Murine Double-Minute 2 Homolog Single Nucleotide Polymorphisms 285 and 309 in Cervical Carcinogenesis

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Abstract

Background and Objective In Caucasians, the MDM2 single nucleotide polymorphism (SNP) 285 G>C (rs117039649) neutralizes the effect of 309 T>G (rs2279744), which increases MDM2 expression and impairs the p53 pathway. In this study, we examined the distribution of these two SNPs in Polish women with squamous cell carcinoma (SCC) (n = 379), adenocarcinoma (n = 59) and other cervical tumor types (n = 18).

Methods The polymerase chain reaction-restriction fragment length polymorphism technique and DNA sequencing were employed in our study.

Results The \( P \) trend value calculated for the MDM2 285 G>C polymorphism was statistically significant (\( P_{\text{trend}} = 0.016 \)) for SCC. Using logistical regression analysis adjusted for the effect of age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status, we observed that the MDM2 285 G>C SNP protected against SCC, with an adjusted odd ratio (OR) for the C carriers versus G/G genotype of 0.536 (\( P = 0.019 \)). Stratified analyses of MDM2 285 G>C revealed a protective role of the C allele against SCC in women with a positive history of oral contraceptive use (age-adjusted OR 0.413, \( P = 0.021 \)) and in premenopausal women (age-adjusted OR 0.362, \( P = 0.022 \)). We also found that the 285GG/309GG vs 285GG/309 TT genotype increased the risk of SCC (adjusted OR 1.890, \( P = 0.005 \)). However, the 285CC/309GG + 285GC/309GG versus 285GG/309GG genotype reduced the risk of SCC (adjusted OR 0.311, \( P = 0.004 \)).

Conclusion Our results demonstrate that the MDM2 285C gene variant and 285CC/309GG ? 285GC/309GG genotypes protect against SCC, most likely by neutralizing the effect of the 309 T>G SNP. The 285GG/309GG genotype increases the risk of SCC possibly due to increased MDM2 expression.

Key Points

- The MDM2 309 T>G (rs2279744) single nucleotide polymorphism (SNP), causes increased MDM2 expression whose action is neutralized by 285 G>C (rs117039649) SNP, located on 24 bps from SNP309 SNP.
- Our genetic assessment demonstrated that the MDM2 285 G>C polymorphism protects against squamous cell carcinoma (SCC), but the 309 T>G does not have the same quality.
- The combined 285CC/309GG + 285GC/309GG genotypes protect against SCC, whereas the 285GG/309GG genotype increases the risk of SCC in the Caucasian populations.
1 Introduction

Cervical tumors are the third most frequent type of neoplasia that causes death among women worldwide [1]. The incidence of cervical neoplasia is especially high in developing countries, accounting for 86 % of all newly diagnosed cases worldwide [1]. Infections with high-risk types of human papillomavirus (HR-HPV) are thought to be the main etiological agents of cervical lesions [2]. HPV infections have been identified in nearly 100 % of all squamous cell carcinoma (SCC) cases [3], and it has been estimated that approximately 15–40 % of sexually active women are infected with HR-HPV [4]. Despite the frequency of HPV infections, only a small percentage of these women exhibit persistent positivity for HR-HPV types [5]. Apart from HPV, other susceptibility variables of cervical lesions have been identified, including social status, tobacco consumption, multi-parity, oral contraceptive use, age of sexual debut, and environmental pollutants [6, 7]. These data indicate that interactions between various susceptibility variables and genetic backgrounds are essential for the cancerous transformation of HR-HPV-infected cervical epithelial cells to cervical malignancies [6–9].

Expression of the HPV E6/E7 oncoproteins leads to the inactivation of tumor suppressor proteins p53 and retinoblastoma tumor suppressor protein (pRB), eventually causing uncontrolled cell cycle progression, increased cell survival, and accumulation of DNA damage [10, 11]. Murine double-minute 2 homolog (MDM2) is a major negative regulator of p53 protein levels [12, 13]. Furthermore, MDM2 interacts with pRB and binds to the activation domain of the E2F1 transcription factor that inhibits pRB regulatory functions [10].

