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Genetic polymorphisms potentially associated with response to metformin in postmenopausal diabetics suffering and not suffering with cancer

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Metformin is a well-known antidiabetic medication, which, besides diabetes, may be involved into modulation of other age-related pathologies, including cancer. The study concerns 12 gene polymorphisms divided into 2 groups consisting of 6 genes each. The first group was composed from so-called "standard" (S) polymorphisms, for which the connection with metabolic response to metformin is already established. The second group included polymorphisms of genes encoding proteins possibly connected with diabetes mellitus type 2 (DM2), impaired glucose tolerance or cancer and entitled here as "associated" (A). A total of 156 postmenopausal women (average age 60.7 ± 0.7) were included, 37 of them healthy, 64 with type DM2 and concurrent treatment-naïve cancer (mostly breast, endometrial or colorectal cancer), 32 with DM2 without cancer, and 23 with treatment-naïve cancer and normal glucose tolerance. The leading metformin response S-marker in combined group of DM2 patients was the CC variant of OCT1-R61C polymorphism of organic cation transporter protein 1 gene. In cancer patients without DM2, this position belonged to AC and AA genotypes of OCT1_rs622342 polymorphism. Among the A-polymorphisms, GA variant of sex hormone-binding globulin gene SHBG_D356N was less frequently observed in DM2 patients with or without cancer. Besides, in diabetics, the same polymorphic variant of SHBG as well as GC genotype of oxidized lipoprotein receptor OLR1_G501C and GG genotype of locus rs11065987 near BRAP gene were carried rather often in combination with "metformin-positive" variant of OCT1_R61C. In addition, carriers of OCT1_R61C and OCT1_rs622342 polymorphisms with potentially positive reaction to metformin had higher insulin resistance score (HOMA-IR) values. Received data lead to the conclusion that postmenopausal diabetics, both with and without cancer, differ in genetic stigmata of potential response to metformin less than they differ from cancer patients without DM2. As genetic polymorphisms associated with metabolic and anticancer metformin (and, possibly, phenformin) effects may be different, this subject requires further investigation.

Introduction

Many countries currently display a trend to population aging, as well as increase in obesity, and diabetes occurrences reaching, in aggregate, epidemic proportions.[1] This, so-called, 'burgeoning elderly population" is regarded as belonging to the group of higher cancer risk,[2] although the contribution of each mentioned separate factor and their connections are not yet completely clear and currently are under investigation.[3,4]

The characteristic model of complex connections is exemplified by diabetes mellitus (DM) and cancer. Although DM is considered to be associated with higher cancer incidence this association greatly varies based on tumor localization, DM type, patient age, gender, and several other factors.[5-7] Some DM-associated factors may have independent effects on cancer risk and disease course,[6-8] and, understandably, antidiabetic treatment is also brought to notice in this regard.

One of the most widely used medications in DM type 2 (DM2) patients are antidiabetic biguanides, of which metformin is currently most common. There are also gradually expanding data on antidiabetic biguanides (metformin and, in in vitro and preclinical studies, phenformin) anticancer activity, although this data continue to be a matter of discussion. Recently, several researchers,[3,9-12] in accord with earlier statements,[13] stressed the necessity of development of adequate criteria for evaluation of antidiabetic biguanides' anticancer effect, as the extent of their influence on cancer morbidity, and mortality in diabetics and non-diabetics of different age groups is not yet fully clear.

Indeed, some of the recently performed cancer risk meta-analyses reveal certain disparities in effects of metformin administration on cancer incidence based on tumor type. Although the overall cancer incidence is lower in diabetics—metformin recipients—it doesn't change or more rarely changes for some tumor localizations.[14,15] Additionally, most of the

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Table 1. Distribution of "standard" polymorphic marker genotypes (%) in studied postmenopausal females

