ORIGINAL ARTICLE

Association between polymorphisms of the DNA repair genes *XRCC1* and *hOGG1* and type 2 diabetes mellitus in the Polish population

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KEY WORDS

DNA repair, gene polymorphism, *hOGG1*, type 2 diabetes mellitus, *XRCC1*

ABSTRACT

INTRODUCTION Elevated oxidative stress in type 2 diabetic patients leads to the accumulation of DNA damage and possibly acceleration of diabetic complications. Numerous studies indicate that diabetic patients may display impaired DNA repair compared to healthy subjects.

OBJECTIVES The aim of the study was to compare the distribution of genotypes of DNA repair genes between type 2 diabetic patients and non-diabetic subjects.

PATIENTS AND METHODS Polymerase chain reaction-based restriction fragment length polymorphism was used to determine the distribution of genotypes and frequency of alleles of polymorphisms of base excision repair genes, including the Arg399Gln polymorphism of the *XRCC1* gene and Ser-326Cys in the *hOGG1* gene. The study population included 195 subjects, including 94 with type 2 diabetes mellitus and 101 with normal glucose metabolism. All study participants were Caucasian and inhabited the city of Łódź, Poland.

RESULTS The frequency of the Gln allele in *XRCC1* gene (41% vs. 47%, odds ratio [OR] 0.80, CI 0.54–1.19) and Cys allele in *hOGG1* gene (19% vs. 18%, OR 1.09, CI 0.65–1.82) did not differ significantly between diabetic patients and subjects with normal glucose metabolism. Linkage analysis revealed that the Arg/Gln–Ser/Ser combination of genotypes of *XRCC1* and *hOGG1*, respectively (not associated with a decreased activity of both genes) occurs more commonly in type 2 diabetic patients.

CONCLUSIONS The results of our study suggest no association between decreased activity of the examined DNA repair genes and type 2 diabetes mellitus in the studied population.

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INTRODUCTION Chronic hyperglycemia in type 2 diabetes mellitus leads to elevated oxidative stress.[1] As a consequence, the accumulation of reactive oxygen species (ROS) may cause additional damage to various biological macromolecules, including DNA. The most often reported nuclear and mitochondrial DNA damage in diabetic patients is 8-hydroxy-2'deoxyguanosine (8OHdG), which can be generated by hydroxyl radicals, singlet oxygen, peroxyl radicals, peroxynitrate and peroxynitrite. ROS are also able to produce various other DNA modifications, including strand breaks.[2-4]

Moreover, glucose at high levels may inhibit the expression of the DNA repair enzyme XPD induced by insulin.[5] We have recently shown that type 2 diabetes mellitus may be associated not only with enhanced oxidative damage but also with increased susceptibility to mutagens and decreased efficacy of DNA repair.[6]

It is accepted that increased ROS generation is an important factor underlying the development of vascular complications in type 2 diabetes, and possibly one of the factors responsible for an increased incidence of cancer in this group of patients.

Harmful consequences of DNA damage could be prevented by efficient repair mechanisms. Oxidized DNA base lesions are removed by 2 types of activity, namely, base excision repair (BER), involving removal of single lesions by glycosylase action and a more complex process involving

TABLE 1 The antropometric data of type 2 diabetic patients and control individuals

	Type 2 diabetic patients (n = 94)					Control individuals (n = 101)				
	N°	Age		BMI		N°	Age		BMI	
		Mean ± SD	Quartiles	Mean ± SD	Quartiles		Mean ± SD	Quartiles	Mean ± SD	Quartiles
Women	45	68.98 ±10.26	45, 61, 70, 75, 98	32.99 ±5.90	20.76, 29.53, 31.25, 35.95, 58.02	63	65.48 ±16.80	20, 60.5, 73, 76, 85	30.50 ±2.97	21, 28.91, 30.11, 32.22, 35.65
Men	49	66.18 ±12.29	33, 56.5, 68.5, 76, 85	30.23 ±4.44	23.38, 26.62, 30.24, 33, 97, 38.47	48	57.73 ±16.96	38, 44.5, 56, 65.5, 93	32.85 ±6.13	26.57, 29.63, 30.80, 32.19, 47.47

Abbreviatons: SD – standard deviation, BMI – body mass index

removal of a lesion-containing oligonucleotide, nucleotide excision repair. The oxidative DNA base modifications, typical of diabetes, are primarily removed in the BER pathway. Therefore, we investigated whether polymorphic variants associated with a decreased activity of 2 genes involved in BER, *XRCC1* and *hOGG1*, occur more frequently in type 2 diabetic patients.

