Mol Diagn Ther (2015) 19:235–244
DOI 10.1007/s40291-015-0153-4

ORIGINAL RESEARCH ARTICLE

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

Andrzej Roszak[1,2] · Matthew Misztal[3] · Anna Sowińska[4] · Pawel P. Jagodziński[3]

Published online: 30 July 2015

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 ($P_{trend} = 0.016$) 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.

Electronic supplementary material The online version of this article (doi:10.1007/s40291-015-0153-4) contains supplementary material, which is available to authorized users.

✉ Pawel P. Jagodziński
pjagodzi@am.poznan.pl

[1] Department of Radiotherapy and Gynecological Oncology, Greater Poland Cancer Center, Poznan, Poland

[2] Department of Electroradiology, Poznan University of Medical Sciences, Poznan, Poland

[3] Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, 6 Święcickiego St., 60-781 Poznan, Poland

[4] Department of Computer Science and Statistics, Poznan University of Medical Sciences, Poznan, Poland

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 SNP309 [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′-GAGCGGTCACTTTTGGGTCT-3′ and 5-CGGAACGTGTCTGAACTTGAC-3′. The PCR-amplified *MDM2* fragment, which is 437 bp in length, was digested using the endonuclease MspA1I (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 FauI 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

Table 1 Clinical and demographic characteristics of patients and controls

Characteristic	Patients ($n = 456$)	Controls ($n = 481$)
Mean age (years) ± SD[a]	48.3 ± 10.8	47.8 ± 9.5
Tumor stage		
IA	62 (13.6 %)	
IB	63 (13.8 %)	
IIA	61 (13.4 %)	
IIB	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® HPV Test Roche Molecular Systems, Inc., (Alameda, CA, USA)

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 χ^2 test. The polymorphism was tested for association with cervical cancer incidence using the Cochran-Armitage *P* trend test (P_{trend}). The χ^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. A

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>G (rs2279744) Polymorphism Among Patients with Cervical Cancer and Controls

The values for the χ^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>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>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>G transition for the cervical cancer patients (Table 2). Furthermore, we did not observe an association of the *MDM2* 309 T>G polymorphism 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>C (rs117039649) Polymorphism Among Patients with Cervical Cancer and Controls

The values for the χ^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>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>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>C SNP with SCC (Table 3). In patients with SCC, the p-trend value calculated for the *MDM2* 285 G>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>C polymorphism with adenocarcinoma,

Table 2 Prevalence of the *MDM2* 309T>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)[b]	P^a	P_{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	
MAF[c]	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	
MAF[c]	0.41	0.37					

[a] χ^2 or Fisher exact test

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

[c] Minor allele frequency

Table 3 Prevalence of the *MDM2* 285 G>C (rs117039649) polymorphism among all patients with cervical cancer, SCC and controls

Genotype	Patients (frequency %)	Controls (frequency %)	Odds ratio (95 % CI)	P^a	Adjusted odds ratio (95 % CI)[b]	P^a	P_{trend}
All							
G/G	430 (94.3)	431 (89.6)	**Referent**	–	**Referent**	–	**0.008**
G/C	25 (5.5)	47 (9.8)	**0.533 (0.322–0.882)**	**0.013**	**0.540 (0.326–0.896)**	**0.017**	
C/C	1 (0.2)	3 (0.6)	0.334 (0.035–3.226)	0.624	0.536 (0.171–1.688)	0.286	
G/C + C/C	26 (5.7)	50 (10.4)	**0.521 (0.319–0.853)**	**0.009**	**0.523 (0.319–0.858)**	**0.010**	
MAF[c]	0.03	0.06					
Squamous cell carcinoma							
G/G	357 (94.2)	431 (89.6)	**Referent**	–	**Referent**	–	**0.016**
G/C	21 (5.5)	47 (9.8)	**0.539 (0.316–0.920)**	**0.022**	0.552 (0.322–1.044)	0.048	
C/C	1 (0.3)	3 (0.6)	0.402 (0.042–3.888)	0.631	0.563 (0.176–1.799)	0.332	
G/C + C/C	22 (5.8)	50 (10.4)	**0.531 (0.316–0.894)**	**0.016**	**0.536 (0.317–0.905)**	**0.019**	
MAF[c]	0.03	0.06					

Significant results are highlighted in bold font

[a] χ^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 *MDM2* 309 T>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 *MDM2* 285 G>C revealed a protective role of this polymorphism among patients of all histological types with a positive history of pregnancy and oral contraceptive use and among women of premenopausal age (Table 4). 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, $P = 0.022$). 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 *MDM2* 285 G>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 *MDM2* 285 G>C SNP and pregnancy, oral contraceptive use, tobacco smoking, or menopausal status in patients with adenocarcinoma and other tumors (data not shown). Moreover, there was no association of either the *MDM2* 285 G>C or 309 T>G polymorphism with HPV genotypes (data not shown).