Group	OCT1 R61C(CT+TT)[1]	OCT1 G401S (GA)[1]	OCT1G465R (GA)[1]	OCT1 rs622342 (CC)[1]	STK11 (GG)[2]	C11orf65 near ATM (CC)[2]
DM2 w/o cancer (32)	9,4 ± 5,1[3]	0	5,9 ± 4,1	15,6 ± 6,4	12,5 ± 5,9	31,3 ± 8,1
Cancer+DM2 (64)	12,5 ± 4,1[3]	3,1 ± 2,1	4,7 ± 2,7	14,1 ± 4,2	14,1 ± 4,2	20,3 ± 5,0
DM all (96)	11,5 ± 3,2[3]	2,1 ± 1,4	5,2 ± 2,2	14,6 ± 3,4	13,5 ± 3,5	24,0 ± 4,3
DM+FH (35)	17,1 ± 6,3	0	5,7 ± 4,0	11,4 ± 5,3	5,7 ± 4,0[4]	22,2 ± 7,0
Cancer w/o DM2 (23)	34,8 ± 9,9	4,4 ± 4,0	8,7 ± 5,9	4,3 ± 3,9	21,7 ± 4,2	21,7 ± 4,2
Healthy (37)	18,9 ± 6,3	2,7 ± 2,7	13,5 ± 5,7	16,2 ± 6,0	18,9 ± 6,4	16,2 ± 6,0

Notes: DM2, diabetes, type 2; w/o, without; FH, family history; in brackets, number of cases [1]Genotypes, the markers of potentially weakened (poor) response to metformin; [2]Genotypes, the markers of potentially positive response to metformin; [3]Difference with group of cancer patients without diabetes is significant ($P < 0.02$); [4]The tendency to difference with group of cancer patients without diabetes (P 0.07)

current data comes from cohort or case-control trials, while 2 recent meta-analyses based on randomized trials didn't reveal any connection (OR 1.02 and 1.01) between cancer incidence and metformin administration in DM patients.[16,17]

These data suggests the importance of individual variations in metformin metabolism and its interaction with cellular targets.[11,12,18,19] This hypothesis is partly verified by accumulated clinical data, e.g., by well-established opinion on metformin's better effect in overweight patients,[18,20] although there are also some contradicting results.[21] Lately, the differences started to be explained by pharmacokinetic distinctions, which are reflected in metformin serum concentrations and utilization rates in organs and tissues and among other methods can be studied on the basis of pharmacogenetic approach.

There are as yet quite few clinical studies employing this approach, most of them concerned organic cation transporter 1 (OCT1) or solute carrier family 22 member 1, SLC22A1 gene polymorphism.[22-24] Hepatic OCT1 and renal OCT2 proteins are involved in metformin metabolism as the substrates for this medicine.[22] Mice with OCT1 gene knockout display decreased metformin capture by hepatic and some other tissues,[25] which suggests the connection between OCT1 polymorphisms and differences in metformin pharmacokinetics leading to different therapeutic response. This concept was tested in several clinical studies.[22-24] The metformin recipients, healthy volunteers with lower functional activity of OCT1 polymorphisms, performed worse in glucose tolerance test.[22] In diabetics, OCT1 rs622342 A > C polymorphism was associated with lower glycosylated hemoglobin values by the end of metformin treatment course.[23,26] In addition, in polycystic ovary syndrome patients with referential OCT1 gene polymorphisms OCT1_R61C (C > T), G401S (G > A), G465R (G > A), and 420del metformin exerted different effect on cholesterol and triglycerides, but not insulin, levels compared with control group.[24]

Besides OCT1, hormonal-metabolic response to metformin depends, in particular, on proteins encoded by C11orf65 (rs11212617) gene located near ataxia-telangiectasia gene (ATM)[27] and, in polycystic ovary syndrome patients, by STK11 gene[28] encoding LKB tumor suppressor kinase. If tumor-suppressing function is inhibited, LKB loses its ability to enhance AMP-activated protein kinase (AMPK) function. This defect can, at least partly, be corrected by metformin administration,[29] which is another reason to study STK11/LKB1 polymorphism.[28] There are also some other gene polymorphisms able to influence metformin pharmacokinetics, e.g., rs2289669 gene encoding multidrug and toxin extrusion 1 (MATE1) protein. Combined with some OCT1 genotypes, it can cause a more evident decrease of glycosylated hemoglobin level in metformin-treated patients with DM2.[23]

There are no published relevant studies concerning biguanides in cancer patients. The only results applicable to cancer settings were obtained by Segal et al.,[30] demonstrating the lower in vitro metformin (but not phenformin) sensitivity of ovarian carcinoma cells in OCT1 knockout mice. Also ovarian carcinoma tissue was characterized in individual patients by very heterogenic OCT1 expression, which may be the cause of metformin effects variability.[30]

