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ORIGINAL ARTICLE

Association Between Single Nucleotide Polymorphism +276G > T (rs1501299) in *ADIPOQ* and Endometrial Cancer

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Abstract Current literature gives evidence of an indisputable role adiponectin plays in adipose tissue metabolism and obesity-related diseases. Moreover, latest research efforts focus on linking genetic markers of this adipocytokine's gene (*ADIPOQ*) with cancer. Aim of this study was to determine the genotype distribution of single nucleotide polymorphism +276G > T (rs1501299) in *ADIPOQ* and an attempt to identify the impact this polymorphism exerts on endometrial cancer risk in obese females. The test group comprised 90 women treated surgically for endometrial cancer between 2000 and 2012 in the Department of Surgical & Endoscopic Gynecology and Gynecologic Oncology, Polish Mothers' Memorial Hospital - Research Institute, Lodz, Poland. 90 individuals treated in the parallel period for uterine fibroids constituted the control group. Patients within both groups were stratified according to BMI into: lean, overweight and obese subjects. Statistical analysis was performed between two major groups and, furthermore, within the abovementioned subgroups. The analysis revealed that allele G of the investigated polymorphism in obese women with endometrial cancer is significantly more frequent, and allele T is significantly less frequent than in lean controls. However, no significant correlation was observed between the polymorphism and endometrial cancer in lean and overweight females. Single nucleotide polymorphism +276G > T (rs1501299) in *ADIPOQ* may be considered to be a risk factor of endometrial cancer. Further research on SNP in EC is warranted to obtain more conclusive outcomes.

Keywords Endometrial cancer · Single nucleotide polymorphism · + 276G > T · Obesity · Adiponectin · *ADIPOQ*

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Introduction

Endometrial cancer (EC) is one of the most common malignancies in women and both morbidity and mortality are still growing [1]. Clinical practice and histology divide this pathology in two independent subgroups: endometrioid endometrial carcinoma (strongly estrogen-dependent) and non-endometrioid endometrial carcinoma [2]. The vast majority of cases are reported in post menopausal patients, whereas obesity, diabetes and arterial hypertension are three major risk factors [3–5]. Not less than 60 % of all endometrial cancers are believed to be obesity-related, therefore obese individuals hold a general six-fold greater risk of death caused by this disease [6, 7]. Genetic phenomena observed in endometrial cancer include mutations in *PTEN*, *K-ras*, *p53*, β-catenin, disturbed mismatch repair, microsatellite instability or even aneuploidies [8].

Indisputable positive correlation of obesity and endometrial cancer combined with the current insight into adipose tissue as a vital endocrine organ rather than just an energy storage compartment have all encouraged researchers to seek for molecular links that interconnect endometrial cancer and obesity. Adipocytes, apart from secretion of proinflammatory cytokines like tumor necrosis factor (TNF-α), interleukins (Il-1, Il-6),

monocyte chemotactic protein (MCP-1) or plasminogen activator inhibitor (PAI-1), produce also adipose tissue specific proteins: adiponectin, leptin, visfatin and resistin, which together are labeled adipocytokines [9]. Among the abovementioned solely adiponectin remains in a negative feedback with the overall body adipose tissue content, moreover, hipoadiponectinemia is identified with many metabolic disorders like type II diabetes, hyperinsulinemia or insulin resistance and with neoplastic diseases including EC [10–13].

Much effort has been lately put into research on genetic polymorphisms and their role in etiopathogenesis and epidemiology of diseases. Up-to-date literature lists numerous polymorphisms in adiponectin gene (*ADIPOQ*) which stand behind versatile metabolic disorders such as obesity, diabetes, insulin resistance, hyperinsulinemia or even coronary heart disease [14].

Single nucleotide polymorphism (SNP) +276G > T (rs1501299) is one of the most widely studied genetic markers in *ADIPOQ* and its role in metabolic disorders – including obesity – is evident [14–17]. However, the correlation of this SNP of *ADIPOQ* and cancer is controversial [18–23]. Yet, to our knowledge, there are no reports that assess the effect of this genetic alteration on the risk of EC. Aim of this study was to analyze the frequency of alleles and genotypes of SNP + 276G > T (rs1501299) in *ADIPOQ* and an attempt to determine the impact this polymorphism exerts on endometrial cancer in obese females.

