Reprint from The Review of DIABETIC STUDIES Vol 7 No 4 2010

The Review of DIABETIC STUDIES

ORIGINAL DATA

A *WFS1* Haplotype Consisting of the Minor Alleles of rs752854, rs10010131, and rs734312 Shows a Protective Role Against Type 2 Diabetes in Russian Patients

Dimitry A Chistiakov[1], Dmitry S. Khodyrev[1], Svetlana A. Smetanina[2], Larisa N. Bel'chikova[2], Lyudmila A. Suplotova[2], and Valery V. Nosikov[1]

[1] *National Research Center GosNIIgenetika, 117545 Moscow, Russia.* [2] *Tyumen State Medical Academy, 625023 Tyumen, Russia. Address correspondence to: Dimitry A. Chistiakov, e-mail: dimitry.chistiakov@lycos.com*

Manuscript submitted December 18, 2010; resubmitted January 7, 2011; accepted January 18, 2011

■ Abstract

BACKGROUND: Rare variants of the *WFS1* gene encoding wolframin cause Wolfram syndrome, a monogenic disease associated with diabetes insipidus, diabetes mellitus, optic atrophy, and deafness. In contrast, common variants of *WFS1* showed association with type 2 diabetes (T2D) in numerous Caucasian populations. **AIM**: In this study, we tested whether the markers rs752854, rs10010131, and rs734312, located in the *WFS1* gene, are related to the development of T2D in a Russian population. **METHODS**: The polymorphic markers were genotyped in Russian diabetic (n = 1,112) and non-diabetic (n = 1,097) patients using a Taqman allele discrimination assay. The correlation between the carriage of disease-associated *WFS1* variants and the patients' clinical and metabolic characteristics was studied using ANOVA and ANCOVA. Adjustment for confounding variables such as gender, age, body mass index, obesity, HbA1c, and hypertension was made. **RESULTS**: Haplotype GAG, consisting of the minor alleles of rs752854, rs10010131, and rs734312, respectively, showed association with decreased risk of T2D (OR = 0.44, 95% CI = 0.32-0.61, p = 4.3 x 10^{-7}). Compared to other *WFS1* variants, non-diabetic individuals homozygous for GAG/CAG had significantly increased fasting insulin ($p_{adjusted}$ = 0.047) and homeostasis model assessment of β-cell function (HOMA-β) index ($p_{adjusted}$ = 0.006). Diabetic patients homozygous for GAG/GAG showed significantly elevated levels of 2-h insulin ($p_{adjusted}$ = 0.029) and HOMA-β = 0.011. **CONCLUSIONS**: Disease-associated variants of *WFS1* contribute to the pathogenesis of T2D through impaired insulin response to glucose stimulation and altered β-cell function.

Keywords: type 2 diabetes · WFS1 · wolframin · polymorphism · endoplasmic reticulum stress · beta-cell function · OGTT · fasting plasma glucose

Introduction

Loss-of-function mutations in the *WFS1* gene encoding wolframin cause Wolfram syndrome (OMIM 222300), which is associated with diabetes insipidus, diabetes mellitus, optic atrophy, and deafness [1]. Wolfram syndrome is a rare autosomal-recessive disorder. Causal variants were mapped by positional cloning on the short arm of chromosome 4 (4p16.1) containing the *WFS1* gene [2, 3]. The disease is accompanied with progressive loss of sensory neurons and pancreatic β-cells. Given the impact of *WFS1* variants on a monogenic form of diabetes, *WFS1* was considered as a likely functional candidate gene for association with a common form of type 2 diabetes (T2D). First evidence for association between *WFS1* and T2D was obtained in a small study involving an analysis of UK families with T2D [4]. The association between *WFS1* and T2D was then repeatedly

replicated by several large-scale studies in various Caucasian populations [5-10]. In *WFS1*, several single nucleotide polymorphisms (SNPs) showed significant associations with T2D, with SNP rs10010131 as the most strongly disease-associated marker (for a minor allele A, odds ratio (OR) = 0.89, 95% confidence interval (95% CI) = 0.86-0.92) [7]. At present, there are no publicly available reports evaluating whether genetic variants of *WFS1* are implicated in the pathogenesis of T2D in Russian patients. However, in one study, an association between SNP rs1801211 and sporadic Parkinson's disease was found in a Russian population [11]. In our study, we examined whether markers rs734312, rs10010131, and 752854, which showed the most robust association in T2D in Europeans, are associated with the development of T2D in a Russian population.