The aim of the present study was to evaluate the frequency of allelic polymorphisms bearing established (called here "standard" or S) genetic markers associated with potentially positive or poor response to metformin in postmenopausal (median age ~60 y) female DM2 patients with or without concurrent cancer. The same groups of patients were studied for genetic markers only presumably associated (A-markers) with reaction to metformin. The distribution of S and A markers was compared, and metabolic and hormonal pattern was studied in order to evaluate its connection with genetic factors predisposing to individual metformin response.

Results

The data on the distribution of S-group genes polymorphisms in studied postmenopausal women are collected in **Table 1**. In a combined group of DM patients (with or without concurrent cancer) CT and TT variants of OCT1-R61C polymorphism, which are the sign of potentially reduced response to metformin,[22,24] were found to be less frequent than in cancer patients without diabetes; this trend was less pronounced in patients with familial form of DM2 type 2. There was no significant difference in occurrence of G401S and G465R polymorphisms of OCT1 gene and rs11212617 variant of C11orf65 located near ATM gene[27] between the individuals of all studied groups. CC variant of

OCT1_rs622342 polymorphism, which is a prognostic marker for weakened response to metformin,[23,26] was less frequent in cancer patients without diabetes (tendency) than in healthy postmenopausal women and in combined DM2-group (χ^2 1.94 and 1.76, accordingly; p 0.16 and 0.18). The same cancer group without diabetes displayed relatively high (p 0.07) occurrence of GG genotype of STK11_ rs8111699 variant, which is considered a potential marker of metformin sensitivity,[28] in comparison to diabetics with family history of DM2 (**Table 1**).

For the majority of A-group gene polymorphisms (DNA repair gene 8-oxoguanine glycosylase OGG1Ser326Cys variant /rs1052134/, oxidized low-density lipoprotein receptor gene OLR1_G501C variant /rs1053646/ and rs11065987 gene located near BRAP) no significant difference between study groups was found. Significant distinctions were found, though, in cancer patients without DM2, which displayed higher occurrence of AG variants of leptin receptor LEPR_Gln223Arg and sex hormone-binding globulin SHBG_D356N genes (especially, in comparison with diabetics with concurrent cancer). Also, the group of cancer patients without diabetes demonstrated tendency toward less frequent occurrence of SHBG_rs6257 TC variant (**Table 2**).

We looked also at A-polymorphisms occurrence in S-polymorphism-expressing groups. Among carriers of the "metformin-positive" variant of OCT1_R61C, the bearing of GC genotype of OLR1_G501C oxidized low-density lipoprotein receptor was found to be higher in all patients with DM2 (χ^2

Table 2. Distribution (%) of the other studied ('associated') polymorphic genotypes in postmenopausal females with and without cancer or diabetes

Group	OGG1 Ser326Cys rs1052134 (CG+GG)	OLR1 G501C rs11053646 (GC)	LEPR Gln223Arg rs1137101 (AG)	SHBG D356N rs6259 (GA)	SHBG c.11217 t > C rs6257 (TC)	rs11065987 (near BRAP) (GG)
DM2 w/o cancer (32)	*46,9 ± 8,8*	21,9 ± 7,2	59,4 ± 8,7	12,5 ± 5,9[2]	12,5 ± 5,9	9,4 ± 5,1
Cancer+DM2 (64)	31,3 ± 5,9	17,2 ± 4,7	46,9 ± 6,1[1]	12,5 ± 4,1[2]	17,2 ± 4,7[3]	18,8 ± 4,8
DM all (96)	36,5 ± 4,8	18,7 ± 4,0	51,0 ± 5,1[1]	12,5 ± 3,4[2]	15,6 ± 3,7[3]	15,6 ± 3,7
DM+FH (35)	31,4 ± 7,8	11,4 ± 5,3	51,5 ± 8,4[1]	20,0 ± 6,8	20,0 ± 6,8[3]	11,4 ± 5,3
Cancer w/o DM2 (23)	39,1 ± 10,1	21,7 ± 8,4	78,3 ± 8,4	34,8 ± 9,8	0	13,0 ± 7,0
Healthy (37)	29,7 ± 7,5	24,3 ± 7,0	43,2 ± 8,1[1]	21,6 ± 6,8	27,0 ± 7,2[3]	18,9 ± 6,4