Abnormal MDM2 levels have been linked to an increase in genetic errors that account for the onset and development of various diseases, including cancer [14, 15]. The T>G transition (rs2279744) at position 309 in the first intron of MDM2 in the promoter region causes up-regulation of both MDM2 mRNA and protein, leading to impairment of the p53 pathway [16]. In Caucasians, a second functional single nucleotide polymorphism (SNP), 285 G>C (rs117039649), has been identified in the promoter region located 24 bps from SNPs09 [17, 18]. This second SNP neutralizes the effect of the 309 T>G transition in MDM2, resulting in decreased MDM2 transcription [18]. There have been controversial findings demonstrating that the 309 MDM2 SNP is a susceptibility factor for the development of cervical cancer in disparate ethnicities [19–23].

The purpose of this study was to investigate the distribution of MDM2 309 T>G and 285 G>C SNPs in women with squamous cell carcinoma (SCC) (n = 379), adenocarcinoma (n = 59) and other cervical tumor types (n = 18) and controls (n = 481) from a Polish population.

2 Patients and Methods

2.1 Study Population

The study population consisted of 456 patients with an assessed stage, histological grade and cervical tumor type based on the International Federation of Gynecology and Obstetrics. Patients’ data were obtained from patients enrolled between July 2008 and August 2014 at the Department of Radiotherapy of the Greater Poland Cancer Center in Poznań, Poland. The patient group included randomly selected women with SCC (n = 379), adenocarcinoma (n = 59) or other histologic types of tumor (n = 18) (Table 1).

The control group consisted of 481 unrelated healthy female volunteers selected during medical examination at the University Hospital, Clinic of Gynecological Surgery at Poznań University of Medical Science (Table 1). Information regarding pregnancy, oral contraceptive use, tobacco smoking, and menopausal status was obtained as part of the patient history. All the patients and controls participating in the study were Caucasians from the Wielkopolska area of Poland. Informed consent was obtained from all participating individuals. The study methods were approved by the Local Ethical Committee of the Poznań University of Medical Sciences (reference number of ethical approval: 1010/07).

2.2 Genotyping

DNA was isolated from peripheral blood leucocytes using the salting out method. We initially sought to identify the MDM2 309 T>G (rs2279744) polymorphism by PCR using the primers 5′-GAGCGGTCACCTTTGGGTCT-3′ and 5′-CGGACGTGCTGAAGTGGC-3′. The PCR-amplified MDM2 fragment, which is 437 bp in length, was digested using the endonuclease MspAI (CMG/CKG; M = A or C; K = G or T) (New England Biolabs, Ipswich, USA) according to the manufacturer’s protocol. The MDM2 309G gene variant was cut into 244, 147 and 46 bp fragments, while the MDM2 309T gene variant was cut into 244 and 193 bp fragments. DNA digestion products were separated by electrophoresis on a 3 % agarose gel and visualized by ethidium bromide staining. Because we did not observe differences in the distribution of the MDM2 309 T>G polymorphism between cases and controls, we subsequently decided to determine the distribution of the 285G>C (117039649) SNP. We found that only the Faul restriction enzyme could recognize the MDM2 285 G>C (117039649) SNP, although this enzyme also recognized several other restriction sites inside the amplified fragment. Therefore, the presence of the MDM2 285 G>C
polymorphism was determined by Sanger sequencing analysis using the same pair of primers used for the MDM2 309 T>G SNP. The presence of the MDM2 309 T>G SNP was also verified by blindly selecting 30% of the samples for sequencing analysis.

### 2.3 Statistical Evaluation

The distinction in genotypic and allelic prevalence between the patients and controls and their genotype deviation from Hardy–Weinberg (HW) equilibrium were evaluated using a \( \chi^2 \) test. The polymorphism was tested for association with cervical cancer incidence using the Cochran-Armitage \( P \) trend test \( (P_{\text{trend}}) \). The \( \chi^2 \) and Fisher exact tests were used to determine the differences in genotypic distributions between the patients and controls. The odds ratio (OR) and 95% confidence intervals (95% CI) were also calculated. A logistic regression analysis was used to adjust for the effect of confounders such as age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status.

