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SHORT COMMUNICATIONS

Association of Variable *rs1801282* Locus of *PPARG2* Gene with Diabetic Nephropathy

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Abstract—The association of the variable *rs1801282* locus of the *PPARG2* gene (peroxisome proliferator-activated receptor gamma) with type 2 diabetes mellitus and its complications was analyzed in inhabitants of the Republic of Bashkortostan. The genotype frequencies of the variable *rs1801282* locus of the *PPARG2* gene did not significantly differ in groups of healthy persons and patients with type 2 diabetes in all three considered inheritance models (codominant, dominant, and recessive). At the same time, it was demonstrated that the risk of one of the diabetic complications, i.e., diabetic nephropathy, was associated with the variable *rs1801282* locus of the *PPARG2* gene. Diabetic nephropathy was more common in patients with the *C/C* genotype (62.7%) compared to the *C/G* and *G/G* genotypes (37.5%), $P = 0.036$. The *G* allele is protective in regard to diabetic nephropathy (OR = 0.36) in patients with type 2 diabetes mellitus.

Keywords: genetic predisposition, peroxisome proliferator-activated receptor gamma, complications of diabetes, polymerase chain reaction, population

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Type 2 diabetes (T2D) is a health and social problem of the modern world. Huge budgets of many states are spent on the treatment of diabetes and its complications. This disease is characterized by a high frequency of complications (retinopathy, nephropathy, neuropathy, and diabetic foot syndrome), which lead to early disability and death. Experts at the International Diabetes Federation (IDF) predict that the number of people with diabetes will reach 380 million by 2025, and most of them will be T2D patients.

T2D results from the interaction of environmental factors (excess food, obesity, physical inactivity, hypertension, etc.) with a multitude of genetic components. The pathogenesis of the disease is, on one hand, the development of insulin resistance and, on the other hand, the defect in insulin secretion. The authors of [1] described the incretin mechanism of impaired glucose homeostasis when so-called incretins are produced in the small intestine in response to oral carbohydrate loading. Incretins enhance the insulin secretion from the beta cells; these hormones are destroyed by the dipeptidyl peptidase 4 (DPP-4). The pathogenesis of diabetes is characterized by the increase in the glucagon secretion in response to hyperglycemia, which maintains a high blood sugar owing to the release of glucose by the liver [1].

A feature of the disease is a high rate of heritability. The risk of T2D is 40% if one of the parents has this disease and 70% if both of them have it [2, 3]. Therefore, it is necessary to study molecular genetic markers of diabetes for better understanding of the pathogenesis of the disease and for the development of new effective medicines against the disease and its prevention.

The approaches to the search for the genes causing multifactorial diseases have been rapidly developing in the last decade. Researchers all over the world have accumulated experience in genetic studies using both the "gene-candidate" approach and the strategy of genome-wide association study (GWAS).

More than 100 genetic markers associated with T2D risk are described to date [4].

A traditional approach to investigate the molecular genetic basis of multifactorial diseases is the study of the linkage disequilibrium (LD) of extended sequences in the families of patients. The loci of the disease can be mapped on a genome-wide level by the genotyping of 400–500 markers. Only two genes of predisposition to diabetes were identified by this way: the calpain 10 gene (*CAPN10*) and the gene of the transcription factor 7-like 2 (*TCF7L2*).

The genes of the disease can be identified by the testing of associations in population studies ("gene-

Table 1. Sequences of primers and fluorescent probes and amplification conditions of *rs1801282* polymorphous locus of the *PPARG2* gene

Sequences of primers, 5'–3'	Sequences of probes, 5'–3'	Annealing temperature, °C
ctcttatgggtgaaactctgg atgaacacgatagcaacgag	r6-gcctattgacgcagaaagcga-bhq fam-cctattgacccagaaagcga-bhq	62

candidate" analysis). The mapping on the genome-wide level requires a very large number of markers because sequences in linkage disequilibrium have a small length in unrelated individuals. Until recently, the study of candidate genes more likely involved in the disease was the only possible strategy.