The *XRCC1* gene is mapped at human chromosome 19q13.2–13.3.[7] A decreased activity of *XRCC1* is associated with an increased sensitivity to ionizing radiation, ultraviolet, hydrogen peroxide, and mitomycin.[8] The *XRCC1* gene is directly involved in the repair of single strand breaks and damaged bases in DNA by the interaction with DNA polymerase β, poly-ADP-ribose polymerase (PARP) and DNA ligase III.[9]

By radiation hybrid panel mapping, *hOGG1* was localized between WI-4179 and AFMA216ZG1 at 3p26, proximal to the VHL gene.[10] The *hOGG1* gene encodes a DNA glycosylase, which repairs 8OHdG in double stranded DNA. The OGG1 protein removes 8OHdG from 8OHdG:C base pairs by DNA glycosylase activity and cleaves the 3'-side of 8OHdG by its AP (apurinic/apirymidynic) lyase activity.[11] A G/A transition in exon 10 of the *XRCC1* gene (the Arg399Gln polymorphism) was correlated with the occurrence of certain types of cancer.[12-14] The Gln/Gln homozygote of this polymorphism may be linked with a decreased effectiveness of BER by an alteration in BRCT (BRCA1 carboxyl-terminal domain) domain of the *XRCC1*. This results in reduced binding of the *XRCC1* by PARP, leading to the accumulation of DNA damage.[15] A T/A transversion was reported in the exon 7 of the *hOGG1* gene (the Ser326Cys polymorphism) and it was linked with the reduced activity of *hOGG1*.[16] Ethnic and interindividual differences in OGG1 activity and several types of polymorphisms at the *hOGG1* gene locus have been observed in the different populations studied.

The aim of the study was to investigate the distribution of genotypes and frequencies of alleles of the DNA repair genes *XRCC1* and the *hOGG1* gene in type 2 diabetic patients compared to patients with normal glucose metabolism.

PATIENTS AND METHODS

Study population

The study population comprised 195 unrelated adult individuals, including 94 with type 2 diabetes mellitus. Subjects with normal glucose metabolism and no family history of diabetes (n = 101) served as the control group. The diagnosis of type 2 diabetes mellitus was made at least 1 year before entering the study upon World Health Organization 1999 criteria.[17] Participants of the study were recruited from patients admitted to the Department of Internal Medicine, Diabetology and Clinical Pharmacology of the Medical University of Łódź, Poland, between 2002 and 2004. All study participants were of Caucasian origin and inhabited the city of Łódź, Poland. The study was approved by the Ethic Committee of the Medical University of Łódź in accordance with the Declaration of Helsinki and each person gave written informed consent before start of the study.

Determination of polymorphisms

Genomic DNA was extracted from peripheral blood lymphocytes by the standard phenol-detergent method. The PCR-restriction fragment length polymorphism method was used to detect the genotypes of the Arg399Gln and Ser326Cys polymorphisms.[18-20] The 20 µl aliquots of polymerase chain reactions (PCR) contained 10 ng genomic DNA, 1,5 U Taq polymerase (InGen – TERPOL, Sieradz, Poland) in 1 × PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM $MgCl_2$, 0.1% gelatin), 1.5 mM $MgCl_2$, 50 mM dNTPs and 250 nM each primer. The PCR was carried out in the MJ Research, INC thermal cycler, model PTC-100 (Waltham, MA, USA). Thermal cycling conditions for the XR*CC1 were*: initial denaturation step for 3 minutes at 95°C followed by 35 cycles composed of 20 seconds at 95°C, 20 seconds at 58°C annealing temperature and 20 seconds at 72°C. The final extension step was performed at 72°C for 5 minutes. For the *hOGG1* the initial denaturation step was performed for 5 minutes at 95°C followed by 34

TABLE 2 Distribution of genotypes, frequency of alleles and odds ratios of the Arg399Gln polymorphism of the *XRCC1* gene in type 2 diabetic patients and non-diabetic controls.