### Table 1 Clinical and demographic characteristics of patients and controls

| Characteristic                      | Patients (n = 456) | Controls (n = 481) |
|-----------------------------------|-------------------|--------------------|
| Mean age (years) \( \pm \) SD\(^a\) | 48.3 \( \pm \) 10.8 | 47.8 \( \pm \) 9.5 |
| Tumor stage                        |                   |                    |
| IA                                | 62 (13.6%)        |                    |
| IB                                | 63 (13.8%)        |                    |
| IIA                               | 61 (13.4%)        |                    |
| IIIB                               | 57 (12.5%)        |                    |
| IIIA                               | 145 (31.8%)       |                    |
| IIIB                               | 53 (11.6%)        |                    |
| IVA                                | 8 (1.8%)          |                    |
| IVB                                | 7 (1.5%)          |                    |
| Histological grade                 |                   |                    |
| G1                                 | 87 (19.1%)        |                    |
| G2                                 | 146 (32.0%)       |                    |
| G3                                 | 98 (21.5%)        |                    |
| Gx                                 | 125 (27.4%)       |                    |
| Histological type                  |                   |                    |
| Squamous cell carcinoma            | 379 (83.1%)       |                    |
| Adenocarcinoma                     | 59 (12.9%)        |                    |
| Other                              | 18 (4.0%)         |                    |
| Pregnancy                          |                   |                    |
| Never                              | 51 (11.2%)        | 51 (10.6%)         |
| Ever                               | 405 (88.8%)       | 430 (89.4%)        |
| Oral contraceptive pill use        |                   |                    |
| Never                              | 247 (54.2%)       | 269 (55.9%)        |
| Ever                               | 209 (45.8%)       | 212 (44.1%)        |
| Tobacco smoking                    |                   |                    |
| Never                              | 293 (64.3%)       | 334 (69.4%)        |
| Ever                               | 163 (35.7%)       | 147 (30.6%)        |
| Menopausal status                  |                   |                    |
| Premenopausal                      | 162 (35.5%)       | 179 (37.2%)        |
| Postmenopausal                     | 294 (64.5%)       | 302 (62.8%)        |
| HPV genotypes\(^b\)               |                   |                    |
| 16 and 18                          | 301 (66.0%)       |                    |
| 16, 18, 31, 33, 35, 39,45,51,52,56,58,59 and 68 | 341 (74.8%)       |

\(^a\) Age at first diagnosis

\(^b\) HPV genotypes were determined by cobas\(^\text{®}\) HPV Test Roche Molecular Systems, Inc., (Alameda, CA, USA)
$P$ value of $<0.05$ was considered statistically significant. Statistical analyses were conducted using Statistica version 10, 2011 (Stat Soft, Inc., Tulsa, USA).

3 Results

3.1 Distribution of the $MDM2$ 309 T$\rightarrow$G (rs2279744) Polymorphism Among Patients with Cervical Cancer and Controls

The values for the $\chi^2$ test of HW equilibrium were 0.396 and 0.154 for the patients and controls, respectively. The distribution and statistical analyses of the $MDM2$ 309 T$\rightarrow$G genotype in the patients and controls are summarized in Table 2. For all patients with cervical cancer, the $P$ trend value calculated for the $MDM2$ 309 T$\rightarrow$G transition was not statistically significant ($P_{\text{trend}} = 0.251$). The logistic regression analysis, which adjusted for the effect of age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status, also did not demonstrate an association with the $MDM2$ 309 T$\rightarrow$G transition for the cervical cancer patients (Table 2). Furthermore, we did not observe an association of the $MDM2$ 309 T$\rightarrow$G SNP with histological type, SCC, adenocarcinoma, other tumors, histological grade or tumor stage (Table 2, Online Resource 1).

