

# Genetic Polymorphism in Patients with Newly Diagnosed Type 2 Diabetes Mellitus

DOI: 10.17691/stm2019.11.2.08

Received April 11, 2018



**Yu.A. Sorokina**, PhD, Associate Professor, Department of General and Clinical Pharmacology<sup>1</sup>;  
**L.V. Lovtsova**, MD, DSc, Associate Professor, Head of the Department of General and Clinical Pharmacology<sup>1</sup>;  
**A.L. Urakov**, MD, DSc, Professor, Head of the Department of General and Clinical Pharmacology<sup>2</sup>;  
 Senior Researcher, Department of Modeling and Synthesis of Technological Processes<sup>3</sup>;  
**O.V. Zanozina**, MD, DSc, Professor, V.G. Vogralick Hospital Therapy and General Practice Department<sup>1</sup>

<sup>1</sup>Privolzhsky Research Medical University, 10/1 Minin and Pozharsky Square, Nizhny Novgorod, 603005, Russia;

<sup>2</sup>Izhevsk State Medical Academy, 281 Kommunarov St., Udmurt Republic, Izhevsk, 426034, Russia;

<sup>3</sup>Udmurt Federal Research Center of the Ural Branch of the Russian Academy of Sciences, 34 T. Baramzina St., Udmurt Republic, Izhevsk, 426067, Russia

**The aim of the research** was to study the potential response to pharmacotherapy in patients with type 2 diabetes mellitus considering the single nucleotide polymorphisms in the genes encoding for endothelial nitric oxide synthase, 8-oxoguanine DNA glycosylase, and p53 protein as well as their combinations.

**Materials and Methods.** A total of 89 patients with newly diagnosed type 2 diabetes mellitus before the start of pharmacotherapy and 80 diabetes-free individuals were examined. Single nucleotide polymorphisms of endothelial nitric oxide synthase, 8-oxoguanine DNA glycosylase, and p53 protein, as well as their combinations of polymorphic genes, were tested.

**Results.** The occurrence rate of the above polymorphic genes in patients with newly diagnosed type 2 diabetes mellitus is close to that in non-diabetic subjects. The most common gene combinations in patients with type 2 diabetes mellitus and the respective controls have been identified. The polygenomic nature of type 2 diabetes mellitus necessitates considering all possible combinations of polymorphic genes.

**Conclusion.** The results substantiate the need to identify combinations of polymorphisms in patients with newly diagnosed type 2 diabetes mellitus in order to personalize drug therapy and increase its efficacy.

**Key words:** genetic polymorphism; type 2 diabetes mellitus; personalized pharmacotherapy; pharmacological response.

## Introduction

The recent technologies for human genetic analysis allow for a fresh look at the efficacy and safety of prescribed medications. Pharmacogenetics of glucose-lowering drugs is an important part of personalized pharmacotherapy of diabetes [1]. This approach is becoming increasingly relevant in light of the newly discovered genes that control the therapeutic efficacy, drug interactions, and adverse reactions related to the drug action [2]. These genes may encode proteins and enzymes directly involved in pharmacokinetics and pharmacodynamics as well as control major metabolic processes. For example, the formation of nitric oxide is catalyzed by a group of enzymes — nitric oxide synthase. Nitric oxide plays a significant role in controlling the vascular tone and the function of the endothelium, which is highly relevant for patients with type 2 diabetes mellitus (DM2) [3].

It has been proposed that the replacement of thymine (T) with cytosine (C) at position 786 of the endothelial

nitric oxide synthase gene is associated with pathology. Specifically, this substitution was implicated in the development of oxidative stress [4] and an increased risk of hypertension [5]. It is accepted that the activity of nitric oxide synthase has an impact on carbohydrate metabolism — turning off the nitric oxide synthase gene leads to impaired glucose uptake by peripheral tissues, which indicates that glucose utilization is nitric oxide-dependent [6]. The control over glucose uptake, in turn, is a major pharmacological action of glucose-lowering drugs.