Materials and Methods

Patients

The test group comprised 90 women treated surgically for endometrial cancer between 2000 and 2012 in the Department of Surgical & Endoscopic Gynecology and Gynecologic Oncology, Polish Mothers' Memorial Hospital - Research Institute, Lodz, Poland. 90 individuals treated in the parallel period for uterine fibroids constituted the control group. Both groups have been stratified accordingly to Body Mass Index (BMI) into: lean (BMI < 25), overweight (25 ≤ BMI < 30) and obese (BMI ≥ 30) and thus six groups (30 patients each) were created for statistical analysis. Due to the role of investigated SNP in metabolic disorders and its potential significance in cancer development, a history of any such comorbidity was an exclusion criterion of the study. The Local Ethic Committee approved the study and each patient gave a written consent (No 56/2012) The characteristics summary of both cases and controls are displayed in Table 1 and Table 2.

Genotype Determination

The genetic assays were performed within the DNA obtained from archival postoperative specimens stored in paraffin blocks in the Department of Clinical Pathology, Polish Mother's Memorial Hospital - Research Institute, Lodz, Poland. Endometrial tissue specimens were fixed in formaldehyde, embedded in paraffin, then sectioned in the microtome at thicknesses of 5 μm and stained with hematoxyline and eosin. The slices were placed in Eppendorf® micro test tubes, shaken five times with xylene, followed by 3-min-long centrifugation (14,000 RPM) after each shaking. The obtained sediment was lavaged in 96 % ethanol and again centrifuged for 3 min and dried in 37 °C. DNA was extracted from the material by DNeasy Blood & Tissue Kit (Qiagen, Germany) according to manufacturer's instruction. PCR-Restriction Fragment Length Polymorphism method (PCR-RFLP) was applied to determine the genotypes of SNP +276G > T (rs1501299) in the analysed probes. Primers (forward: 5′ TCTCTCCATGGCTGACAGTG 3′, reverse: 5′ AGATGCAGCAAAGCCAAAGT 3′) were applied to assess SNP +276G > T (rs1501299). The PCR-RFLP was performed in PTC-100 TM (MJ Research, INC, Waltham, MA, USA) thermal cycler. The amplification took place in 50 μl of reaction mixture of the following composition: genomic DNA, PCR buffer (TaKaRa, Japan), dNTP (TaKaRa, Japan), Taq Polymerase (TaKaRa, Japan), primers (Polgen, Poland) and H_2O. PCR cycler conditions were as follows: 95 °C for 30s, 62 °C for 30s and 72 °C for 30s, repeated in 35 cycles. The product set in 20 μl of reaction mixture was incubated for 14 h with restriction enzyme (*Bsm*I, New England BioLabs Inc., USA) in 65 °C. PCR-RFLP products were electrophoresed in a 2 % agarose gel (Sigma, Saint Louis,USA) and then visualised by ethidium bromide staining (Sigma, Saint Louis,USA). DNA Ladder 100 bp (Polgen, Poland) was used as mass ruler. The agarose gel was studied in ultraviolet light (Kodak Edas 290). The reaction produced fragments of 468 bp (homozygous: GG), 468, 320 and 148 bp (heterozygous: GT) and 320 and 148 bp (homozygous: TT).

Table 1 Cases

	Age: mean (median, SD)	BMI: mean (median, SD)
Group I	58,6 (54; ± 12,5)	22,7 kg/m2 (23,3 kg/m2; ± 1,6)
Group II	60,9 (57,5; ± 11,2)	27,9 kg/m2 (28,1 kg/m2; ± 1,3)
Group III	63,8 (64; ± 9,5)	34,9 kg/m2 (34,5 kg/m2; ± 1,9)
In total	61,1 (62; ± 11,2)	28,6 kg/m2 (28,1 kg/m2; ± 5,2)

Table 2 Controls

	Age: – mean (median; SD)	BMI – mean (median; SD)
Group I	54,3 (54; ± 4,2)	22,6 kg/m2 (23,1 kg/m2; ± 1,9)
Group II	57,5 (56; ± 4,6)	27,2 kg/m2 (27,5 kg/m2; ± 0,2)
Group III	57,5 (55; ± 6,2)	33,4 kg/m2 (33,2 kg/m2; ± 2,5)
In total	56,4 (55; ± 5,3)	27,9 kg/m2 (27,5 kg/m2; ± 5,0)

Statistical Analysis

For the investigated SNPs standard χ2-test was applied to assess the departure from Hardy-Weinberg equilibrium. Genotype and allele frequencies in cases and controls were compared by χ2-test. Specific risks were depicted as odds ratios (ORs) with associated 95 % intervals (CIs) by unconditional logistic regression. *P*-values <0.05 were considered significant.