3.2 Distribution of the $MDM2$ 285 G$\rightarrow$C (rs117039649) Polymorphism Among Patients with Cervical Cancer and Controls

The values for the $\chi^2$ test of HW equilibrium were 0.612 and 0.403 for the patients and controls, respectively. The prevalence and statistical analyses of the $MDM2$ 285 G$\rightarrow$C genotypes in the patients and controls are presented in Table 3. For all patients with cervical cancer, the p-trend value calculated for the $MDM2$ 285 G$\rightarrow$C polymorphism was statistically significant ($P_{\text{trend}} = 0.008$). Adjusting for the effect of age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status, the logistic regression analysis demonstrated that the G/C versus G/G genotype has a significant protective role in cervical carcinogenesis, with an adjusted OR 0.540 (95 % CI 0.326–0.896, $P = 0.017$). There was also a protective effect of the C/C and G/C versus G/G genotype, with an adjusted OR 0.523 (95 % CI 0.319–0.858, $P = 0.010$).

There was also an association of the $MDM2$ 285 G$\rightarrow$C SNP with SCC (Table 3). In patients with SCC, the p-trend value calculated for the $MDM2$ 285 G$\rightarrow$C polymorphism was statistically significant ($P_{\text{trend}} = 0.016$). We observed a protective effect of the C/C and G/C versus G/G genotype, with an adjusted OR 0.536 (95 % CI 0.317–0.905, $P = 0.019$). However, we did not observe an association of the $MDM2$ 285 G$\rightarrow$C polymorphism with adenocarcinoma,

| Table 2 Prevalence of the $MDM2$ 309T$\rightarrow$G (rs2279744) polymorphism among all patients with cervical cancer, SCC and and controls |
| Genotype | Patients (frequency %) | Controls (frequency %) | Odds ratio (95 % CI) | $P^a$ | Adjusted odds ratio (95 % CI) | $P^a$ | $P_{\text{trend}}$ |
|----------|------------------------|------------------------|----------------------|-------|-----------------------------|-------|------------------|
| All      |                        |                        |                      |       |                             |       |                  |
| T/T      | 174 (38.2)             | 202 (42.0)             | Referent             | –     | Referent                    | –     | –                |
| T/G      | 204 (44.7)             | 204 (42.4)             | 1.161 (0.877–1.537)  | 0.297 | 1.174 (0.885–1.588)         | 0.265 | 0.251            |
| G/G      | 78 (17.1)              | 75 (15.6)              | 1.207 (0.829–1.759)  | 0.326 | 1.099 (0.910–1.328)         | 0.326 |                  |
| T/G + G/ G | 282 (61.8)         | 279 (58.0)             | 1.173 (0.903–1.525)  | 0.231 | 1.180 (0.907–1.535)         | 0.217 |                  |
| MAFc     | 0.39                   | 0.37                   |                      |       |                             |       |                  |
| Squamous cell carcinoma |                  |                        |                      |       |                             |       |                  |
| T/T      | 139 (36.7)             | 202 (42.0)             | Referent             | –     | Referent                    | –     | 0.086            |
| T/G      | 169 (44.6)             | 204 (42.4)             | 1.204 (0.895–1.620)  | 0.221 | 1.212 (0.898–1.637)         | 0.208 |                  |
| G/G      | 71 (18.7)              | 75 (15.6)              | 1.376 (0.932–2.032)  | 0.108 | 1.174 (0.964–1.429)         | 0.110 |                  |
| T/G + G/ G | 240 (63.3)         | 279 (58.0)             | 1.250 (0.948–1.648)  | 0.113 | 1.257 (0.951–1.660)         | 0.107 |                  |
| MAFc     | 0.41                   | 0.37                   |                      |       |                             |       |                  |

$^a$ $\chi^2$ or Fisher exact test

$^b$ ORs were adjusted by age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status

$^c$ Minor allele frequency
other tumors, histological grade or tumor stage (Online Resource 2).

### 3.3 Stratified Analyses Between the MDM2 309 T>G and MDM2 285 G>C Genotypes and Cervical Cancer Risks

Stratified analyses did not reveal any association of the \( \text{MDM2} \, 309 \, \text{T} \rightarrow \text{G} \) genotypes with pregnancy, oral contraceptive use, tobacco smoking, or menopausal status in patient groups with SCC, adenocarcinoma, other tumors, different histological grades and tumor stage (data not shown).