Abbreviations:
ANOVA - analysis of variance
ANCOVA - analysis of covariance
ATF6 - activating transcription factor 6
BMI - body mass index
BP - blood pressure
CI - confidence interval
DBP - diastolic blood pressure
DNA - deoxyribonucleic acid
ER - endoplasmic reticulum
FFA - free fatty acids
FPG - fasting plasma glucose
HbA1c - glycated hemoglobin
HDL - high-density lipoprotein
HDL-C - high-density lipoprotein cholesterol
HOMA-beta - homeostasis model assessment of beta-cell function
HOMA-IR - homeostasis model assessment of insulin resistance
ISI - insulin sensitivity index
LD - linkage
LDL - low-density lipoprotein
LDL-C - low-density lipoprotein cholesterol
Na^+/K^+-ATPase - sodium-potassium adenosine triphosphatase (also known as sodium-potassium pump)
NEFA - non-esterified fatty acids assay
OGTT - oral glucose tolerance test
OR - odds ratio
PG - plasma glucose
SBP - systolic blood pressure
SD - standard deviation
SMAD - homolog of drosophila protein, mothers against decapentaplegic (MAD), and *Caenorhabditis elegans* protein SMA
Smurf1 - SMAD ubiquitination regulatory factor 1
SNP - single nucleotide polymorphism
STRAP structure-based sequences alignment program
T2D - type 2 diabetes
TG - triglycerides
WFS1 - wolfram syndrome 1 gene
WHO - World Health Organization

Materials and methods

Patients

We studied a total of 2,209 unrelated patients aged 50 years and older. 1,112 of whom were affected by T2D. The remaining 1,097 control individuals were normoglycemic, and had no clinical diabetes. According to the patients' questionnaires, 1,944 (88% of 2,209) participants had four grandparents of Russian ancestry, the remaining 265 patients had three grandparents of Russian ancestry, and one of either Ukrainian, or Belarusian descent. A total of 794 (402 affected and 392 non-affected) individuals living in Moscow, and neighboring areas, were recruited by the Endocrinology Research Center. The second cohort (710 diabetic and 705 non-diabetic residents of Tymen) was collected by the Tyumen State Medical Academy. T2D was defined according to WHO diagnostic criteria. Thus, participants were regarded as diabetic if either their fasting plasma glucose concentration was ≥7.8 mmol/l, or their plasma glucose concentration was ≥11.1 mmol/l 2 h after a 75-g oral glucose tolerance test (OGTT) [12]. Another criterion was treatment by glucose-lowering agents. Hypertension was defined as systolic pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg in at least two separate measurements, or a history of hypertension. Obesity was defined as BMI ≥30 kg/m^2 [13]. All study participants resided in Moscow, or Moscow region. Controls had no past history of glucose intolerance, a glycated hemoglobin (HbA1c) level of <6.4%, or a normal OGTT, and no family history of diabetes. To avoid interference from biological variables, people with a previous diagnosis of type 1 diabetes, gestational diabetes, rare forms of T2D, secondary diabetes (pancreatitis, hemochromatosis), and hypercholesterolemia, or those undergoing treatment with cholesterol-lowering drugs, were excluded from the study. The study protocol was approved by the Review Board of the Endocrinology Research Center, and all participants provided written informed consent.