Notes: See text for explanation of terminology and **Table 1**, for abbreviations [1,2,3]Difference with group of cancer patients without diabetes is significant ($P < 0.01–0.05$)

Table 3. Distribution, in %, of "associated (A) genotypes" in the groups of postmenopausal diabetics–carriers of genetic markers of metformin response

A-genotypes	Group of patients	Variants of S-genotypes related to potentially positive (+) or weakened (–) response to metformin					
		OCT1 R61C (+) CC	OCT1 R61C (–) CT+TT	OCT rs622342 (+) AC+AA	OCT rs622342 (–) CC	C11Orf65 (+) CC	C11Orf65 (–) AA
OGG1 Ser326Cys (CG+GG)	DM + cancer (64)	31,6 ± 6,2 (56)	25,0 ± 11,3 (8)	30,3 ± 6,1 (55)	33,3 ± 15,7 (9)	*8,3 ± 7,5 (12)*[3]	*36,5 ± 6,9 (52)*
	DM, all (96)	38,4 ± 5,2 (85)	18,2 ± 11,5 (11)	32,5 ± 5,2 (82)	57,1 ± 13,2 (14)	30,4 ± 8,6 (22)	37,8 ± 5,5 (74)
OLR1 G501C (GC)	DM + cancer (64)	19,3 ± 5,2 (56)	0 (8)	17,9 ± 5,1 (55)	11,1 ± 10,2 (9)	23,1 ± 11,5 (12)	15,4 ± 4,8 (52)
	DM, all (96)	20,9 ± 4,3*(85)*	0 (11)	20,5 ± 4,3 (82)	7,1 ± 6,8 (14)	26,0 ± 9,2 (22)	16,2 ± 4,2 (74)
LEPR Gln223Arg (AG)	DM + cancer (64)	42,1 ± 6,5 (56)	75,0 ± 15,2 (8)	*50,9 ± 6,8 (55)*	*22,2 ± 13,8 (9)*	33,3 ± 13,6 (12)	50,0 ± 6,9 (52)
	DM, all (96)	46,5 ± 5,4 (85)[1]	81,8 ± 11,5 (11)	51,8 ± 5,4 (82)	42,8 ± 13,2 (14)	39,1 ± 10,2 (22)	54,1 ± 5,9 (74)
SHBG D356N (GA)	DM + cancer (64)	14,0 ± 4,6(56)	0 (8)	12,7 ± 4,3 (55)	11,1 ± 10,3 (9)	25,0 ± 12,5 (12)	9,6 ± 4,1 (52)
	DM, all (96)	12,9 ± 3,7 (85)	9,1 ± 8,6 (11)	13,4 ± 3,7 (82)	7,1 ± 6,8 (14)	18,1 ± 10,1 (22)	10,8 ± 3,8 (74)
SHBG, rs6257 (TC)	DM + cancer (64)	16,0 ± 4,8 (56)	25,0 ± 15,2 (8)	20,0 ± 5,4 (55)	0 (9)	25,0 ± 12,5 (12)	15,4 ± 4,8 (52)
	DM, all (96)	15,3 ± 3,9 (85)	18,2 ± 11,5 (11)	18,3 ± 4,1 (82)[2]	0 (14)	18,2 ± 8,1 (22)	14,9 ± 4,1 (74)
rs11065987 (near BRAP) (GG)	DM + cancer (64)	21,4 ± 5,4(56)	0 (8)	18,2 ± 5,2 (55)	22,2 ± 13,9 (9)	33,3 ± 13,4 (12)	15,4 ± 5,0(52)
	DM, all (96)	17,6 ± 4,1 (85)	0 (11)	15,8 ± 4,1 (82)	14,3 ± 9,3 (14)	22,7 ± 8,9 (22)	13,5 ± 4,0 (74)

Notes: See text for explanation of terminology and **Table 1**, for abbreviations; (+) potentially responsive to metformin; (–) potentially weakened (poor) response to metformin. [1]Difference with the data in group OCT1_R61C(–) is significant, p 0.03 [2]The tendency to difference with the data in group OCT1_ rs622342 (–) [3]The tendency to difference with the data in group C11Orf65 (–) Additional statistical information in relation to notes 2 and 3 is given in section "Results".