DNA is among the most important biomolecules sensitive to active forms of oxygen. The oxidative damage to DNA results in 8-oxoguanine, which plays a role in mutagenesis, carcinogenesis, age-related diseases, and diabetes mellitus [7]. The enzyme 8-oxoguanine DNA glycosylase acts to remove residues of 8-oxoguanine from human DNA. It has been demonstrated that enzyme 8-oxoguanine DNA glycosylase plays a key role in restoring the molecule of DNA from the damage caused by oxidative stress and it also improves glucose utilization by cells [8].

**Corresponding author:** Yuliya A. Sorokina, e-mail: zwx@inbox.ru

Other reports indicated a relationship between the type of diabetes mellitus, its course and duration and the 8-oxoguanine DNA glycosylase gene polymorphism (Ser326Cys rs1052133 C977G) [9, 10].

The p53 protein and the respective *TP53* gene are involved in the cellular response to stress by stopping the cell cycle at control points to repair DNA or inducing apoptosis in case it is impossible to repair the damage [11].

Congenital hyperinsulinism caused by a mutation of glucokinase is associated with apoptosis of pancreatic beta cells. Genetically altered glucokinase activates the p53 suppressor protein. Damage to DNA and p53 plays a key role in the development of hyperinsulinemia and hyperglycemia in DM2 [12].

When studying the gene polymorphisms, one should bear in mind the high probability of their combinations in one individual, which increases the chance of a particular trait, disease, or metabolic disorder [13]. For example, the combination of the *TP53* gene polymorphism with the nuclear repair factor 1 polymorphism increases the risk of developing DM2 by 3 times [14].

**The aim of the research** was to study the potential response to pharmacotherapy in patients with type 2 diabetes mellitus considering the single nucleotide polymorphisms in the genes encoding for endothelial nitric oxide synthase, 8-oxoguanine DNA glycosylase, and p53 protein as well as their combinations.

**Materials and Methods**

The study was based on Nizhny Novgorod Regional Clinical Hospital named after N.A. Semashko and the laboratories of the Privolzhsky Research Medical University. A total of 89 patients with newly diagnosed DM2 and 80 subjects with no signs of diabetes (the comparison group) were examined.

**Frequency of gene polymorphism haplotypes in patients with type 2 diabetes mellitus and diabetes-free subjects**

Genotype	DM2 (n=89)	Group of comparison (n=80)	$\chi^2$	p	Odds ratio	
					Value	95% CI
<i>Endothelial nitric oxide synthase gene</i>						
CC	0.133	0.192	0.87	0.65	1.24	0.37–4.12
CT	0.284	0.308			1.42	0.51–3.97
TT	0.583	0.500			0.63	0.24–1.68
<i>8-oxoguanine DNA glycosylase gene</i>						
CC	0.621	0.625	2.95	0.09	0.98	0.82–1.18
GC	0.379	0.330			1.24	1.03–1.49
GG	—	0.045			0.01	0–0.17
<i>TP53 gene</i>						
CC	0.149	0.124	2.19	0.33	0.35	0.08–1.48
GC	0.362	0.313			1.10	0.49–2.46
GG	0.489	0.563			1.26	0.59–2.71

Here: CI — confidence interval; p is the level of statistical significance of the differences between the main group and the comparison group.

The study was conducted in accordance with the Helsinki Declaration (2013) and approved by the Ethics Committee of the Privolzhsky Research Medical University.

The patients were included in the study according to the following criteria: the diagnosis of DM2 made within 1 year prior to the study; age — from 40 to 70 years; glycated hemoglobin — from 6.5 to 8.0%; body mass index — up to 40 kg/m<sup>2</sup>; voluntary consent to participate in the study.

The exclusion criteria were: DM1; impaired glucose tolerance and impaired fasting glucose; severe complications of DM2; impaired liver, kidney or cardiovascular function; exacerbation of chronic disease; inconsistency with the inclusion criteria.