Biochemical measurements

Fasting cholesterol, HDL, cholesterol, triglycerides, and plasma glucose were measured by standard enzymatic assays. Fasting plasma FFA concentrations were assayed by an enzymatic colorimetric method using a commercially available kit (WAKO NEFA C-test; Waco Chemicals, Neuss, Germany). LDL cholesterol was derived using the

Table 1. Clinical and metabolic characteristics of the patients

Parameter	Type 2 diabetes (n = 1,112)	Control (n = 1,097)	p
Gender (m/f)	501/611	530/567	NS
Age (yr)	60.7 ± 7.3	61.2 ± 10.4	NS
Diabetes duration (yr)	11.5 ± 7.6	-	-
BMI (kg/m^2)	28.9 ± 5.5	27.2 ± 4.8	NS
Systolic BP (mmHg)	136.9 ± 17.7	128.0 ± 15.6	NS
Diastolic BP (mmHg)	98.9 ± 14.2	88.2 ± 12.2	0.03
HbA1c (%)	7.9 ± 1.3	5.2 ± 0.5	0.024
FPG (mmol/l)	9.8 ± 1.7	5.7 ± 0.5	< 0.002
2-h PG (mmol/l)	12.4 ± 1.2	6.8 ± 0.7	< 0.001
FS insulin (mU/l)	14.8 ± 7.7	10.2 ± 6.2	< 0.002
2-h serum ins. (mU/l)	84.0 ± 32.1	48.2 ± 19.9	< 0.001
HOMA-β	46.2 ± 22.4	92.7 ± 46.3	< 0.001
HOMA-IR	6.5 ± 1.6	2.6 ± 0.6	0.008
ISI	5.1 ± 1.1	6.4 ± 1.3	0.026
HDL-C (mmol/l)	1.3 ± 0.3	1.2 ± 0.3	NS
LDL-C (mmol/l)	3.3 ± 1.1	3.4 ± 0.9	NS
TC (mmol/l)	5.0 ± 1.3	5.0 ± 1.1	NS
TG (mmol/l)	2.2 ± 0.6	1.6 ± 0.5	0.022
FFA (mmol/l)	0.5 ± 0.2	0.5 ± 0.1	NS
Hypertension (n, %)	418 (37.6)	349 (31.8)	0.043
Obesity (%)	234 (21.0)	178 (16.2)	< 0.008

Legend: Data are mean ± SD. Data compared using chi-squared test (gender, hypertension, obesity), or unpaired Student's t-test (other). BMI: body mass index. BP: blood pressure. HbA1c: glycated hemoglobin. FPG: fasting plasma glucose. PG: plasma glucose. FS: fasting serum. HOMA-β: homeostasis model assessment of β-cells. HOMA-IR: homeostasis model assessment of insulin resistance. ISI: insulin sensitivity index. HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol. TC: total cholesterol. TG: triglycerides. FFA: free fatty acids. NS: not significant.

Friedewald equation [14]. A standard 75-g OGTT was performed after a 12-h overnight fast according to WHO recommendations. HbA1c was measured using ion-exchange high performance liquid chromatography (normal reference range: 4.1-6.4%). Plasma insulin levels were determined by means of an enzymatic immunoassay. Homeostasis model assessment of β-cell function (HOMA-β) was calculated as 20 x fasting plasma insulin (mU/l)/(fasting glucose (mmol/l) - 3.5). Homeostasis model assessment of insulin resistance (HOMA-IR) was computed as fasting plasma insulin (mU/l) x fasting glucose (mmol/l)/ 22.5 [15]. Insulin sensitivity index (ISI) was assessed as ISI = exp (2.63 - 0.28 x ln (fasting plasma insulin (mU/L)) - 0.31 x ln (serum triglycerides (mmol/l))) [16]. HOMA-IR ≥ 3.8 and ISI ≤ 6.3 were considered as threshold scores reflecting the presence of IR. Clinical and metabolic characteristics of diabetic and non-diabetic participants are summarized in Table 1.

DNA analysis

Total DNA was isolated from whole-blood samples pretreated with proteinase K using a standard protocol for extraction with phenol-chloroform. Genotyping of rs734312, rs10010131, and rs752854 in *WFS1* was performed by a Taqman-based allelic discrimination on a Real-Time PCR System 7500 (Applied Biosystems, Foster City, CA, USA) using the recommended protocol [17]. Overall, genotype calling rate was 99.0% for rs10010131, 98.8% for rs734312, and 98.1% for rs752854.