Table 4. Hormonal-metabolic status of postmenopausal females with new onset diabetes: comparison of groups with potentially different response to metformin

Polymorphisms	Potential response to metformin in carriers of genotypes presented in brackets	BMI, cond. un.	Waist, cm	HbA1c, %	Triglycerides, mmol/L	HOMA-IR, cond.un.	Estradiol, pmol/L
OCTR61C rs12208357	Weakened [CT+TT]	33,6 ± 2,3 (7)	102,0 ± 3,6 (7)	6,13 ± 0,47 (6)	2,06 ± 0,25 (2)	3,23 ± 0,27 (3)	92,5 ± 77,3 (4)
	potentially positive [CC]	32,1 ± 0,9 (56)	97,4 ± 2,0 (57)	6,40 ± 0,18 (42)	1,73 ± 0,07 (54)	5,18 ± 0,74 (35)[1]	84,6 ± 23,2 (39)
OCT1 rs622342	Weakened [CC]	31,5 ± 2,0 (5)	93,2 ± 5,7 (5)	6,15 ± 0,15 (2)	1,73 ± 0,31 (4)	3,31 ± 0,66 (5)	46,0 ± 27,7 (4)
	potentially positive [others]	32,3 ± 0,9 (58)	98,3 ± 1,9 (59)	6,38 ± 0,17 (46)	1,77 ± 0,07 (57)	5,29 ± 0,77 (33)[1]	89,4 ± 24,0 (39)
C11orf65 rs11212617	Weakened [others]	32,1 ± 1,0 (48)	97,0 ± 2,0 (49)	6,50 ± 0,21 (36)	1,75 ± 0,08 (46)	5,28 ± 0,92 (28)	94,5 ± 27,4 (34)
	potentially positive [CC]	32,9 ± 1,5 (15)	101,0 ± 4,4 (15)	5,97 ± 0,13 (12)	1,81 ± 0,11 (15)	4,32 ± 0,47 (10)	51,0 ± 13,3 (9)
LKB1/STK11 rs8111699	Weakened [CC]	33,7 ± 1,5 (19)	102,1 ± 3,8 (19)	6,37 ± 0,22 (15)	1,88 ± 0,10 (19)	5,77 ± 1,06 (10)	118,6 ± 50,9 (17)
	potentially positive [others]	31,6 ± 1,0 (44)	96,2 ± 2,0 (45)	6,37 ± 0,22 (33)	1,71 ± 0,08 (42)	4,76 ± 0,86 (28)	63,7 ± 14,2 (26)

Notes: In round brackets, number of cases; BMI, body mass index; HbA1c, glycosylated hemoglobin; HOMA-IR, insulin resistance score value [1]Difference with respective group with potentially weakened (poor) response to metformin is significant ($P < 0.05$)

2.87; p 0.09), including diabetics with cancer (χ^2 2.59; p 0.11) (**Table 3**). Similar relations were observed for GG variant of BRAP-associated rs11065987 gene (χ^2 respectively 2,27 and 2,11; p 0.13 и 0.14), and much less strongly (χ^2 1.31; p 0.25) in diabetics suffering with cancer, for GA variant of sex hormone-binding globulin gene SHBG_D356N. Contrariwise, in combined DM2 group (in diabetics with and without cancer), AG type of leptin receptor gene LEPR_Gln223Arg was found less frequently in "metformin-positive" than in "metformin-negative" group of OCT1_R61C polymorphism carriers (χ^2 4.71; p 0.03), while in the group of diabetics with cancer genotypes CG + GG of 8-oxoguanine glycosylase gene were discovered more rarely in carriers of "metformin-positive" rather than "metformin-negative" polymorphic variant of C11Orf65, χ^2 3,61; p 0.06 (**Table 3**).