The *PPARG2* gene (peroxisome proliferator-activated receptor gamma) encoding the nuclear PPAR-gamma receptor was shown in different studies to be the first gene associated with T2D [5, 6]. The PPAR-gamma receptor is the molecular target for thiazolidine compounds, which have hypoglycemic activity. The *PPARG2* gene is expressed in the fat tissue. The variable *rs1801282* locus of this gene contains a single nucleotide substitution of guanine for cytosine in codon 12, which results in the replacement of proline by alanine (*Pro12Ala*) in the protein. About 15% of Caucasoids have alanine at position 12 of the protein product of the *PPARG2* gene. This leads to enhanced transcription activity, enhanced sensitivity to insulin, and protection from the risk of T2D [6]. Although an initial report was followed by a series of negative results, the family studies with the use of the transmission disequilibrium test (TDT) showed, nevertheless, that the *Pro* allele was more frequently transmitted to descendants with T2D [7]. The combination of these studies with meta-analysis confirmed the association between *Pro12Ala* polymorphism and T2D [8, 9].

Molecular genetic studies of T2D on the variable *rs1801282* locus were not previously performed in the Republic of Bashkortostan. The goal of this work is to analyze the associations of the polymorphic *rs1801282* region of the *PPARG2* gene with T2D, as well as with some clinical and metabolic characteristics of patients with T2D and with complications of T2D in inhabitants of the Republic of Bashkortostan.

In total, 620 inhabitants of the Republic of Bashkortostan were examined. There were 294 T2D patients and 326 individuals without clinical and laboratory signs of diabetes and without a family history of diabetes. Samplings of patients and control groups were comparable by sex, age, and ethnicity.

DNA was isolated from venous blood by phenol-chloroform extraction. The DNA regions were amplified by real-time PCR using the TaqMan probes (TestGen, Russia) (Table 1).

The level of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) cholesterol was evaluated by the spot metering method on an Olympus biochemical analyzer (Abbott, Germany) using the Beckman Coulter kits. The content of the C-peptide in the blood serum was evaluated by enzyme-enhanced chemiluminescence on an automatic IMMULITE analyzer (DPC, United States) using reagents from the same company.

The glomerular filtration rate (GFR) was calculated by the standard formula MDRD (Modification of Diet in Renal Disease Study) (http://mdrd.com).

The insulin resistance index HOMA-IR (homeostasis model assessment of insulin resistance) was calculated using the HOMA2 Calculator v2.2.3 program (http://dtu.ox.ac.uk/homa).

The data were statistically processed using the SNPStats program (http://bioinfo.iconcologia.net/snpstats/start.htm?).

Associations were considered significant at $P < 0.05$. The strength of associations was quantitatively evaluated by the odds ratio (OR) using a 95% confidence interval (CI). The genetic marker is considered to be protective or predisposing to the disease at $OR < 1.0$ and $OR > 1.0$, respectively.

All patients signed their informed consent before entering the study. The study was approved at a meeting of the expert council on biomedical ethics in clinical disciplines at Bashkir State Medical University of the Ministry of Health of the Russian Federation (November 15, 2013).

The genotype frequencies of the variable *rs1801282* locus of the *PPARG2* gene did not statistically differ between groups of healthy persons and patients with type 2 diabetes (Table 2). The *C/C* genotype was revealed in 69.7% of the control group and in 68.4% of patients with diabetes; the *C/G* genotype was revealed in 26.5 and 29.8%, respectively ($P = 0.260$).

We performed an analysis of associations between the variable *rs1801282* locus of the *PPARG2* gene and clinical and laboratory characteristics of T2D patients (Table 3). Significant differences in carriers of the *C/C*, *C/G*, and *G/G* genotypes were not revealed by age, age at the moment of the diagnosis of the disease, duration of diabetes, and a family history of diabetes. The groups of patients did not differ in anthropometric characteristics, parameters of carbohydrate and lipid metabolism, the level of creatinine in blood, and GFR.