All subjects were divided into groups according to the type of single nucleotide gene polymorphisms (SNP). The SNP of the endothelial nitric oxide synthase (eNOS3) gene (C786T rs2070744) was identified using the real-time polymerase chain reaction. The SNP of the 8-oxoguanine DNA glycosylase (hOGG1) gene (Ser326Cys rs1052133 C977G) and the *TP53*-encoding gene (Pro72Arg rs1042522 C215G) were determined by polymerase chain reaction with electrophoresis. The analytic procedures were performed using diagnostic kits for detecting human genome polymorphisms (SNP-EXPRESS; Litekh, Russia). Each sample of isolated DNA underwent two amplification reactions in parallel — with two pairs of allele-specific primers. A DT-Prime-5M detection amplifier (DNA-Technology, Russia) was used.

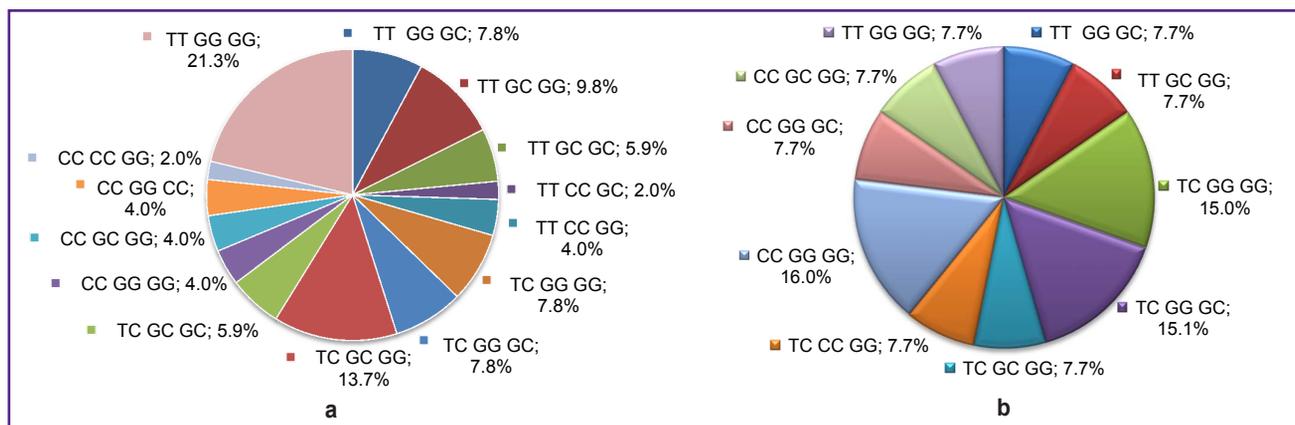
The results obtained in the study were processed using the Statistica 10.0 software package (StatSoft Inc., USA). To assess the statistical significance of the differences between the values, criterion  $\chi^2$  with the Yates correction was used. The critical level of significance when testing the statistical hypotheses was assumed to be 0.05 [15].

To compute the odds ratio, a certified calculator for statistics in case-control studies (Gen-Expert; URL: [http://gen-exp.ru/calculator\\_or.php](http://gen-exp.ru/calculator_or.php)) was used.

**Results**

First, we analyzed the occurrence rates of SNPs of the endothelial nitric oxide synthase gene (C786T rs2070744). There, the CC genotype (homozygous for allele 1) was as frequent in patients with DM2 as in non-diabetic controls. The frequency of heterozygotes (CT) and homozygotes for allele 2 (TT) genotypes practically did not differ between the two groups (see the Table).

No statistically significant difference was found between the frequencies of alleles C and T in the examined groups of patients/subjects.



### The prevalence of polymorphism combinations of the selected genes:

(a) in patients with newly diagnosed type 2 diabetes mellitus; (b) in non-diabetic subjects; the SNP values are shown in the order: genes encoding for eNOS3, TP53, and hOGG1

The data indicated no association between diabetes and the C or T alleles of the *C786T* gene polymorphism. By the parameters of gender, age, body mass index, or the concomitant diseases, the carriers of CC, CT, and TT haplotypes were comparable.