Statistical analysis

Data were analyzed with SPSS/Win programs (version 13.0; SPSS Inc., Chicago, IL, USA). Results are given as mean ± SD, or percentages. Skewed variables for the continuous traits were log-transformed before statistical comparisons were made. For comparison of quantitative data in groups of affected and non-affected patients, unpaired Student's t-test was used. Tests for Hardy-Weinberg equilibrium and comparison of genotype and allele frequencies in the T2D subjects and controls were performed using the chi-squared test with Yates' correction. OR and 95% CI were used to assess the extent of association of the various genotypes with T2D. A wild-type genotype was used as a reference group to provide separate OR for each genotype.

Pair-wise linkage disequilibrium (LD) between markers and haplotype estimation was calculated using ARLEQUIN v.2.0 software [18]. The significance of interaction between clinical characteristics and haplogenotypes was assessed by two-way ANOVA. Observed relationships were adjusted for patients' conventional risk factors by ANCOVA using age, gender, HbA1c, BMI, obesity, and hypertension as covariates. P-values of less than 0.05 were considered significant. To align species-specific protein sequences of wolframin, the web-based interactive structure-based sequences alignment program (STRAP) was used (available at http://3d-alignment.eu/).

Results

Observed genotype frequencies of all polymorphic markers of *WFS1* were in accordance with the Hardy-Weinberg equilibrium (data not shown). Among three SNPs studied, markers rs10010131 and rs752854 showed significant associations with T2D in the Russian population (Table 2). For SNP rs10010131, a minor allele A was associated with reduced disease risk (OR = 0.77, 95% CI = 0.68-0.87, p = 2.4 x 10^{-5}). Similarly, the minor allele G of rs752854 was also related to decreased risk of T2D (OR = 0.86, 95% CI = 0.75-0.96, p = 0.013). The minor allele G of marker rs752854 showed borderline association with a lower diabetes risk (OR = 0.87, 95% CI = 0.76-0.99, p = 0.05). However, after conditioning on effect of SNP rs10010131, associations between two other markers and the disease became non-significant (for rs752854, OR = 0.98, 95% CI = 0.83-1.14, p = 0.91; for rs734312, OR = 0.95, 95% CI = 0.82-1.1, p = 0.79). Therefore, rs10010131 is the only marker strongly associated with diabetes. The association of two other SNPs is rather secondary and influenced by rs10010131.

The marker rs752854 was in moderate LD with the SNP rs10010131 (D' = 0.75, p = 0.009), while rs10010131 showed mild but significant LD with the marker rs734312 (D' =0.69, p = 0.039). No significant pair-wise LD were found between rs752854 and 734312 (D' = 0.48, p = 0.39). Major alleles of these markers constituting the most frequent haplotype AGA were significantly associated with a higher diabetes risk (OR = 1.21, 95% CI = 1.06-1.38, p = 0.0056) (Table 3). In contrast, the rarest haplotype GAG consisting of the minor alleles of the markers showed highly significant association with decreased risk of T2D (OR = 0.44, 95% CI = 0.32-0.61, p = 4.3 x 10^{-7}). We studied whether the carriage of disease-associated *WFS1* variants is correlated with various metabolic traits associated with metabolic syndrome and T2D. Since the association between markers rs734312 and rs752854 and diabetes is dependent on the effect from rs10010131, we focused on the relation between SNP rs10010131 and metabolic traits. Compared to other *WFS1* variants, both diabetic and non-diabetic carriers of the protective genotype AA of rs10010131 had higher HOMA-β, suggesting improved homeostasis of β-cells in these carriers (Table 4). We also considered whether the carriage of *WFS1* haplogenotypes is associated with diabetes-related metabolic traits. Each of the groups of diabetic and non-diabetic patients was divided into three subgroups carrying one, two, or no copies of the protective haplogenotype GAG/GAG. After adjustment for confounding variables, we observed significantly increased levels of fasting insulin ($p_{adjusted}$ = 0.047) and HOMA-β ($p_{adjusted}$ = 0.006) in the controls homozygous for GAG/GAG, compared with the carriers of other *WFS1* variants (Table 5). In T2D patients, homozygotes GAG/GAG had significantly elevated 2-h insulin ($p_{adjusted}$ = 0.027) and HOMA-β ($p_{adjusted}$ = 0.011) as