The analysis of associations of the variable *rs1801282* locus of the *PPARG2* gene with the development of chronic complications of diabetes and comorbidities showed no statistical significance (Table 3). The association was demonstrated in

Table 2. Frequency distribution of genotypes by variable *rs1801282* locus of the *PPARG2* gene in the T2D patients and control groups

Inheritance model	Genotype	Distribution of genotypes, *n*		OR (95% CI)	*P*
		control	T2D		
Codominant	*C/C*	205 (69.7%)	223 (68.4%)	1.00	0.260
	C/G	78 (26.5%)	97 (29.8%)	1.14 (0.80–1.63)	
	G/G	11 (3.8%)	6 (1.8%)	0.50 (0.18–1.38)	
Dominant	*C/C*	205 (69.7%)	223 (68.4%)	1.00	0.720
	C/G-G/G	89 (30.3%)	103 (31.6%)	1.06 (0.76–1.50)	
Recessive	*C/C-C/G*	283 (96.3%)	320 (98.2%)	1.00	0.150
	G/G	11 (3.7%)	6 (1.8%)	0.48 (0.18–1.32)	

Table 3. Dependence of clinical and metabolic parameters on polymorphic variants of *rs1801282 PPARG2* (dominant model)

Parameters, $m \pm \sigma$[1]	Genotypes		*P*
	C/C	*C/G-G/G*	
Age, years	63.0 ± 1.0	63.4 ± 1.6	0.820
T2C debut age, years	55.3 ± 1.0	55.7 ± 1.5	0.810
T2C duration, years	7.5 ± 0.6	7.8 ± 0.8	0.830
Family history of diabetes, %	27.1	34.3	0.430
Weight, kg	76.6 ± 1.5	76.0 ± 2.2	0.620
Body mass index, kg/m^2	30.4 ± 0.6	29.7 ± 0.8	0.500
Waist circumference, cm	99.3 ± 1.3	98.3 ± 1.7	0.680
Thigh circumference, cm	109.0 ± 2.3	108.6 ± 2.8	0.930
Fasting glucose, mmol/L	7.2 ± 0.2	7.0 ± 0.3	0.620
Postprandial glucose, mmol/L	9.8 ± 0.3	9.4 ± 0.4	0.260
Glycohemoglobin $HbA1_c$, %	7.5 ± 0.2	7.4 ± 0.1	0.590
C-peptide, ng/mL	2.46 ± 0.24	2.20 ± 0.21	0.510
HOMA-IR	2.25 ± 0.22	1.80 ± 0.18	0.200
Cholesterol, mmol/L	5.6 ± 0.2	5.7 ± 0.2	0.830
Triglycerides, mmol/L	1.77 ± 0.23	1.75 ± 0.20	0.970
LDL, mmol/L	3.20 ± 0.20	3.62 ± 0.26	0.250
HDL, mmol/L	1.31 ± 0.10	1.22 ± 0.08	0.630
Creatinine, μmol/L	90.5 ± 2.2	86.9 ± 3.1	0.370
GFR (MDRD), mL/min/1.73 m^2	56.2 ± 1.4	56.6 ± 1.2	0.880
Polyneuropathy, %	38.4	38.9	0.960
Macroangiopathy, %	50.0	36.1	0.160
Retinopathy, %	50.0	55.6	0.580
Nephropathy, %	62.7	37.5	**0.036**
Obesity, %	50.0	48.6	0.890
Arterial hypertension, %	70.9	75.0	0.650

[1] *m* is the mean; σ is the standard deviation. Statistically significant differences are in bold.

patients with diabetic nephropathy with the *C/C* genotype ($P = 0.036$) (Table 3).

No association between the variable *rs1801282* locus of the *PPARG2* gene and T2D was revealed in our study in any inheritance model. The variable *rs1801282* locus of the *PPARG2* gene was shown to be associated with the development of diabetic nephropathy according to the dominant model. The presence of the *G* allele in the genotype (*C/G*, *G/G* genotypes) marks a reduced risk of diabetic nephropathy (OR = 0.36; 95% CI 0.13–0.95).