Attempt to match genetic and hormonal-metabolic patterns in patients with new onset (treatment-naïve) DM2 revealed 2 main relations. First, the carriers of potentially "metformin-positive" OCT1_R61C or OCT1rs622342 gene polymorphisms displayed a significant increase of insulin resistance score value compared with "metformin-negative" genotype carriers (*P*, respectively, 0.04 and 0.05). The second trend, requiring further study, is a tendency to relatively lower serum estradiol level in carriers of such polymorphisms of STK11/LKB1 and C11orf65 genes which are considered to be "metformin-positive"[27,28] (**Table 4**).

Discussion

Metformin is a well-known antidiabetic medication, and during the last 4–5 decades at least twice it has drawn attention as a presumably active anticancer drug.[3,9-11,13,31] Although potential selectiveness of metformin efficiency[10,11,19] and the need for further search of its effect prediction methods were brought to the notice, pharmacogenetic approach to this topic is still on the rather earlier stages.[22,23,27,28] According to our knowledge, never before has the study of metformin pharmacogenetics in cancer patients, and among others in the ones with diabetes, been conducted. Phamacogenetics is often considered a literal reflection of genetic diversity in metabolic pathways regulation, including the response to individual drugs and their therapeutic effects. Therefore, the bearing of innate single nucleotide polymorphisms (SNPs) associated with certain functions may serve as a marker for actual or potential (as in this study) response to a drug, specifically, metformin.

The results obtained are assembled in 4 sections (presented in **Tables 1–4**). Most of the data are new, and it deserves consideration in several aspects. In particular, the occurrence of different well-known by present genetic polymorphisms with high probability of metformin-response prediction,[22,26-28] which we ranked as "standard", is different in the same study groups (cancer patients with or without DM2; diabetics without cancer and healthy controls), as can be seen in **Table 1**. Important examples in this regard can be derived, in particular, from groups of patients with familial diabetes and cancer patients without DM2 (see data on genotype GG STK11/LKB1 in the first of these groups and on the pattern of OCT1 R61C and OCT1 rs 622342 variants, in the second, **Table 1**). Therefore, the response to metformin can likely be predicted by using different or combined pharmacogenetic patterns, while single pharmacogenetic markers may be, at some point, ineffective. Also, the "standard" polymorphisms sometimes may not have the required predictive strength, requiring the enhancement of prediction model with "associated" polymorphisms (**Table 3**), the occurrence of which, while being studied separately, didn't differ much in most study groups, except the group of cancer patients without diabetes (**Table 2**).

Table 5. Primer sequences, annealing temperatures, and lengths of amplicons used in the study

Gene	SNP	5′–3′ sequence of the primer	Amplicon length, bp	*T*ann, °C
OCT1	R61C	AGG GCT CCA GCC ACA GCG (OCT1–61-C)	120	66 °C
		AGG GCT CCA GCC ACA GCA (OCT1–61-T)		
		CTG CTG TCG GCT GCC TTT G (OCT1–61-F)		
OCT1	G401S	TCA CCA TTG ACC GCG AGG G (OCT1–401-G)	156	60 °C
		TCA CCA TTG ACC GCG AGA G (OCT1–401-A)		
		CAA CAC TTT CCC CAC ACT TC (OCT1–401-R)		
OCT1	G465R	TGT ATT TTA TCA GGA ACC TCG (OCT1–465-G)	189	60 °C
		TGT ATT TTA TCA GGA ACC TCA (OCT1–465-A)		
		TGC TGA GCC CAC TGC CGA (OCT1–465-R)		
OCT1	A > C (rs622342)	AGA TTG TTA GAT CTA TGT ATT TG (OCT1-A)	153	60 °C
		AGA TTG TTA GAT CTA TGT ATT GG (OCT1-C)		
		GAA AGA CAG AGA GAA TCA GTG (OCT1-com)		
STK11	C > G (rs8111699)	TGT GAG AGT GAG CCC CCT (STK11-C)	179	65 °C
		TGT GAG AGT GAG CCC CGT (STK11-G)		
		CCT CCC TGC CTC CGT GTT (STK11-R)		
C11orf65	G > T rs11212617	TAC AAA GGG CAG ATC AGA GAC (C11orf65-G)	160	60 °C
		TAC AAA GGG CAG ATC AGA GAA (C11orf65-T)		
		TGC GTG GAG TCA GAG TCTA A (C11orf65-R)		
OGG1	Ser326Cys	TGC CGA CCT GCG CCA ATC (OGG1-G)	89	65 °C
		TGC CGA CCT GCG CCA ATG (OGG1-C)		
		GGT GCC CCA TCT AGC CTT (OGG1-R)		
OLR1	G501C	GCTCATTTAACTGGGAAAAGA (OLR1–501-G)	164	60 °C
		GCTCATTTAACTGGGAAAACA (OLR1–501-C)		
		ATTCCTCCAGTGACAGTTTA (OLR1–501-R)		
LEPR	Gln223Arg	AAC TGA CAT TAG AGG TGA CC (LEPR-223-G)	122	63 °C
		AAC TGA CAT TAG AGG TGA CT (LEPR-223-A)		
		ATG TTG TGA ATG TCT TGT GC (LEPR-223-com)		
SHBG	D356N (rs6259)	GCA AAA AGA GGT GGA AGA GTC (SHBG-6259-G)	184	60 °C
		GCA AAA AGA GGT GGA AGA GTT (SHBG-6259-A)		
		TCG GAG GGA AGA AGA ATA GG (SHBG-6259-F)		
SHBG	t > C (rs6257)	TCC CTA CTC AGC TTT GTT TGT (SHBG-6257-T)	177	65 °C
		TCC CTA CTC AGC TTT GTT TGC (SHBG-6257-C)		
		AGA GGG CAG AAC CAG GGG A (SHBG-6257-R)		
Locus near BRAP	rs11065987	GTC CAC CAC ACT CAG TCA AT ('BRAP'-A)	131	60 °C
		GTC CAC CAC ACT CAG TCA AC ('BRAP'-G)		
		TCG AAC TAG GAG CTG TGT CT ('BRAP'-F)		

