 WHO diagnostic criteria. Among 240 type 2 diabetic patients 154 had a complication determined as diabetic foot syndrome (DFS). It was established on the basis of clinical examination, impaired sensitivity detection using Neuropathy Disability Score and instrumental methods including Doppler ultrasonography, rheovasography and angiography of the lower extremities. Only individuals without T2DM or impaired glucose tolerance were included to the control group. BMI, fasting glucose level, systolic, diastolic, pulse and mean blood pressure, anamnesis data were collected in both groups. The present study was complied with the Declaration of Helsinki and was approved by the Ethnic Committee of the Medical Institute of Sumy State University. Written informed consent regarding the participation to the present investigation with following venous blood selection was obtained from all subjects. Genomic DNA was extracted from venous blood using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, USA). The determination of MTHFR gene C677T (rs1801133) variant was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. PCR was carried out in Thermocycler GeneAmp PCR System 2700 (Thermo Fisher Scientific, USA). Statistical Package for Social Science software (SPSS, version 17.0, Chicago, IL, USA) was used for most statistical analysis. The comparison of means between the control and study groups was performed using ANOVA or two-tailed Student’s t-test. The χ² test was used to check correspondence to Hardy-Weinberg equilibrium and to compare MTHFR C677T alleles and genotype distributions between case and control groups. Logistic and multivariable regression were used to calculate odds ratio (OR) and 95% confidence interval (CI) for different models of inheritance. All statistical tests were two-sided. Value of P < 0.05 was considered significant.

Results. In T2DM and control groups there were following allele (C – major and T – minor allele) frequencies and genotype distributions: C-allele – 0.64, T-allele – 0.36, C/C – 41.7%, C/T – 44.2%, T/T – 14.1% and C-allele – 0.69, T-allele – 0.31, C/C – 46.0%, C/T – 47.5%, T/T – 6.5% consequently. Obtained allele and genotype frequencies in both T2DM and control group were not deviate from Hardy-Weinberg equilibrium expectations significantly (P = 0.034 and P > 0.05). It was also showed that allele frequencies were not significantly different in compared groups (P = 0.60), but the difference in genotype variants distribution between T2DM and control groups was statistically reliable (P = 0.034).

According to the binary logistic regression results the significant association was in recessive (P = 0.021) and additive (P = 0.025 for T/T genotype) models. It was shown that TT-genotype has in 2.2 (95% CI = 1.128-4.434) times higher risk of type 2 diabetes emergence compared to carriers of major C-allele. After adjusted for sex, age, BMI, obesity, arterial blood pressure and smoking the statistical reliability was retained (P = 0.035; OR = 2.096; 95% CI = 1.054–4.165 and P = 0.047; OR = 2.072; 95% CI = 1.011–4.246 for recessive and T/T-genotype of additive models respectively). It was investigated that TT-genotype males had in 2.6 (95% CI = 1.119–6.155; P = 0.027) times higher...
T2DM risk emergence in comparison with major C-allele carries (according to the recessive model) and after adjusted to other factors association remained significant ($P_{\text{adj}} = 0.029$; $\text{OR}_{\text{adj}} = 2.615$; 95% CI = 1.102–6.208). However, there was no significant association between C677T genotype and risk for T2DM development in female group (both $P_{\text{adj}}$ and $P_{\text{adj}} > 0.05$). It was investigated the following genotypes frequencies among DFS patients: C/C – 37.7%, C/T – 46.8%, T/T – 15.5%. It was also shown that T-allele occurs significantly more frequently in diabetic foot patients compared to control ($P = 0.002$). At the same time the difference in genotype distribution was significant ($P = 0.016$). According to the binary logistic regression results individuals with TT-genotype had in 2.4 (95% CI = 1.166–4.930; $P_{\text{adj}} = 0.017$) times higher risk of diabetic foot emergence comparing with thus carrying C-allele (according to the recessive model) and in 2.6 (95% CI = 1.235–5.640; $P_{\text{adj}} = 0.012$) times compared to C/C-homozygotes (according to the additive model). After adjusted for age, sex, BMI, obesity, arterial blood pressure and smoking the following result was obtained: T/T-genotypes had in 2.5 (95% CI = 1.168–5.164; $P_{\text{adj}} = 0.018$) times and in 2.7 (95% CI = 1.218–5.852; $P_{\text{adj}} = 0.014$) higher risk of diabetic foot development comparing with major allele carries and C/C-homozygates respectively. This is the first study elucidated MTHFR gene C677T-variant distribution among Ukrainian diabetic patients. It was identified that C677T polymorphism of the fourth exon of MTHFR gene is associated with increased T2DM risk development in male subjects. There was no association between MTHFR gene C677T-polymorphic variant and T2DM risk in females. It was also revealed that mentioned locus is related to increased risk of diabetic foot syndrome development regardless of sex. Further studies related to investigation of other genes polymorphism contribution in T2DM development are needed for better understanding of its pathogenesis as well as for improving diagnostic methods, pharmacotherapy and prevention directions on the basis of patient individual features.

References:


Keywords: Single nucleotide polymorphism, methylenetetrahydrofolate reductase, type 2 diabetes mellitus, diabetic foot syndrome.