Genotype	Number	Frequency 95% CI	Number	Frequency 95% CI	OR 95% CI	p two-sided	corr χ^2 p two-sided	„power" [a]
Arg/Arg	35	0.37	29	0.29	1.47		1.24	0.428
		0.27–0.47		0.20–0.38	0.81–2.69	0.2246	0.2654	
Arg/Gln	40	0.43	49	0.49	0.79		0.4779	0.653
		0.33–0.53		0.39–0.58	0.45–1.36	0.4722	0.4892	
Gln/Gln	19	0.2	23	0.23	0.86		0.067666	
		0.12–0.28		0.15–0.31	0.43–1.70	0.7288	0.7948	0.493
Arg	110	0.59	107	0.53	1.105		0.996923	
		0.51–0.66		0.46–0.60	0.84–1.87	0.3079	0.3181	0.527
Gln	78	0.41	95	0.47	0.88		0.996923	
		0.34–0.49		0.40–0.54	0.54–1.19	0.3079	0.3181	0.527

Abbreviations: OR – odds ratio

a The so-called *post hoc* "retrospective statistical power" was computed based on the observed effect size and sample size (using real sample size, significance and χ^2 statistics)

cycles of 30 seconds at 95°C, 30 seconds at the 57°C annealing temperature, 60 seconds at 72°C for and the final extension for 7 minutes at 72°C. The *XRCC1*–Arg399Gln polymorphism was determined using the following primers (TIB MOLBIOL, Poznań, Poland) – sense: 5'-CAA GTA CAG CCA GGT CCT AG-3', antisense: 5'-CCT TCC CTC ATC TGG AGT AC-3'. The 248 base pairs (bp) PCR product was for 16 hours with 5 U of the restriction enzyme NciI. The Arg allele was digested into 89 and 159 bp fragments and the Gln allele remained intact. The *hOGG1*–Ser326Cys polymorphism was determined using following primers (EUROGENTEC, Seraing, Belgium) – sense: 5'-GGA AGG TGC TTG GGG AAT-3', antisense: 5'-ACT GTC ACT AGT CTC ACC AG-3'. The 200 bp PCR product was digested for 16 hours with 4 U of the restriction enzyme Fnu*4H1*. The Cys/Cys homozygotes gave fragment length of 100 bp, heterozygotes – 200 bp and 100 bp, whereas the Ser/Ser homozygotes – 200 bp. Restriction fragments were analyzed on 8% acrylamide gel stained with ethidium bromide.

Data analysis The statistical significance of the differences of observed alleles and phenotypes between groups was tested using the χ^2 test. Potential linkage between genotype and diabetes was assessed by the logistic regression. A statistical power of the results was also calculated. In all tests p values of less than 0.05 were considered statistically significant. Analyses were performed using the STATISTICA 6.0 package (Statsoft, Tulsa, OK, USA).

RESULTS The demographic characteristic did not differ significantly between both groups (TABLE 1). There were no significant differences in genotype distribution and allele frequencies of the Arg399Gln polymorphisms in the *XRCC1* gene (TABLE 2). Similarly, no significant differences were found between genotype distribution and allele frequencies of the Ser326Cys polymorphism in the *hOGG1* gene (TABLE 3).

The Arg/Arg genotype associated with normal activity of the *XRCC1* gene occurred more commonly in type 2 diabetic patients (OR: 1.47, 95% CI 0.81–2.69), although the difference was not statistically significant.

Gene–gene interactions for both polymorphisms are shown in TABLE 4.

The linkage analysis revealed that the combination of Arg/Gln and Ser/Ser genotypes of the two *XRCC1* and *hOGG1* genes respectively occurred more commonly in diabetic patients.

Additionally, the Gln/Gln–Cys/Cys combination of genotypes that usually correlated with a decreased activity of both *XRCC1* and *hOGG1* was absent in diabetic patients as opposed to control subjects.

Genotype distributions in type 2 diabetes mellitus patients and those with normal glucose metabolism were in Hardy-Weinberg equilibrium.