The comparison of genetic, hormonal and metabolic data in patients with treatment-naïve DM2 (with or without cancer) revealed some differences (see **Table 4**) between carriers of polymorphic variants of organic cation transporter 1 (OCT1) and 2 other genes, C11orf65 and STK11, included also, based on published data,[27,28] into "standard" group. The trend discovered in this section, to association of hyperestrogenemia with variants of C11orf65 and STK11 pointing on potentially poor response to metformin, is another clue for a more complex action of this biguanide, which, apart from its antidiabetic properties, has other targets, e.g., aromatase (estrogen synthetase) complex.[32] At the same time, carrying of polymorphic sex hormone binding globulin variants, which are associated with DM2 risk and affect serum estradiol level,[33] may, as it turned out, concur with "metformin-positive"

OCT1_R61C and OCT1_ rs622342 genotypes (**Table 3**). This fact is an additional argument in favor of comparative pharmacogenetic analysis of metformin response markers, incorporating simultaneously the antimetabolic, estrogen-modulating, and antineoplastic aspects of its action. Data of this kind will let us see if the statement, that "multifactorial nature of hypoglycemic metformin response can, at least partly, mask the role of polymorphisms involved in biguanide utilization and elimination,"[34] is correct for the other aspects of this drug action.

The important trait of the postmenopausal women studied was the fact that a significant part of them had a new onset DM2. Many such females were characterized by compensated glucose metabolism disturbance and rather moderate increase of glycosylated hemoglobin concentration (**Table 4**). The healthy controls without diabetes or cancer were younger than patients with DM2 (without or with cancer), but of the same age as the patients with cancer and without diabetes (see "Materials and Methods" section). The published data gives more evidence on cancer patients with concurrent DM2 being older, than cancer patients without diabetes,[5,35] which is in accord with our observations. Although it is still not completely clear if the fact of relatively small (3–5 y) age difference between mentioned groups can really affect the results, the further investigation of this subject can have practical significance, considering, e.g., more often occurrence of NRXN3 (neurexin group) gene polymorphism, associated with obesity and cell adhesion, in the youngest group of breast cancer patients.[36]

The drawback of the current study is relatively small number of probands in each group, which leads to the conclusion that it should be viewed as a pilot study. Nevertheless, the data obtained is significant and possibly stimulating for further investigation in this area, incorporating the evaluation of not only potential but also actual effects of metformin in comparison with the pattern of studied polymorphisms. Supposedly, phenformin should undergo the similar investigation, as it can evidently inhibit the development of age-related pathology (including clinical cancer),[3,10,12,13] and these effects may also be controlled by pharmacokinetic and pharmacogenetic mechanisms.