In contrast, the stratified analysis for \( \text{MDM2} \, 285 \, \text{G} \rightarrow \text{C} \) revealed a protective role of this polymorphism among patients of all histological types with a positive history of pregnancy and oral contraceptive use, tobacco smoking, or menopausal status. The age-adjusted OR for women with a history of pregnancy possessing the C allele was 0.547 (95 % CI 0.318–0.881, \( P = 0.034 \)). The age-adjusted OR for women with a history of contraceptive use possessing the C allele was 0.541 (95 % CI 0.223–0.901, \( P = 0.034 \)). The age-adjusted OR for premenopausal women possessing the C allele was 0.362 (95 % CI 0.151–0.956, \( P = 0.018 \)). However, no significant association was observed between \( \text{MDM2} \, 285 \, \text{G} \rightarrow \text{C} \) and patients with a positive history of tobacco smoking.

We also found a protective role of the C allele against SCC in women with a positive history of oral contraceptive use (age-adjusted OR 0.413; 95 % CI 0.191–0.985, \( P = 0.021 \)) and in premenopausal women (age-adjusted OR 0.362; 95 % CI 0.137–0.928, \( P = 0.022 \)) (Table 5). However, we did not observe an association between \( \text{MDM2} \, 285 \, \text{G} \rightarrow \text{C} \) and patients with a positive history of smoking.

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### 3.4 Distribution of the MDM2 285 G>C and 309 T>G Combined Genotypes Among Patients with Cervical Cancer and Controls

The distribution and logistic regression analyses of the \( \text{MDM2} \, 285 \, \text{G} \rightarrow \text{C} \) and \( \text{MDM2} \, 309 \, \text{T} \rightarrow \text{G} \) combined genotypes in the patients and controls are presented in Table 6. Adjusting for the effect of age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status, the logistic regression analysis demonstrated that the 285GG/309GG versus the 285GG/309TT genotypes are significantly associated with all histological types of cervical cancer, with an adjusted OR 1.753 (95 % CI 1.136–2.703, \( P = 0.011 \)) (Table 6). Moreover, for all patients with cervical cancer, we observed a protective effect of the 285CC/309GG + 285GC/309GG versus the 285GG/309GG genotypes, with an adjusted OR 0.306 (95 % CI 0.142–0.660, \( P = 0.002 \)) (Table 6).
The reanalysis based on the histological type demonstrated that the 285GG/309GG versus the 285GG/309 TT genotype is a significant risk factor for SCC with an adjusted OR 1.890 (95 % CI 1.208–2.957, \( P = 0.005 \)) (Table 6). There was also a protective role of the 285CC/309GG versus the 285GG/309GG genotype in SCC with an adjusted OR 0.311 (95 % CI 0.141–0.689, \( P = 0.004 \)). There was no association between the \( MDM2 \) 285 G>C and 309 T>G combined genotypes with adenocarcinoma, other tumors, histological grade or tumor stage (Online Resource 3).

4 Discussion

\( MDM2 \) is considered an oncogene whose overexpression results in malignant transformations. The overexpression of \( MDM2 \) has been observed in various human malignancies,
including sarcoma, melanoma, breast carcinoma, glioblastoma leukemia and others [24]. Studies conducted in animal models and in cells in vitro have demonstrated that MDM2 also displays p53-independent oncogenic properties that regulate proliferation, apoptosis, tumor invasion and metastasis [25–29]. MDM2 protein levels and function are precisely controlled at the transcriptional, translational [30–33] and post-translational levels [34–40]. Therefore, various SNPs occurring in the MDM2 gene could potentially dysregulated both transcription and translation.

The MDM2 309 T>G SNP augments the binding of transcriptional factor Sp1 to the 309 G allele. This transition increases MDM2 protein levels by 2- to 4-fold and reduces p53 function [16].