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 *MDM2* 285 G>C and 309 T>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/309 TT 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).

Table 4 Stratified analyses between the distribution of the *MDM2* 285G>C (rs117039649) genotypes and all histological types of cervical cancer risks: pregnancy, oral contraceptive use, tobacco smoking, and menopausal status

High risk exposure	Patients			Controls			Adjusted allelic odds ratio (95 % CI)[a]	P^b
Genotype	G/G	G/C	C/C	G/G	G/C	C/C		
Pregnancy								
Ever	383	22	0	387	42	1	**0.547 (0.318–0.881)**	**0.034**
Never	47	3	1	44	5	2	0.574 (0.191–1.757)	0.518
Oral contraceptive use								
Ever	198	11	0	189	21	2	**0.541 (0.223–0.901)**	**0.022**
Never	232	14	1	242	26	1	0.671 (0.367–1.161)	0.161
Smoking								
Ever	155	8	0	133	12	2	0.464 (0.196–1.068)	0.066
Never	275	17	1	298	35	1	0.583 (0.364–0.995)	0.061
Menopausal status								
Premenopausal	155	7	0	160	17	2	**0.363 (0.151–0.956)**	**0.018**
Postmenopausal	275	18	1	271	30	1	0.659 (0.369–1.216)	0.237

Significant results are highlighted in bold font

[a] Odds Ratio were adjusted by age

[b] Chi square analysis

Table 5 Stratified analyses between the distribution of the *MDM2* 285G>C (rs117039649) genotypes and squamous cell carcinoma risk: pregnancy, oral contraceptive use, tobacco smoking, and menopausal status

High risk exposure	Patients			Controls			Adjusted allelic odds ratio (95 % CI)[a]	P^b
Genotype	G/G	G/C	C/C	G/G	G/C	C/C		
Pregnancy								
Ever	318	20	0	387	42	1	0.584 (0.361–0.988)	0.052
Never	39	1	1	44	5	2	0.401 (0.124–1.461)	0.273
Oral contraceptive use								
Ever	174	9	0	189	21	2	**0.413 (0.191–0.985)**	**0.021**
Never	183	12	1	242	26	1	0.784 (0.372–1.257)	0.287
Smoking								
Ever	138	7	0	133	12	2	0.441 (0.191–1.121)	0.071
Never	219	14	1	298	35	1	0.713 (0.345–1.122)	0.109
Menopausal status								
Premenopausal	126	5	0	160	17	2	**0.362 (0.137–0.928)**	**0.022**
Postmenopausal	231	16	1	271	30	1	0.696 (0.383–1.274)	0.201

Significant results are highlighted in bold font

[a] Odds Ratio were adjusted by age

[b] Chi square analysis

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 + 285GC/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,

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^a	Adjusted odds ratio (95 % CI)[d], P^a
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**[b]	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[c]	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[c]	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

[a] χ^2 test

[b] (GC/GG + CC/GG vs GG/TT)

[c] (GC/TG + GC/GG + CC/GG vs GG/TT)

[d] ORs were adjusted by age, pregnancy, oral contraceptive use, tobacco smoking, and menopausal status

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

[21]. 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 [43]. 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.

Acknowledgments We gratefully acknowledge the technical assistance of Ms. Agnieszka Mikuczewska.

Compliance with Ethical Standards

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

Funding This study, presented by RA, MM, SA and JPP, was funded by Grant No. 502-01-01124182-07474 from Poznan University of Medical Sciences.

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.