Materials and Methods

Patients

The study was approved by the local Ethic Committee. A total of 156 women, age 43 to 88 y (mean ± SE, 60,7 ± 0,7) were included. All patients were menopausal for at least one year. There were patients with DM2 without cancer (n = 32, mean age 62,0 ± 1,5, var 52–84), patients with diabetes and concurrent cancer, mostly breast, endometrial, or colorectal (n = 64, mean age 62,5 ± 1,1, var 46–88), patients with cancer without signs of DM2 (n = 23, mean age 59,7 ± 2,0, var 43–81), and healthy females (n = 37, mean age 57,1 ± 1,1, var 49–79). Among all patients with diabetes (n = 96) 65 were treatment-naïve (in 40 patients DM2 was diagnosed simultaneously with cancer), the other 31 patients (24 with DM2 and cancer, 7 with DM2 only) have already received some form of antidiabetic therapy. None of the cancer patients started anticancer treatment by the moment of the study.

Polymorphic markers

The polymorphisms studied belonged to 2 groups. The first, "standard" (S) one was composed of gene polymorphisms with proven relation to metformin response (see above), namely, polymorphic variants of organic cation transporter 1 gene (R61C/rs12208357; G401S/rs34130495; G465R/rs45476695 and intronic variant A > C/rs622342), serine/threonine kinase 11 or liver kinase B1 (STK11/LKB1 – OMIM 602216) as well as C11orf65 (rs11212617) gene in the locus which includes ATM gene. Besides, another group of metformin response-associated (A) polymorphisms was studied. These genes are supposed to be associated with such processes as glucose intolerance/DM, metabolic syndrome, chronic inflammation, and/or cancer. The group included polymorphisms of DNA repair gene, 8-oxoguanine glycosylase OGG1Ser326Cys (rs1052134),[37] oxidized low-density lipoprotein receptor gene OLR1_G501C (rs1053646),[38] leptin receptor gene LEPR_Gln223Arg (rs1137101),[39] 2 sex hormone-binding globulin gene variants - SHBG_D356N (rs6259) and SHBG_T > C(rs6257),[33,40] and rs11065987 gene located near BRAP locus, associated with BRCA1 and involved into modulation of cellular growth and differentiation and inflammatory signal pathways.[41]

Genotyping

DNA was obtained from peripheral blood mononuclears collected in the morning before meal. After plasma separation DNA was extracted with modified NaCl-chloroform protocol.[42] Genotypes for the polymorphic markers were determined by allele-specific real-time polymerase chain reaction (PCR) using iCycler iQ (Bio-Rad) and SYBR Green I intercalating dye. Primers, annealing temperatures and length values of fragments are presented in **Table 5**. PCR amplification volume was 20 μl. Reaction mixture was composed of 1 unit of hot-start Taq DNA polymerase, one-step PCR buffer, 50 ng of DNA, 1.5–3.0 mM $MgCl_2$, 200 μmol of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), 100 nmol of each primer, 0.2 μl 20× SYBR-Green I solution. The reaction started with Taq-polymerase activation phase (95 °C, 7 min). The further 45 cycles of PCR consisted of denaturation phase (95 °C, 30 s), annealing (60–66 °C, 60 s) and elongation (72 °C, 60 s).

Hormonal-metabolic status

This part of the study was performed only for 65 treatment-naïve patients with DM2, among those 40 had concurrent untreated cancer and 25 didn't have cancer diagnosed. The cubital vein blood was taken in the morning 10–12 h after the last meal. Besides anthropometry, blood glucose, glycosylated hemoglobin (HbA1c), serum lipids, insulin, and estradiol levels were evaluated (by enzyme colorimetric and immune enzyme assays), and insulin resistance score value (HOMA) was calculated.[43]

Statistical analysis

Analysis of the data was performed with SigmaPlot for Windows and Statistica 8.0 software. Comparison of hormonal and metabolic parameters values (M ± m) between separate groups of patients was based on Student *t* test. The heterogeneity test was performed by comparison of genotype distribution for each polymorphism between groups using Pearson χ-square test

(χ^2 with one degree of freedom). The significance level value used throughout the study was 0.05.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Note

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