In our study, we did not find a significant association between cervical cancer development or clinicopathological features and the MDM2 309 T>G SNP in our sample of the Polish population. Previous studies have shown that the MDM2 309 T>G polymorphism is not a risk factor for cervical cancer in northeastern Brazilian, Caucasian or African-American ethnicities [19, 20]. However, the MDM2 309 polymorphism has been shown to contribute to high-grade squamous intraepithelial lesions and HR-HPV-related cervical carcinogenesis in a Japanese population.

| Table 6 Prevalence of the MDM2 285G>C and 309T>G combined genotypes |
|--------------------------|--------------------------|--------------------------|--------------------------|
| SNP 285/309 MDM2 genotype | Patients (frequency %) | Controls (frequency %) | Odds ratio (95 % CI), P<sup>a</sup> | Adjusted odds ratio (95 % CI)<sup>d</sup>, P<sup>a</sup> |
| All | | | | |
| GG/TT | 174 (38.2) | 202 (42.0) | Referent | Referent |
| GG/TG | 191 (41.9) | 184 (38.3) | 1.205 (0.905–1.605), 0.202 | 1.296 (0.972–1.726), 0.076 |
| GC/TG | 13 (2.9) | 20 (4.2) | 0.755 (0.365–1.562), 0.447 | 0.834 (0.399–1.740), 0.627 |
| GG/GG | 65 (14.3) | 45 (9.4) | 1.677 (1.090–2.580), 0.018 | 1.753 (1.136–2.703), 0.011 |
| GC/GG | 12 (2.6) | 27 (5.6) | 0.503 (0.254–0.995), 0.045<sup>b</sup> | 0.438 (0.043–4.464), 0.485 |
| CC/GG | 1 (0.2) | 3 (0.6) | | |
| GC/TG + (GC + CC)/GG | 26 (5.7) | 50 (10.4) | 0.604 (0.361–1.011), 0.053<sup>c</sup> | 0.628 (0.373–1.059), 0.081 |
| CC/GG + GC/GG vs GG/GG | 13 (2.9) vs 65 (14.3) | 30 (6.2) vs 45 (9.4) | 0.300 (0.141–0.638), 0.001 | 0.306 (0.142–0.660), 0.002 |
| GC/TG vs GG/TG | 13 (2.9) vs 191 (41.9) | 20 (4.2) vs 184 (38.3) | 0.626 (0.303–1.296), 0.204 | 0.632 (0.304–1.316), 0.219 |

Squamous cell carcinoma

| GG/TT | 139 (36.7) | 202 (42.0) | Referent | Referent |
| GG/TG | 159 (41.9) | 184 (38.3) | 1.256 (0.928–1.700), 0.140 | 1.262 (0.930–1.714), 0.135 |
| GC/TG | 10 (2.6) | 20 (4.2) | 0.727 (0.330–1.600), 0.426 | 0.739 (0.333–1.641), 0.455 |
| GG/GG | 59 (15.6) | 45 (9.4) | 1.905 (1.222–2.971), 0.004 | 1.890 (1.208–2.957), 0.005 |
| GC/GG | 11 (2.9) | 27 (5.6) | 0.581 (0.288–1.175), 0.127 | 0.573 (0.280–1.171), 0.125 |
| CC/GG | 1 (0.3) | 3 (0.6) | | |
| GC/TG + (GC + CC)/GG | 22 (5.8) | 50 (10.4) | 0.639 (0.370–1.104), 0.107<sup>c</sup> | 0.643 (0.370–1.118), 0.116 |
| CC/GG + GC/GG vs GG/GG | 12 (3.2) vs 59 (15.6) | 30 (6.2) vs 45 (9.4) | 0.305 (0.141–0.662), 0.002 | 0.311 (0.141–0.689), 0.004 |
| GC/TG vs GG/TG | 10 (2.6) vs 159 (41.9) | 20 (4.2) vs 184 (38.3) | 0.579 (0.263–1.273), 0.169 | 0.578 (0.261–1.283), 0.177 |