References

1. Jemal ADVM, Bray F, Center MM, Ferley J, Ward E, Forman D. Global cancer statistic. CA Cancer J Clin. 2011;61:69–90.
2. Zur Hausen H. Papillomavirus infections—a major cause of human cancers. Biochim Biophys Acta. 1996;1288:F55–78.
3. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189:12–9.
4. Villa LL. Human papillomaviruses and cervical cancer. Adv Cancer Res. 1997;71:321–41.
5. de Freitas AC, Gurgel AP, Chagas BS, Coimbra EC, do Amaral CM. Susceptibility to cervical cancer: an overview. Gynecol Oncol. 2012;126:304–11.
6. Castellsague X, Munoz N. Cofactors in human papillomavirus carcinogenesis—role of parity, oral contraceptives, and tobacco smoking, Chapter 3. J Natl Cancer Inst Monogr. 2003;20–8.
7. Moreno V, Bosch FX, Muñoz N, Meijer CJ, Shah KV, Walboomers JM, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case–control study. Lancet. 2002;359:1085–92.
8. Magnusson PK, Lichtenstein P, Gyllensten UB. Heritability of cervical tumours. Int J Cancer. 2000;88:698–701.
9. Wei L, Griego AM, Chu M, Ozbun MA. Tobacco exposure results in increased E6 and E7 oncogene expression, DNA damage and mutation rates in cells maintaining episomal human papillomavirus 16 genomes. Carcinogenesis. 2014;35:2373–81.
10. Münger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res. 2002;89:213–28.
11. Williams VM, Filippova M, Filippov V, Payne KJ, Duerksen-Hughes P. Human papillomavirus type 16 E6* induces oxidative stress and DNA damage. J Virol. 2014;88:6751–61.
12. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell. 1992;69:1237–45.
13. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature. 1992;358:80–3.
14. Zhao Y, Yu H, Hu W. The regulation of MDM2 oncogene and its impact on human cancers. Acta Biochim Biophys Sin (Shanghai). 2014;46:180–9.
15. Eischen CM, Lozano G. The Mdm network and its regulation of p53 activities: a rheostat of cancer risk. Hum Mutat. 2014;35:728–37.
16. Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, et al. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell. 2004;119:591–602.
17. Paulin FE, O'Neill M, McGregor G, Cassidy A, Ashfield A, Ali CW, et al. MDM2 SNP309 is associated with high grade node positive breast tumours and is in linkage disequilibrium with a novel MDM2 intron 1 polymorphism. BMC Cancer. 2008;8:281.
18. Knappskog S, Bjornslett M, Myklebust LM, Huijts PE, Vreeswijk MP, Edvardsen H, et al. The MDM2 Promoter SNP285C/309G Haplotype Diminishes Sp1 Transcription Factor Binding and Reduces Risk for Breast and Ovarian Cancer in Caucasians. Cancer Cell. 2011;19:273–82.
19. Meissner Rde V, Barbosa RN, Fernandes JV, Galvão TM, Galvão AF, Oliveira GH. No association between SNP309 promoter polymorphism in the MDM2 and cervical cancer in a study from northeastern Brazil. Cancer Detect Prev. 2007;31:371–4.
20. Hu X, Zhang Z, Ma D, Huettner PC, Massad LS, Nguyen L, et al. TP53, MDM2, NQO1, and susceptibility to cervical cancer. Cancer Epidemiol Biomarkers Prev. 2010;19:755–61.
21. Nunobiki O, Ueda M, Yamamoto M, Toji E, Sato N, Izuma S, et al. MDM2 SNP 309 human papillomavirus infection in cervical carcinogenesis. Gynecol Oncol. 2010;118:258–61.
22. Zhuo X, Ren J, Li D, Wu Y, Zhou Q. MDM2 SNP309 variation increases cervical cancer risk among Asians. Tumour Biol. 2014;35:5331–7.
23. Knappskog S, Lønning PE. MDM2 SNP309 and risk of cervical cancer. Tumour Biol. 2014;35:6185–6.
24. Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. Nucleic Acids Res. 1998;26:3453–9.
25. Xiao ZX, Chen J, Levine AJ, Modjtahedi N, Xing J, Sellers WR, et al. Interaction between the retinoblastoma protein and the oncoprotein MDM2. Nature. 1995;375:694–8.
26. Yang JY, Zong CS, Xia W, Yamaguchi H, Ding Q, Xie X, et al. ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. Nat Cell Biol. 2008;10:138–48.
27. Fu W, Ma Q, Chen L, Li P, Zhang M, Ramamoorthy S, et al. MDM2 acts downstream of p53 as an E3 ligase to promote FOXO ubiquitination and degradation. J Biol Chem. 2009;284:13987–4000.
28. Yang JY, Zong CS, Xia W, Wei Y, Ali-Seyed M, Li Z, et al. MDM2 promotes cell motility and invasiveness by regulating E-cadherin degradation. Mol Cell Biol. 2006;26:7269–82.
29. Huart AS, MacLaine NJ, Meek DW, Hupp TR. CK b1alpha plays a central role in mediating MDM2 control of p53 and E2F-1 protein stability. J Biol Chem. 2009;284:32384–94.
30. Mendrysa SM, Perry ME. The p53 tumor suppressor protein does not regulate expression of its own inhibitor, MDM2, except under conditions of stress. Mol Cell Biol. 2000;20:2023–30.
31. Zhang J, Sun Q, Zhang Z, Ge S, Han ZG, Chen WT. Loss of microRNA-143/145 disturbs cellular growth and apoptosis of human epithelial cancers by impairing the MDM2-p53 feedback loop. Oncogene. 2013;32:61–9.
32. Suh SS, Yoo JY, Nuovo GJ, Jeon YJ, Kim S, Lee TJ, et al. MicroRNAs/TP53 feedback circuitry in glioblastoma multiforme. Proc Natl Acad Sci USA. 2012;109:5316–21.
33. Xiao J, Lin H, Luo X, Wang Z. miR-605 joins p53 network to form a p53:miR-605:mdm2 positive feedback loop in response to stress. EMBO J. 2011;30:524–32.
34. Maya R, Balass M, Kim ST, Shkedy D, Leal JF, Shifman O, et al. ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage. Genes Dev. 2001;15:1067–77.
35. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, Shieh SY, et al. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev. 1999;13:152–7.
36. Chen L, Li Z, Lane WS, Chen J, Shinozaki T, Nota A, et al. Functional role of Mdm2 phosphorylation by ATR in attenuation of p53 nuclear export. Oncogene. 2003;22:8870–80.
37. Lohrum MA, Ashcroft M, Kubbutat MH, Vousden KH. ATM activates p53 by regulating MDM2 oligomerization and E3 processivity. EMBO J. 2009;28:3857–67.
38. Ashcroft M, Kubbutat MH, Vousden KH. Identification of a cryptic nucleolar-localization signal in MDM2. Nat Cell Biol. 2000;2:179–81.
39. Gajjar M, Candeias MM, Malbert-Colas L, Mazars A, Fujita J, Olivares-Illana V, et al. The p53 mRNA-Mdm2 interaction controls Mdm2 nuclear trafficking and is required for p53 activation following DNA damage. Cancer Cell. 2012;21:25–35.
40. Goldberg Z, Vogt Sionov R, Berger M, Zwang Y, Perets R, Van Etten RA, et al. Tyrosine phosphorylation of Mdm2 by c-Abl: implications for p53 regulation. EMBO J. 2002;21:3715–27.
41. Singhal P, Hussain S, Thakur N, Batra S, Salhan S, Bhambani S, et al. Association of MDM2 and p53 polymorphisms with the advancement of cervical carcinoma. DNA Cell Biol. 2013;32:19–27.