DISCUSSION Given that inefficient DNA repair processes may contribute to the development of diabetic complications, the purpose of the present study was to investigate the distribution of polymorphisms of genotypes and frequencies of alleles of 2 DNA repair genes in diabetic patients compared to subjects with normal glucose metabolism. We studied 2 relatively common polymorphisms such as *XRCC1* Arg399Gln and *hOGG1* Ser326Cys. To our knowledge, none of the previous studies has evaluated an association between polymorphisms of the DNA repair genes *XRCC1* and *hOGG1* and type 2 diabetes.

Because the number of individuals, both patients and controls, enrolled in the present study was limited, we estimated a statistical power of the study. The obtained results are useful to design further studies in larger populations.

TABLE 3 Distribution of genotypes, frequency of alleles and odds ratios of the Ser326Cys polymorphism of the *hOGG1* gene in type 2 diabetic patients and non-diabetic controls.

Genotype	Number	Frequency 95% CI	Number	Frequency 95% CI	OR 95% CI	p two-sided	corr χ^2 p two-sided	„power"[a]
Ser/Ser	59	0.63	66	0.65	0.89		0.079686	
		0.53–0.73		0.56–0.75	0.50–1.61	0.7649	0.7777	0.437
Ser/Cys	34	0.36	34	0.34	1.12		0.046949	
		0.26–0.46		0.24–0.43	0.62–2.01	0.7645	0.8285	0.437
Cys/Cys	1	0.01	1	0.01	1.08			
		–0.01–0.03		–0.01–0.03	0.07–17.44	>0.9999	>0.9999	N/E
Ser	152	0.81	166	0.82	0.92		0.042827	
		0.75–0.86		0.77–0.87	0.55–1.53	0.7944	0.8361	0.390–0.400
Cys	36	0.19	36	0.18	1.09		0.042827	
		0.14–0.25		0.13–0.23	0.65–1.82	0.7944	0.8361	0.390–0.400

Abbreviations: N/E – non estimated, OR – odds ratio

a The so-called *post hoc* "retrospective statistical power" was computed based on the observed effect size and sample size (using real sample size, significance and chi square statistics)

In the subgroup of the Polish population, genotype and allele frequencies obtained from diabetic patients did not differ significantly from those found in control subjects with the *XRCC1* Arg399Gln and the *hOGG1* Ser326Cys polymorphisms. However, these data indicated that in the studied population the genotype and allele frequencies of homozygous polymorphic variants of *XRCC1* differed from those obtained from other Caucasian populations.[12,19-23] Compared to other populations the 1.5–2.0 fold higher frequency of Arg/Arg genotype and the 1.5–2.0 fold lower frequency of Gln/Gln genotype were observed. It seems worth noting that the Arg/Arg genotype is associated with normal function of the *XRCC* gene. In case of *hOGG1* Ser326Cys polymorphism allele frequencies did not differ from other Caucasian populations, but they were 2 fold lower for Cys allele than those in Hawaiian and Japanese populations.[24-26] Additionally, in the examined population the Gln/Gln-Cys/Cys combination of genotypes, which usually correlated with a decreased activity of both *XRCC1* and *hOGG1*, was absent in diabetic patients compared to control subjects. It might be tempting to speculate that those polymorphic variants were abolished in the diabetic population. However, a larger population would be required to confirm this hypothesis.

As it was already mentioned the number of cases in the current study was rather low. We decided to publish the results of this study, since they may be helpful for other currently ongoing or planned research. We would not have done so if the results had been positive.

Cellular metabolism is a source of endogenous ROS and accounts for the background level of oxidative DNA damage detected in normal tissues. In diabetic patients, hyperglycemia is believed to augment disproportionately free radical production, due to the increased mitochondrial oxidative metabolism that is secondary to the high intracellular glucose level, autooxidation of glucose and its metabolic intermediates, sorbitol pathway activation, and oxidative degradation of advanced glycation end-products.[27-29] It has been shown that DNA damage in lymphocytes and leucocytes can be used as a marker of oxidative stress in diabetes.[2,30] It has been additionally demonstrated that DNA damage was significantly higher in the poorly-controlled diabetic patients compared to well-controlled subject, regardless of sex.[31]

In both type 1 and type 2 diabetes, the role of free radicals in diabetic complications is relatively well investigated. Abnormally high levels of ROS in diabetic patients can alter the structure and function of various extra- and intracellular components, including lipids, proteins and DNA. DNA damage is regarded as an important element of cellular dysfunction and death, and is crucial in the pathogenesis of diabetic complications. It has been shown that ROS could promote DNA damage and both formation and progression of atherosclerotic plaques.[32] One may assume that the effective DNA repair system would be able to reduce at least in part the risk arising from DNA damage and inhibit the progression of atherosclerosis in diabetic patients.