In computing the occurrence of SNP of the 8-oxoguanine DNA glycosylase gene (Ser326Cys rs1052133 C977G), it was found that the CC (homozygote for allele 1) and GC (heterozygote) genotypes were frequent in patients with DM2 as well as in non-diabetic individuals; no GG genotype was detected at all (see the Table). The carriers of these haplotypes were also comparable in terms of gender composition, age, body mass index, and the presence of associated diseases.

Analysis of the occurrence of SNP of the p53 protein gene (Pro72Arg rs1042522 C215G) showed that the CC genotype (homozygous for allele 1) was frequent in patients with DM2 at almost the same level as in the control group of subjects. The frequencies of heterozygotes (GC) and homozygotes for allele 2 (GG) did not significantly differ (see the Table).

The frequencies of alleles C and G in this SNP gene were similar in both the main and comparison groups. No differences were found between the carriers of the p53 gene polymorphisms in terms of gender distribution, age, body mass index, and concomitant diseases.

However, when analyzing the results of individual genotyping, we also considered the variety of combinations of polymorphisms and mutations co-existing simultaneously in the human body. Therefore, after identifying the SNPs of the main genes, we conducted a study on the occurrence of SNP combinations in DM2 patients and in subjects of the comparison group.

The diagrams (see the Figure) show the occurrence rates of gene polymorphism combinations in the following sequence: the first genotype in each combination reflects the polymorphism of the endothelial

eNOS3 (rs2070744), the next genotype reflects the *TP53* gene polymorphism (rs1042522), and the third genotype shows the polymorphism of the hOGG1 gene (rs1052133).

The most frequent combination in patients with DM2 is homozygous for allele 2 of the *C786T* SNP, homozygous for allele 2 of the Pro72Arg SNP, and homozygous for allele 1 of Ser326Cys SNP (21.3% of cases). This is followed by the combination: heterozygous *C786T* SNP, heterozygous Pro72Arg SNP, and homozygote for allele 1 Ser326Cys SNP (13.7% of cases). The rarest combinations are homozygous for allele 1 *C786T* SNP, homozygous for allele 1 Pro72Arg SNP, and homozygous for allele 1 Ser326Cys SNP (2% of cases), and also: homozygote for allele 2 of *C786T* SNP, homozygote for allele 1 of Pro72Arg SNPs, and homozygotes for allele 1 of Ser326Cys SNP (4% of cases) (see the Figure (a)).

In non-DM subjects, the three most common combinations were also identified. The first is heterozygous *C786T* SNP, homozygous for allele 2 of Pro72Arg SNP, and homozygote for allele 1 of Ser326Cys SNP (15% of cases). The second combination is heterozygous *C786T* SNP, homozygous for allele 2 of Pro72Arg SNP, and heterozygote Ser326Cys (15.1% of cases). The third combination is homozygous for allele 1 of *C786T* SNP, homozygous for allele 2 of Pro72Arg SNP, and homozygote for allele 1 of Ser326Cys (16% of cases) (see the Figure (b)).

The presented results on the prevalence of polymorphic gene combinations can be the basis for further monitoring of biochemical processes, metabolism, and pharmacological response in patients with DM2, and non-diabetic individuals.

### Discussion

The findings suggest that the frequencies of polymorphic genes encoding for endothelial nitric oxide

synthase, 8-oxoguanine DNA glycosylase, and p53 protein in patients with recently diagnosed DM2 (without severe diabetic complications) are similar to those in diabetes-free individuals.