Table 2. Association of *WFS1* variants with type 2 diabetes

Allele / genotype	T2D (n = 1,112)	Control (n = 1,097)	OR (95% CI)	p
rs752854				
A/A	594 (54.6)	550 (51.2)	1	
A/G	425 (39.2)	436 (40.6)	0.90	NS
G/G	68 (6.2)	88 (8.2)	0.72	0.061
Allele A	1617 (74.0)	1536 (71.5)	1	
Allele G	563 (26.0)	612 (28.5)	0.87 (0.76-0.99)	0.05
rs10010131				
A/A	398 (36.2)	316 (29.1)	1	
A/G	527 (47.9)	537 (49.4)	0.78 (0.64-0.94)	0.012
G/G	175 (15.9)	234 (21.5)	0.59 (0.46-0.76)	< 0.001
Allele G	1323 (60.1)	1169 (53.8)	1	
Allele A	877 (39.9)	1005 (46.2)	0.77 (0.68-0.87)	< 0.001
rs734312				
A/A	440 (40.1)	392 (36.1)	1	
A/G	502 (45.7)	502 (46.3)	0.89	NS
G/G	156 (14.2)	191 (17.6)	0.73 (0.57-0.94)	0.016
Allele A	1384 (62.9)	1286 (58.4)	1	
Allele G	814 (37.1)	884 (41.6)	0.86 (0.75-0.96)	0.013

Legend: Data are n (%). A wild-type genotype is used as a reference group (OR =1). OR: odds ratio. CI: confidence interval.

Table 3. Association of *WFS1* haplotypes with type 2 diabetes

Haplotype	T2D (n = 1,112)	Control (n = 1,097)	OR (95% CI)	p
AGA	648 (29.8)	556 (26.0)	1.21 (1.06-1.38)	< 0.006
AGG	307 (14.1)	275 (12.9)	1.11	NS
AAA	391 (18.0)	394 (18.4)	0.97	NS
AAG	267 (12.3)	305 (14.2)	0.84	0.062
GGA	180 (8.3)	150 (7.0)	1.20	NS
GAA	151 (6.9)	168 (7.8)	0.88	NS
GGG	173 (8.0)	169 (7.9)	1.01	NS
GAG	57 (2.6)	123 (5.8)	0.44 (0.32-0.61)	< 0.001

Legend: Data are n (%). Haplotype AGA includes allele A of rs752854, allele G of rs10010131, and allele A of rs734312. OR: odds ratio. CI: confidence interval.

Table 4. Association of SNP rs10010131 of *WFS1* with metabolic characteristics of type 2 diabetic patients and non-diabetic controls