Whereas there is growing evidence for the involvement of ROS in atherosclerotic plaque development, the role of DNA damage and involvement of DNA repair systems are less clear. There are relatively few reports examining the relationship between the efficacy of DNA repair systems and metabolic and cardiovascular diseases. Existing evidence indirectly indicates the possibility of such a relationship, since increased amounts of 8OHdG were found in diabetic subjects with advanced carotid atherosclerosis.[33] Elevated urinary 8OHdG and leukocyte DNA were also detected in diabetic patients with hyperglycemia, and the level of urinary 8OHdG correlated with

TABLE 4 Gene-gene interaction of the Arg399Gln polymorphism of the *XRCC1* gene and Ser326Cys polymorphism of the *hOGG1* gene it type 2 diabetic patients and non-diabetic individuals

Genotype	Number	Frequency 95% CI	Number	Frequency 95% CI	OR 95% CI	p two-sided	corr χ^2 p two-sided	„power" [a]
Arg/Arg–Ser/Ser	24	0.26	15	0.15	1.97		2.835705	
		0.17–0.34		0.08–0.22	0.96–4.03	0.074	0.0922	0.475
Arg/Arg–Ser/Cys	11	0.12	14	0.14	0.82		0.055849	
		0.05–0.18		0.07–0.21	0.35–1.92	0.6748	0.8132	0.412
Arg/Arg–Cys/Cys	0	0	0	0	N/E			
Arg/Gln–Ser/Ser	22	0.23	39	0.39	0.48		4.555788	
		0.15–0.32		0.29–0.48	0.26–0.91	0.0301	0.0328	0.531
Arg/Gln–Ser/Cys	17	0.18	10	0.1	2.01		2.090698	
		0.10–0.26		0.04–0.16	0.87–4.64	0.1454	0.1482	0.478
Arg/Gln–Cys/Cys	1	0.01	0	0	N/E			
Gln/Gln–Ser/Ser	13	0.14	12	0.12	1.19		0037001	
		0.07–0.21		0.06–0.18	0.51–2.76	0.8307	0.8475	0.494
Gln/Gln–Ser/Cys	6	0.06	10	0.1	0.62		0.40112	
		0.01–0.11		0.04–0.16	0.22–1.78	0.4399	0.5265	0.492
Gln/Gln–Cys/Cys	0	0	1	0,01	0			
				–0.01–0.2		0.5179	>0.9999	

Abbreviations: N/E – non estimated, OR – odds ratio

a The so-called *post hoc* "retrospective statistical power" was computed based on the observed effect size and sample size (using real sample size, significance and χ^2 statistics)

the severity of diabetic nephropathy and retinopathy.[34] The study performed by Collins et al. on a mixed European population revealed a strong association between premature coronary heart disease in men and the lymphocyte 8OHdG level.[3] On the other hand, Guven et al. did not find any association between *XRCC1* Arg399Gln polymorphism and both the presence and severity of coronary artery disease.[35]

Free radical-mediated DNA damage and impaired antioxidant defense have also been implicated as contributors to the development of cancer. Recent evidence indicates that type 2 diabetes is associated with increased incidence and mortality from a number of cancers, including those of the colon, breast, endometrium, liver, bladder and pancreas.[36-38] It is of interest that cancer and atherosclerosis-related cardiovascular diseases often share common pathogenic determinants, such as DNA damage, oxidative stress and chronic inflammation.[39] This is in agreement with the results of our previous study suggesting that type 2 diabetes mellitus may be associated not only with the elevated level of oxidative DNA damage, but also with the increased susceptibility to mutagens and the decreased efficacy of DNA repair.[6]

Taking into account the results of this study and the previous work,[6] we conclude that the elevated level of oxidative DNA damage observed in type 2 diabetic patients is not derived from disturbed DNA repair processes associated with the decreased activity of the examined DNA repair genes – *XRCC1* and *hOGG1* genes. However, further studies on larger populations are required to determine the consistency of these observations.

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