There are reports implicating polymorphisms of the above three genes in the risk of developing DM2 and its complications, while other authors do not confirm this association [16, 17]. Likewise, our study found no correlation between the two events. However, the conflicting literature data and the present results suggest the need for dynamic monitoring of patients with different haplotypes of polymorphic genes in order to clarify the influence of genetic diversity on the severity and progression of diabetes. This is due to two reasons: 1) the selected polymorphic genes can be associated not only with the risk of developing diabetes, but rather with its severity, subsequent complications and ineffective glucose-lowering therapy; 2) the diversity of gene SNP combinations may be a factor of tolerability and safety of the sugar-lowering drugs in individual patients [2].

Earlier [18], we identified the so-called “response” and “failure” phenotypes among the polymorphic genes described above, i.e., the gene combinations correlating with the patients’ reaction to therapy with metformin. It was then proposed that the pre-treatment phenotype identification would be important for personalized pharmacotherapy, its efficacy [19] and safety [20]. Nevertheless, the obtained data do not negate the need to dynamically monitor the efficacy and safety of the prescribed pharmacotherapy, as well as the progression of the disease.

## Conclusion

The results of the study demonstrate the presence of genetic polymorphism in patients with newly diagnosed type 2 diabetes mellitus. Combinations of polymorphic genes play a role in progression of the disease and to the long-term effects of pharmacotherapy. These combinations should be taken into account when the strategy of personalized treatment is developed.

**Research funding.** The authors received no specific funding for this project.

**Conflict of interest.** The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

## References

1. Kononenko I.V., Mayorov A.Y., Koksharova E.O., Shestakova M.V. Pharmacogenetics of hypoglycemic agents. *Diabetes Mellitus* 2015; 18(4): 28–34, <https://doi.org/10.14341/dm7681>.
2. Staiger H., Schaeffeler E., Schwab M., Häring H.-U. Pharmacogenetics: implications for modern type 2 diabetes therapy. *Rev Diabet Stud* 2015; 12(3–4): 363–376, <https://doi.org/10.1900/rds.2015.12.363>.
3. Banerjee M., Vats P. Reactive metabolites and

antioxidant gene polymorphisms in type 2 diabetes mellitus. *Br J Biomed Sci* 2014; 20(1): 10, <https://doi.org/10.4103/0971-6866.132747>.

4. Fortis M.F., Fraga L.R., Boquett J.A., Kowalski T.W., Dutra C.G., Gonçalves R.O., Vianna F.S.L., Schüler-Faccini L., Sanseverino M.T.V. Angiogenesis and oxidative stress-related gene variants in recurrent pregnancy loss. *Reprod Fertil Dev* 2018; 30(3): 498–506, <https://doi.org/10.1071/rd17117>.

5. Seidlerová J., Filipovský J., Mayer O. Jr., Kučerová A., Pešta M. Association between endothelial NO synthase polymorphisms and arterial properties in the general population. *Nitric Oxide* 2015; 44: 47–51, <https://doi.org/10.1016/j.niox.2014.11.016>.

6. Miranda J.A., Belo V.A., Souza-Costa D.C., Lanna C.M., Tanus-Santos J.E. eNOS polymorphism associated with metabolic syndrome in children and adolescents. *Mol Cell Biochem* 2013; 372(1–2): 155–160, <https://doi.org/10.1007/s11010-012-1456-y>.

7. Torres-Gonzalez M., Gawlowski T., Kocalis H., Scott B.T., Dillmann W.H. Mitochondrial 8-oxoguanine glycosylase decreases mitochondrial fragmentation and improves mitochondrial function in H9C2 cells under oxidative stress conditions. *Am J Physiol Cell Physiol* 2014; 306(3): C221–C229, <https://doi.org/10.1152/ajpcell.00140.2013>.

8. Yuzefovych L.V., Solodushko V.A., Wilson G.L., Rachek L.I. Protection from palmitate-induced mitochondrial DNA damage prevents from mitochondrial oxidative stress, mitochondrial dysfunction, apoptosis, and impaired insulin signaling in rat L6 skeletal muscle cells. *Endocrinology* 2012; 153(1): 92–100, <https://doi.org/10.1210/en.2011-1442>.