Parameter	Type 2 diabetes (n = 1,100)				Control (n = 1,087)			
	G/G (n = 398)	A/G (n = 527)	A/A (n = 175)	p	G/G (n = 398)	A/G (n = 527)	A/A (n = 175)	p
BMI (kg/m^2)	28.9 ± 5.5	29.9 ± 6.1	29.2 ± 5.8		27.8 ± 5.6	26.5 ± 4.5	27.2 ± 5.0	
HbA1c (%)	7.9 ± 1.3	7.5 ± 1.3	7.9 ± 1.5		5.3 ± 0.6	5.0 ± 0.5	5.4 ± 0.5	
SBP (mmHg)	135.4 ± 17.1	138.0 ± 16.2	131.4 ± 17.5	NS	123.3 ± 15.5	128.1 ± 16.1	126.8 ± 14.2	NS
DBP (mmHg)	99.3 ± 14.8	97.7 ± 15.2	101.4 ± 16.1	NS	84.4 ± 12.5	87.2 ± 13.1	86.9 ± 12.8	NS
FPG (mmol/l)	10.2 ± 1.7	9.4 ± 1.7	9.6 ± 1.9	NS	5.9 ± 0.6	5.5 ± 0.5	5.2 ± 0.5	NS
2-h PG (mmol/l)	12.9 ± 1.3	11.7 ± 1.1	11.8 ± 1.3	NS	7.0 ± 0.8	6.5 ± 0.6	6.3 ± 0.8	NS
FS insulin (mU/l)	14.1 ± 7.4	14.4 ± 7.7	15.4 ± 8.1	NS	9.0 ± 5.5	9.4 ± 5.8	10.7 ± 6.2	0.08
2-h SI (mU/l)	79.7 ± 31.5	84.8 ± 28.6	90.5 ± 31.1	0.077	46.6 ± 18.8	47.7 ± 19.2	51.2 ± 21.2	NS
HOMA-β	42.1 ± 23.3	48.8 ± 20.6	50.5 ± 21.5	0.036	85.0 ± 48.2	94.0 ± 45.5	125.9 ± 50.6	0.009
HOMA-IR	6.4 ± 1.4	6.0 ± 1.7	6.6 ± 1.5	NS	2.4 ± 0.5	2.3 ± 0.6	2.5 ± 0.6	NS
ISI	5.3 ± 1.2	4.8 ± 1.3	4.9 ± 1.2	NS	6.4 ± 1.2	6.2 ± 1.3	6.3 ± 1.2	NS
HDL-C (mmol/l)	1.2 ± 0.4	1.2 ± 0.3	1.3 ± 0.3	NS	1.3 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	NS
LDL-C (mmol/l)	3.2 ± 1.1	3.1 ± 1.1	2.7 ± 1.3	NS	3.3 ± 1.2	3.4 ± 1.1	3.3 ± 1.0	NS
TC (mmol/l)	5.1 ± 1.2	4.9 ± 1.4	4.8 ± 1.5	NS	5.0 ± 1.3	5.1 ± 1.1	4.8 ± 1.2	NS
FFA (mmol/l)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	NS	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	NS
TG (mmol/l)	2.2 ± 0.4	2.2 ± 0.6	2.4 ± 0.6	NS	1.5 ± 0.6	1.6 ± 0.5	1.3 ± 0.4	NS

Legend: Data are mean ± SD. P-values adjusted for gender, age, BMI, obesity, HbA1c, and hypertension. BMI: body mass index. HbA1c: glycated hemoglobin. SBP: systolic blood pressure. DBP: diastolic blood pressure. FPG: fasting plasma glucose. PG: plasma glucose. FS: fasting serum. HOMA-β: homeostasis model assessment of β-cells. HOMA-IR: homeostasis model assessment of insulin resistance. ISI: insulin sensitivity index. HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol. TC: total cholesterol. TG: triglycerides. FFA: free fatty acids. NS: not significant.

well. These observations suggest that the protective role of the *WFS1* haplogenotype GAG/GAG may be related to a higher production of insulin and better function of pancreatic β-cells, as reflected by the HOMA-β value. The SNP rs10010131 itself is associated with improved function of β-cells. However, taking minor alleles of rs734312 and rs752854 into account results in the formation of the protective haplogenotype GAG/GAG, whose association with better β-cell function is even stronger than that of rs10010131 alone.

Discussion

We found associations between *WFS1* genetic variants and T2D in Russian patients, thereby confirming results of earlier studies that showed the involvement of *WFS1* polymorphisms in conferring susceptibility/resistance to T2D in multiple populations of Caucasians. In our study, diabetic patients homozygous for the protective *WFS1* haplogenotype GAG/GAG containing alleles of rs752854, rs1001013, and 734312 showed increased levels of 2-h insulin and HOMA-β. In contrast, carriers of a predisposing *WFS1* variant such as the haplogenotype AGA/AGA had reduced serum insulin 2-h post-OGTT and HOMA-β (data not shown). Therefore, carriage of the susceptibility *WFS1* variant is related to impaired glucose-induced insulin response resulting from β-cell dysfunction. These results are in accordance with findings in other populations reporting the relationship between the carriage of various disease-associated variants of *WFS1* and altered insulin secretion in response to glucose stimulation [10, 19-21] and lower pancreatic β-cell function [22].