42. Amaral CM, Cetkovská K, Gurgel AP, Cardoso MV, Chagas BS, Paiva Júnior SS, et al. MDM2 polymorphism associated with the development of cervical lesions in women infected with Human papillomavirus and using of oral contraceptives. Infect Agent Cancer. 2014;9:24.
43. Vargas-Torres SL, Portari EA, Klumb EM, Guillobel HC, Camargo MJ, Russomano FB, et al. Effects of MDM2 promoter polymorphisms on the development of cervical neoplasia in a Southeastern Brazilian population. Biomarkers. 2014;19:637–45.
44. Knappskog S, Lønning PE. Effects of the MDM2 promoter SNP285 and SNP309 on Sp1 transcription factor binding and cancer risk. Transcription. 2011;2:207–10.
45. Neo SJ, Su X, Thomsen JS. Surface plasmon resonance study of cooperative interactions of estrogen receptor alpha and transcriptional factor Sp1 with composite DNA elements. Anal Chem. 2009;81:3344–9.
46. Knappskog S, Trovik J, Marcickiewicz J, Tingulstad S, Staff AC, MoMaTEC study group, et al. SNP285C modulates oestrogen receptor/Sp1 binding to the MDM2 promoter and reduces the risk of endometrial but not prostatic cancer. Eur J Cancer. 2012;48:1988–96.
47. Son J, Park JW, Lambert PF, Chung SH. Requirement of estrogen receptor alpha DNA-binding domain for HPV oncogene-induced cervical carcinogenesis in mice. Carcinogenesis. 2014;35:489–96.
48. Cortés-Malagón EM, Bonilla-Delgado J, Díaz-Chávez J, Hidalgo-Miranda A, Romero-Cordoba S, Uren A, et al. Gene expression profile regulated by the HPV16 E7 oncoprotein and estradiol in cervical tissue. Virology. 2013;447:155–65.
49. Renaux-Petel M, Sesboüé R, Baert-Desurmont S, Vasseur S, Fourneaux S, Bessenay E, et al. The MDM2 285G-309G haplotype is associated with an earlier age of tumour onset in patients with Li-Fraumeni syndrome. Fam Cancer. 2014;13:127–30.