Significant results are highlighted in bold font

<sup>a</sup> χ<sup>2</sup> test
<sup>b</sup> (GC/GG + CC/GG vs GG/TT)
<sup>c</sup> (GC/TG + GC/GG + CC/GG vs GG/TT)
<sup>d</sup> ORs were adjusted by age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status.
Singhal et al. (2013) found that the MDM2 309 SNP in an Indian population is associated with cervical neoplasia, HPV infection and age at the time of neoplasia diagnosis [41]. Amaral et al. (2014) suggested MDM2 309 as a marker for the progression from low to high squamous intraepithelial lesions in a northeastern Brazilian population. This group also demonstrated that oral contraceptives, HPV infections and the MDM2 309 SNP synergistically contributed to cervical lesions [42]. The MDM2 309 SNP has also been shown to be a biomarker of cervical neoplasia in non-smoking women and in those with a family history of cancer in a southeastern Brazilian population [48]. A recently published meta-analysis did not demonstrate a significant association of the MDM2 309 SNP with cervical cancer risk in the overall population. However, a stratification-based ethnicity study revealed that the MDM2 309 SNP is a significant risk factor for cervical cancer in Asian populations [22].

Our results demonstrate that the MDM2 285G>C polymorphism may protect against SCC development in a sample of the Polish population.

The MDM2 285 G>C polymorphism exists in complete linkage disequilibrium with MDM2 309 T>G [17]. Knappskog et al. (2011) demonstrated that the 285 G>C SNP in the MDM2 promoter region did not exist in Asian populations but is present in Caucasian populations with an allele frequency of approximately 8% [18]. Moreover, they found that the 285C/309G haplotype reduces the binding of Sp1 to the MDM2 promoter region and contributes to a reduced risk for breast and ovarian carcinomas in Caucasian populations [18]. Employing a plasmon resonance assay, Knappskog et al. (2011) demonstrated that the MDM2 309G allele increased the strength of binding to Sp1 by 22% compared to that observed with the MDM2 309T allele [18]. Additionally, the MDM2 285C allele led to a 51% decrease in the binding of Sp1 to the promoter region [18]. They showed that the MDM2 285C/309G haplotype, which is found in approximately 12% of all MDM2 309G alleles in Caucasians, displayed approximately 10% lower binding affinity to Sp1 than the MDM2 285G/309T haplotype. They also demonstrated that the MDM2 285G/309G haplotype exhibits the strongest binding between Sp1 and the MDM2 promoter region [44] and is likely responsible for the highest transcription rate of MDM2. These findings may partially explain the results of our study demonstrating that patients possessing the 285GG/309GG combined genotype exhibit an increased risk of SCC development. Moreover, the work of Knappskog et al. [18] is in agreement with our findings that indicate a protective role of the 285CC/309GG + 285GC/309GG combined genotype against the development of SCC.

Our study also demonstrated that the MDM2 285 G>C polymorphism may protect women who have used oral contraceptives and women of premenopausal age from SCC. These results are in agreement with previously published data suggesting a possible causative role of contraceptive use and menopausal status in cervical cancer development [6, 7] as well as a protective role of the MDM2 285G>C SNP in some female estrogen-related cancers [17, 18]. Oral contraceptives are used by premenopausal women and may affect the increase in MDM2 expression, which can be further reduced by the MDM2 285C gene variant. It is notable that transcription factor Sp1 binds cooperatively with an estrogen receptor, and the 285 G>C transition is situated within the estrogen receptor binding site [45, 46]. It should also be noted that cervical cancer has also been recognized as an estrogen-affected malignancy [47, 48].

Moreover, Renaux-Petel et al. (2014) recently found that Li-Fraumeni syndrome patients possessing the 285G/309G haplotype display an onset of tumors 5 years earlier compared to patients possessing other haplotypes [49].

Our genetic assessment is the first to demonstrate that the MDM2 285 G>C polymorphism and the 285CC/309GG + 285GC/309GG combined genotype may protect against SCC and that the 285GG/309GG combined genotype may increase the risk of SCC in Caucasian populations. However, this study is characterized by low statistical power for using these gene variants as a major predictor factor of cervical cancer development in clinical practice. Therefore, this study should be replicated in other larger independent cohorts.

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Compliance with Ethical Standards

Conflict of interest RA, MM, SA and JPP have no conflict of interest to report.

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Ethical Approval and Informed Consent The study procedures were approved by the local Ethical Committee of the Poznan University of Medical Sciences. Informed consent was obtained from all participating individuals.

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