SNP rs734312 is a missense mutation that is situated in the last exon (exon 8) of *WFS1*, and leads to an amino acid (aa) substitution of histidine to arginine in codon 611 (H611R). The H611R polymorphism resides in the cytoplasmic loop between the eighth and ninth transmembrane segments [23]. There are no data reporting whether this SNP is functionally relevant. However, the alignment of the human *WFS1* aa sequence against protein sequences of wolframin from other mammals showed that arginine-611 is

Table 5. Association of haplogenotypes of *WFS1* with metabolic characteristics of type 2 diabetic patients and non-diabetic controls

Parameter	Type 2 diabetes (n = 1,087)				Control (n = 1,070)			
	GAG/GAG (n = 20)	GAG/X (n = 17)	X/X (n = 1,050)	p	GAG/GAG (n = 42)	GAG/X (n = 39)	X/X (n = 989)	p
BMI (kg/m^2)	28.6 ± 5.3	29.3 ± 5.9	29.4 ± 5.9		26.4 ± 4.6	27.4 ± 4.9	27.9 ± 5.2	
HbA1c (%)	8.0 ± 1.2	7.8 ± 1.4	7.7 ± 1.3		5.3 ± 0.5	5.1 ± 0.5	5.3 ± 0.5	
SBP (mmHg)	137.2 ± 18.2	135.0 ± 19.2	136.3 ± 16.8	NS	128.7 ± 15.0	126.9 ± 16.9	128.3 ± 15.7	NS
DBP (mmHg)	102.2 ± 15.9	96.6 ± 13.3	94.5 ± 14.9	NS	86.7 ± 11.5	89.9 ± 13.4	85.6 ± 12.5	NS
FPG (mmol/l)	8.9 ± 1.1	9.3 ± 1.5	9.7 ± 1.7	NS	5.3 ± 0.6	5.6 ± 0.6	5.8 ± 0.5	NS
2-h PG (mmol/l)	12.2 ± 1.0	12.8 ± 1.3	12.5 ± 1.2	NS	6.5 ± 0.8	6.2 ± 0.8	7.0 ± 0.7	NS
FS insulin (mU/l)	15.9 ± 8.9	15.0 ± 7.5	14.5 ± 7.7	NS	11.7 ± 6.5	11.5 ± 5.8	10.4 ± 6.0	0.047
2-h SI (mU/l)	93.4 ± 26.7	86.6 ± 29.1	80.4 ± 31.5	0.027	50.6 ± 20.5	46.5 ± 19.2	47.3 ± 19.4	NS
HOMA-β	58.9 ± 27.5	51.7 ± 22.2	46.8 ± 21.2	0.011	130.4 ± 54.3	109.5 ± 50.3	90.4 ± 44.2	0.006
HOMA-IR	6.3 ± 1.7	6.2 ± 1.5	6.3 ± 1.6	NS	2.8 ± 0.5	2.9 ± 0.6	2.7 ± 0.6	NS
ISI	4.8 ± 1.0	4.9 ± 1.3	5.2 ± 1.3	NS	6.4 ± 1.4	6.4 ± 1.5	6.2 ± 1.3	NS
HDL-C (mmol/l)	1.3 ± 0.3	1.3 ± 0.4	1.1 ± 0.2	NS	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	NS
LDL-C (mmol/l)	3.4 ± 1.2	3.0 ± 1.0	2.9 ± 1.1	NS	3.2 ± 1.0	3.3 ± 1.2	3.5 ± 0.9	NS
TC (mmol/l)	4.9 ± 1.2	5.2 ± 1.4	5.0 ± 1.4	NS	4.9 ± 1.1	5.3 ± 1.2	5.1 ± 1.2	NS
FFA (mmol/l)	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	NS	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	NS
TG (mmol/l)	2.5 ± 0.6	2.5 ± 0.5	2.1 ± 0.7	0.059	1.8 ± 0.5	1.4 ± 0.4	1.6 ± 0.4	NS

Legend: Data are mean ± SD. P-values adjusted for gender, age, BMI, obesity, HbA1c, and hypertension. Haplotype GAG includes allele G of rs752854, allele A of rs10010131, and allele G of rs734312. X designates any haplotype other than the haplotype GAG. BMI: body mass index. HbA1c: glycated hemoglobin. SBP: systolic blood pressure. DBP: diastolic blood pressure. FPG: fasting plasma glucose. PG: plasma glucose. FS: fasting serum. HOMA-β: homeostasis model assessment of β-cells. HOMA-IR: homeostasis model assessment of insulin resistance. ISI: insulin sensitivity index. HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol. TC: total cholesterol. TG: triglycerides. FFA: free fatty acids. NS: not significant.

evolutionary preserved in primates and rodents (Figure 1). Markers rs752854 and rs10010131 are located in non-coding regions (intron 2 and intron 4, respectively) of the *WFS1* gene. Therefore, these markers tag the etiological marker of *WFS1*, which is still unknown.