The largest meta-analysis of associations of the variable *rs1801282* locus of the *PPARG2* gene with T2D, which included 32849 patients and 47456 individuals of the control group, confirmed that the *12Pro* allele was responsible for the increased risk of the disease [9]. At the same time, the variable *rs1801282* locus of the *PPARG2* gene showed no correlation with T2D in a number of studies [10]. This is probably due to population genetic characteristics of the examined samples.

For example, it has been demonstrated in some studies that the *G* allele (*Ala12*) causes the resistance to T2D development in Caucasoids and Indians and is not associated with risk of the disease in Chinese [11–14].

One of the most serious complications of diabetes is diabetic nephropathy (DN). Among the causes of death, DN in T2D patients is in second place after cardiovascular diseases. DN is caused by the joint effect of metabolic, hemodynamic, and genetic factors. Renal disease does not occur in all T2D patients, which suggests the presence of a genetic component of the DN.

In 1989, the first works appeared on the possibility of a family inheritance of diabetic kidney disease [15]. Modern studies of the genetic markers of renal disease in patients with type 2 diabetes are few in number and contradictory.

Association of the *rs1801282* locus of the *PPARG2* gene with the development of diabetic nephropathy was shown in the Caucasoid and Chinese populations but not in the Taiwan residents [16–18]. The meta-analysis of 18 studies including 3361 T2D patients and 5825 control individuals demonstrated that the *rs1801282* locus (*Pro12Ala*) of the *PPARG2* gene is associated with the risk of diabetic nephropathy [19].

The mechanism of the association between the *rs1801282* locus of the *PPARG2* gene and the risk of diabetic nephropathy is still not fully understood.

There are published data that individuals having the *G* allele (*Ala12*) in the genotype show enhanced resistance to oxidative stress, which is one of the pathogenetic causes of diabetic nephropathy [20, 21].

We have demonstrated that the *rs1801282* locus of the *PPARG2* gene is not associated with type 2 diabetes mellitus among residents of the Republic of Bashkortostan, but is associated with the development of diabetic nephropathy in T2D patients. It is also revealed that the *G* allele (*Ala12*) is protective against the development of diabetic nephropathy (OR = 0.36, $P = 0.036$).

Further molecular genetic studies are needed for understanding the pathogenesis of type 2 diabetes and its complications and developing a strategy for the prevention and treatment of the disease.

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REFERENCES

1. Dedov, I.I. and Shestakova, M.V., *Inkretiny: novaya vekha v lechenii sakharnogo diabeta 2-go tipa: prakticheskoe rukovodstvo dlya vrachei* (Incretins: a New Milestone in the Treatment of Type 2 Diabetes: A Practical Guide for Physicians), Moscow: Dipak, 2010.
2. Kobberling, J. and Tillilm, H., Empirical risk figures for first-degree relatives of non-insulin dependent diabetics, in *The Genetics of Diabetes Mellitus,* London, 1982, pp. 201–209.
3. Groop, L., Forsblom, C., Lehtovirta, M., et al., Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects, *Diabetes,* 1996, vol. 45, pp. 1585–1593.
4. Sanghera, D. and Blackett, P., Type 2 diabetes genetics: beyond GWAS, *J. Diabetes Metab.,* 2012, vol. 3, no. 5, pp. 2–17. doi 10.4172/2155-6156.1000198
5. Saxena, R., Voight, B.F., Lyssenko, V., et al., Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels, *Science,* 2007, vol. 316, pp. 1331–1336. doi 10.1126/science.1142358
6. Deeb, S., Fajas, L., Nemoto, M., et al., A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity, *Nat. Genet.,* 1998, vol. 20, pp. 284–287.
7. Altshuler, D., Hirschhorn, J., Klannemark, M., et al., The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes, *Nat. Genet.,* 2000, vol. 26, pp. 76–80. doi 10.1038/79839
8. Tonjes, A., Scholz, M., Loeffler, M., and Stumvoll, M., Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals, *Diabetes Care,* 2006, vol. 29, pp. 2489–2497. doi 10.2337/dc06-0513
9. Gouda, H., Sagoo, G., Harding, A., et al., The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis, *Am. J. Epidemiol.,* 2010, vol. 171, pp. 645–655. doi 10.1093/aje/kwp450
10. Ludovico, O., Pellegrini, F., Di Paola, R., et al., Heterogeneous effect of peroxisome proliferator-activated receptor gamma2 Ala12 variant on type 2 diabetes risk, *Obesity,* 2007, vol. 5, pp. 1076–1081. doi 10.1038/oby.2007.617