Results

The primary statistical comparison of cases ($n = 90$) and controls ($n = 90$) showed no difference in genotype/allele distribution of SNP +276G > T (rs1501299) in *ADIPOQ* in these groups. Moreover, BMI-adjusted analysis of subgroups within cases and controls (lean cases Vs. lean controls, overweight cases Vs. overweight controls, obese cases Vs. obese controls) neither revealed any statistically significant outcomes. However, statistical analysis revealed that allele G in obese cases is significantly more frequent (67 Vs. 48 %), and allele T significantly less frequent (33 Vs. 52 %) than in lean controls (see Table 3). No significant correlation was observed between the polymorphism and EC in lean and overweight females

Discussion

Increasing morbidity and mortality in EC have encouraged researchers to seek for efficient tools to reverse this negative trend. Ginecological Oncology already provides an effective screening standard that influences both morbidity and mortality in cervical cancer. Even genetics can direct the physician towards an appropriate therapeutic pathway (e.g. BRCA mutations in breast/ovarian cancer). Although endometrial cancer is one of the major malignancies in women, it can already now be diagnosed relatively early: pathologic examination of tissue specimens in women with classical symptoms is not only limited to clinical reference centers but has become a common agenda for every practitioner, thus enabling therapy introduction comparatively early. Therefore, major efforts should be put into research on diseases that lack such a screening or routine diagnostic pattern, or where these still remain inadequate. Ovarian cancer would be a proper example of such one. The abovementioned reasoning obviously refers to endometrioid endometrial cancer, which was the subject of the study, and cannot be whatsoever accredited to non endometrioid endometrial cancer, which is defined by a completely different symptomatology and clinical course.

Table 3 Genotypes and alleles distributions of SNP +276G > T (rs1501299) in *ADIPOQ* in obese cases *versus* lean controls

	Obese cases ($n = 30$)		Lean controls ($n = 30$)		OR (95 % CI)[a]	p[b]
Genotype/Allele	number	%	number	%		
G/G	12	40	5	17	**1.00 Ref.**	
G/T	16	53	19	63	0.35 [0.10–1.21]	0.163
T/T	2	7	6	20	**0.14 [0.02–0.94]**	**0.043**
G	40	**67**	29	48	**1.00 Ref.**	
T	20	**33**	31	52	**0.47 [0.22–0.97]**	**0.042**

[a] odds ratio analysis [OR – odds ratio, CI - Confidence Interval 95 %].

[b] χ2 for the departure from Hardy-Weinberg equilibrium.

This study depicts a comparison between endometrial cancer patients and cancer free controls with regard to clinical features of obesity i.e. BMI. Cases ($n = 90$) and controls ($n = 90$) have been equally divided into 6 quantitatively equivalent groups: lean cases, overweight cases, obese cases, lean controls, overweight controls, obese controls. After extended statistical analysis the only finding was that allele G in obese cases is significantly more frequent (67 Vs. 48 %) and allele T significantly less frequent (33 Vs. 52 %) than in lean controls. Thus allele G of SNP +276G > T (rs1501299) in *ADIPOQ* may be considered to be a risk factor of endometrial cancer, whereas allele T may be a protective factor of the disease.

Drawing conclusions from obtained data should be done with a substantial level of caution due to the limitations affecting this study: test group and controls may be quantitatively unsatisfactory, SNPs linkage disequilibrium was not considered, circulating adiponectin levels in patients were unknown and the relation between SNP +276G > T (rs1501299) in *ADIPOQ* and uterine fibroids has not been yet stated. Moreover, one cannot be sure if the discovered statistical finding is indeed due to EC, or rather due to obesity itself.

Although great interest has been put into SNPs and despite the abundance of such DNA markers, until now none of these has entered clinical practice neither in screening nor diagnostics. Taking into consideration the complex nature of cancer, authors dare to claim, that multicomponent genetic assays calculating a cumulative risk derived from a resultant of numerous variables could be much more useful in cancer risk stratification.

Conclusions

SNP +276G > T (rs1501299) in *ADIPOQ* may be considered to be a risk factor of endometrial cancer, whereas allele T may be a protective factor of the disease. Further research on SNP in EC is warranted to obtain more conclusive outcomes.

Conflict of Interest We declare that we have no conflict of interest.

Authors' Contribution Jan Bieńkiewicz: protocol and project development, data collection and management, manuscript writing and editing.
Beata Smolarz: genetical assays and data analysis.
Andrzej Malinowski: protocol and project development.

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