Wolframin is a 100 kDa transmembrane glycoprotein expressed in neural tissues and β-cells. The hypothetical structure of this protein comprising 890 aa includes the N-terminal cytoplasmic domain, nine transmembrane segments (aa 314-652), and a C-terminal tail [23]. Wolframin is incorporated into the endoplasmic reticulum (ER) membrane, and its C-terminus is exposed to the ER lumen. Using a yeast two-hybrid system, the *WFS1* C-terminal and transmembrane domains were shown to interact with Na^+/K^+-ATPase β1 subunit [24]. Wfs1-deficient mice exhibit typical phenotypic outcomes of diabetes, such as progressive glucose intolerance and concomitant insulin deficiency, as a consequence of reduced β-cell mass resulting from impaired cell cycle progression, increased apoptosis, and ER stress [25-27].

The proposed functions of wolframin include the regulation of membrane trafficking, protein processing and calcium homeostasis in the ER of neurons and pancreatic β-cells [28, 29]. This protein negatively regulates ER stress by suppressing the expression of activating transcription factor 6α (ATF6α)-dependent target genes, stabilizing the E3 ubiquitin ligase HRD1, and enhancing ubiquitination and proteasome-mediated degradation of ATF6α [30]. In β-cells, wolframin was recently found to be involved in insulin secretion through intragranular acidification that is necessary for the priming of secretory granules preceding exocytosis [31].

In both diabetes and Wolfram syndrome, *WFS1* expression in β-cells is reduced [32]. Furthermore, in β-cells with impaired intracellular signaling, wolframin may become the subject of enhanced ubiquitination and proteasomal degradation mediated by E3 ligase Smurf1 [33]. Therefore, upon *WFS1* deficiency, accumulation of misfolded and unfolded proteins result in ER stress that may lead to increased apoptotic β-cell death. Also, insu-


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Human_WFS1                  VTVAVCSVPLLLRWWTKASFSVVGMVKSLTRSSMVKLILVWLTAIVLFCW
White-cheeked_gibbon_WFS1   VTVAVCSVPLLLRWWTKASFSVVGMVKSLTRSSMVKLILVWLTAIVLFCW
Sumatran_orangutan_WFS1     VTMAVCSVPLLLRWWTKASFSVVGMVKSLTRSSMVKLILVWLTAIVLFCW
Mouse_WFS1                  VTTVICGVPLLFRWWTKANFSVMGMVKSLTKSSMVKLILVWLTAILLFCW
Rat_WFS1                    VTTVICSVPLLFRWWTKANFSVVGMVKSLTRSSIVKLILVWLTAILLFCW
                            ** .:*.****:******.***:*******:**:***********:****
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Figure 1. Alignment of the protein sequence of human wolframin (aa 599-648) against wolframin aa sequences of other mammals. Arginine (R) at codon 611 is highlighted in gray.

lin processing and secretion may be impaired in diabetes, and may result in increased circulating proinsulin levels due to the disturbance in wolframin-dependent acidification of secretory granules [31].

Further studies are required to identify an etiological marker in *WFS1*. A recent comprehensive fine-mapping analysis of the *WFS1* gene performed in UK Caucasians with the consideration of rare variants (minor allele frequency < 0.01) revealed several putative causal variants for T2D association [9]. However, it was impossible to distinguish between their effects on disease risk due to strong LD between the SNPs within the candidate interval. To distinguish between their effects, it is necessary to study very large population datasets with size exceeding 100,000 records.

Acknowledgements: The work was supported by grant no. 10-04-01496-a from the Russian Foundation for Basic Research.

Disclosures (conflict of interests statement): The authors report no conflict of interests.

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