11. Bondar', I.A., Filipenko, M.L., Shabel'nikova, O.Yu., et al., Association of polymorphic markers rs7903146 of the TCF7L2 gene and rs1801282 of the PPARG gene (Pro12Ala) with type 2 diabetes in Novosibirsk oblast, *Sakh. Diabet,* 2013, no. 4, pp. 17–22. doi 10.14341/DM2013417-22
12. Chauhan, G., Spurgeon, C., Tabassum, R., et al., Impact of Common Variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1 on the risk of type 2 diabetes in 5164 Indians, *Diabetes,* 2010, vol. 59, pp, 2068–2074.
13. Radha, V., Vimaleswaran, K.S., Babu, H.N., et al., Role of genetic polymorphism peroxisome proliferator-activated receptor-2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: evidence for heterogeneity, *Diabetes Care,* 2006, vol. 29, pp. 1046–1051. doi 10.2337/dc05-1473
14. Li, L.L., Ma, X.L., Ran, J.X., et al., Genetic polymorphism of peroxisome proliferator-activated receptor-2 Pro12Ala on ethnic susceptibility to diabetes in Uygur, Kazak and Han subjects, *Clin. Exp. Pharmacol. Physiol.,* 2008, vol. 35, pp. 187–191.
15. Seaquist, E.R., Goetz, F.C., Rish, S., and Barbosa, J., Familial clustering of diabetic kidney disease: evidence for genetic susceptibility to diabetic nephropathy, *N. Engl. J. Med.,* 1989, vol. 320, pp. 1161–1165. doi 10.1056/NEJM198905043201801
16. Liu, L., Zheng, T., Wang, F., et al., Pro12Ala polymorphism in the PPARG gene contributes to the development of diabetic nephropathy in Chinese type 2 diabetic patients, *Diabetes Care,* 2010, vol. 33, pp. 144–149. doi 10.2337/dc09-1258
17. De Cosmo, S., Motterlini, N., Prudente, S., et al., BENEDICT Study Group: impact of the PPAR-gamma2 Pro12Ala polymorphism and ACE inhibitor therapy on new-onset microalbuminuria in type 2 diabetes: evidence from BENEDICT, *Diabetes,* 2009, vol. 58, pp. 2920–2929.
18. Wu, L.S., Hsieh, C.H., Pei, D., et al., Association and interaction analyses of genetic variants in ADIPOQ, ENPP1, GHSR, PPARgamma and TCF7L2 genes for diabetic nephropathy in a Taiwanese population with type 2 diabetes, *Nephrol. Dial. Transplant.,* 2009, vol. 24, pp. 3360–3366.
19. Zhang, H., Zhu, S., Chen, J., et al., Peroxisome proliferator-activated receptor gamma polymorphism Pro12Ala is associated with nephropathy in type 2 diabetes: evidence from meta-analysis of 18 studies, *Diabetes Care,* 2012, vol. 35, pp. 1388–1393. doi 10.2337/dc11-2142
20. Luo, W., Cao, J., Li, J., et al., Adipose tissue specific PPAR-deficiency increases resistance to oxidative stress, *Exp. Gerontol.,* 2008, vol. 43, pp. 154–163.
21. Forbes, J.M., Coughlan, M.T., and Cooper, M.E., Oxidative stress as a major culprit in kidney disease in diabetes, *Diabetes,* 2008, vol. 57, pp. 1446–1454.

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