9. Milić M., Kišan M., Rogulj D., Radman M., Lovrenčić M.V., Konjevoda P., Domijan A.M. Level of primary DNA damage in the early stage of metabolic syndrome. *Mutat Res* 2013; 758(1–2): 1–5, <https://doi.org/10.1016/j.mrgentox.2013.07.013>.

10. Hara M., Nakamura K., Nanri H., Nishida Y., Hishida A., Kawai S., Hamajima N., Kita Y., Suzuki S., Mantjoro E.M., Ohnaka K., Uemura H., Matsui D., Oze I., Mikami H., Kubo M., Tanaka H.; Japan Multi-Institutional Collaborative Cohort (J-MICC) Study Group. Associations between hOGG1 Ser326Cys polymorphism and increased body mass index and fasting glucose level in the Japanese general population. *J Epidemiol* 2014; 24(5): 379–384, <https://doi.org/10.2188/jea.je20140002>.

11. Zhang Z., Tang P. Genomic pathology and biomarkers in breast cancer. *Crit Rev Oncog* 2017; 22(5–6): 411–426, <https://doi.org/10.1615/critrevoncog.v22.i5-6.60>.

12. Bonfigli A.R., Sirolla C., Testa R., Cucchi M., Spazzafumo L., Salvioli S., Ceriello A., Olivieri F., Festa R., Procopio A.D., Brandoni G., Boemi M., Marra M., Franceschi C. The p53 codon 72 (Arg72Pro) polymorphism is associated with the degree of insulin resistance in type 2 diabetic subjects: a cross-sectional study. *Acta Diabetol* 2012; 50(3): 429–436, <https://doi.org/10.1007/s00592-012-0450-x>.

13. Murk W., DeWan A.T. Exhaustive genome-wide search for SNP-SNP interactions across 10 human diseases. *G3 (Bethesda)* 2016; 6(7): 2043–2050, <https://doi.org/10.1534/g3.116.028563>.

14. Tornovsky-Babeay S., Dadon D., Ziv O., Tzipilevich E., Kadosh T., Schyr-Ben Haroush R., Hija A., Stolovich-Rain M., Furth-Lavi J., Granot Z., Porat S., Philipson L.H., Herold K.C., Bhatti T.R., Stanley C., Ashcroft F.M., In't Veld P., Saada A., Magnuson M.A., Glaser B., Dor Y. Type 2 diabetes and

congenital hyperinsulinism cause DNA double-strand breaks and p53 activity in  $\beta$  cells. *Cell Metab* 2014; 19(1): 109–121, <https://doi.org/10.1016/j.cmet.2013.11.007>.

15. Rebrova O.Yu. *Statisticheskiy analiz meditsinskikh dannykh. Primenenie paketa prikladnykh programm STATISTICA* [Statistical analysis of medical data. Application of the software package STATISTICA]. Moscow: MediaSfera; 2006; 312 p.

16. Strycharz J., Drzewoski J., Szemraj J., Sliwinska A. Is p53 involved in tissue-specific insulin resistance formation? *Oxid Med Cell Longev* 2017; 2017: 1–23, <https://doi.org/10.1155/2017/9270549>.

17. Kung C.-P., Murphy M.E. The role of the p53 tumor

suppressor in metabolism and diabetes. *J Endocrinol* 2016; 231(2): R61–R75, <https://doi.org/10.1530/joe-16-0324>.

18. Sorokina Y.A. Pharmacogenetic aspects of oral hypoglycemic therapy. Response and failure phenotypes. *Meditsinskiy sovet* 2015; 8: 82–85.

19. Sorokina Yu.A., Zanozina O.V., Lovtsova L.V., Seropyan M.Yu. *A method of flow prediction efficiency and therapy of patients with type 2 diabetes*. Patent RU 2626670. 2017.

20. Urakov A., Urakova N. Rheology and physical-chemical characteristics of the solutions of the medicines. *Journal of Physics: Conference Series* 2015; 602: 012043, <https://doi.org/10.1088/1742-6596/602/1/012043>.