Polymorphisms in TP53 and MDM2 combined are associated with high grade endometrial cancer

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Keywords: TP53, MDM2, Endometrial Cancer, Polymorphisms

Abstract

Objectives: Determinants of endometrial cancer grade have not been precisely defined, however, cell cycle control is considered to be integrally involved in endometrial cancer development. TP53 and MDM2 are essential components for cell cycle arrest and apoptosis. Polymorphisms in these genes cause TP53 inactivation and MDM2 over-expression, leading to accumulation of genetic errors.

Methods: One polymorphism in MDM2, rs2279744 (SNP309) and three polymorphisms in TP53 rs1042522 (R72P), rs17878362 and rs1625895 were genotyped in 191 endometrial cancer cases and 291 controls using PCR-based fragment analysis, RFLP analysis and real-time PCR.

Results: The results showed no associations of the three TP53 polymorphisms and MDM2 SNP309 alone or in combination with endometrial cancer risk. However, the combination of MDM2 SNP309 and the three TP53 polymorphisms was significantly associated with a higher grade of endometrial cancer (Wild-type genotypes versus variant genotypes: OR 4.15, 95% CI 1.82-9.46, p=0.0003). Analysis of family history of breast cancer revealed that the variant genotypes of the three TP53 polymorphisms were significantly related to a higher frequency of family members with breast cancer in comparison to endometrial cancer cases without a family history of breast cancer (Wild-type genotypes versus variant genotypes: OR 2.78, 95% CI 1.36-5.67, p=0.004).

Conclusions: The combination of the MDM2 SNP309 and the three TP53 polymorphisms appear to be related to a higher grade of endometrial cancer. The association of the endometrial cancer cases with family history of breast cancer and the three TP53 polymorphisms suggests that this constellation of malignancies may represent a low-risk familial cancer grouping.

Introduction

Endometrial cancer is a common malignancy in the industrialised world. Many studies have identified environmental factors associated with endometrial cancer, yet the interaction between environmental and genetic factors remains poorly defined. Only a minority of endometrial cancer cases have been shown to be a result of an inherited condition, hereditary non-polyposis colorectal cancer (HNPCC) [1]. In the setting of HNPCC, endometrial cancer and colorectal cancer are the most common epithelial malignancies in women with this syndrome at 54% and 52%, respectively [2]. The underlying genetic defect of HNPCC is an autosomal dominantly inherited germline mutation in any one of four DNA mismatch repair (MMR) genes, hMLH1, hMSH2, hMSH6 and PMS2. Other heritable causes of endometrial cancer remain to be elucidated however given that other highly penetrant genes have not been identified for endometrial cancer; it is likely that a number of low penetrant genes with additive effects relate to disease risk. Since the completion of the Human Genome Project, a large number of polymorphisms have been identified and several have been associated with changes in cell cycle control.

One important pathway for the maintenance of genomic integrity involves the TP53 tumour suppressor gene and its negative regulator, mouse double-minute 2 homologue (MDM2). TP53 activation is induced in response to kinase signalling pathways that recognises DNA damage and it functions to regulate expression of genes involved in cell cycle arrest, apoptosis, DNA repair and angiogenesis to prevent the accumulation of genetic errors [3-5]. MDM2 has the ability to inactivate the function of TP53 through ubiquitinization and degradation, by direct binding to the protein [6, 7]. MDM2 over-expression has been associated with many types of cancer where it has been shown to be involved in the inactivation of wild-type TP53 thereby obliterating cell cycle checkpoint control [8]. Specifically for endometrial cancer, a direct relationship has been observed between increasing proliferation and progressive derailment of TP53 and MDM2 [9, 10].

Numerous polymorphisms have been reported in TP53; however three appear to have functional effects that have been related to a change in malignant potential. These polymorphisms are R72P, a 16bp insertion in intron 3 and a G>A polymorphism in intron 6.

Wu et al. (2002) performed functional studies on cell lines expressing at least one variant allele of the three polymorphisms and found that the ability of TP53 to regulate DNA repair processes was significantly reduced [11]. The TP53 Arg (72R) allele is more prone to human papillomavirus oncoprotein (E6) mediated degradation than the Pro (72P) allele [12]. Furthermore, the R72P polymorphism has been shown to alter the efficiency of p73, which is a TP53 homolog and transcription factor that responds to DNA damage and initiates apoptotic signalling pathways [13, 14]. A polymorphism located in the promoter region of MDM2, SNP309, results in increased MDM2 levels and reduces the activity of TP53 [15]. Since TP53 and MDM2 are central components in the maintenance of genomic integrity, these polymorphisms may be associated with endometrial cancer.

The TP53 R72P polymorphism has been studied in a number of cancers but association studies involving this polymorphism and endometrial cancer risk have shown varying results (reviewed in [16]). The R72P polymorphism has been suggested to be in linkage disequilibrium with the 16bp insertion in intron 3 and the G>A polymorphism in intron 6. Ueda et al. (2006) studied the relationship between the R72P polymorphism and the risk of developing endometrial cancer and found an increased risk of disease in patients harbouring the Arg/Arg genotype compared to those with combined Arg/Pro and Pro/Pro genotypes [17]. Conversely, a study by Roh et al. (2004) found an increased risk of endometrial cancer in carriers of the Pro allele [18]. Both studies were conducted among Asian populations (Japanese and South Korean). Due to their small sizes (108 cases, 95 controls; 95 cases, 285 controls) the statistical power of these studies however was weak. Moreover, three studies reported no associations [19-21].

Furthermore, a study by Saffari et al. (2005) examined the relationship between twelve TP53 genetic alterations including the R72P polymorphism in 59 endometriod carcinomas and lower overall survival and responsiveness to adjuvant radiotherapy [22]. The R72P polymorphism was identified in seven of the twelve variants identified and women carrying the Arg/Pro genotype had a lower overall survival than those with the wild type Arg/Arg genotype. Additionally, women harbouring the Arg/Pro genotype who did not receive adjuvant radiation therapy had a significantly lower survival rate than those with the Arg/Pro genotype whom received treatment. Treated women with the Arg/Pro genotype had a similar

survival rate to those women with the wild-type Arg/Arg genotype. These results suggested that women with the TP53 Arg/Pro genotype have an altered response to radiation induced DNA damage.

Recently, two reports have studied the association of the MDM2 SNP309 T>G polymorphism and the risk of developing endometrial cancer. The first report published by Walsh et al. (2007) found that women with the GG genotype were at a greater risk of developing endometrial cancer (OR 2.76, 95%CI (1.06-7.20), p=0.03) compared to those carrying the TT and TG genotypes [23]. This study however was relatively small (n=73 cases, n=79 controls), which potentially could result in a lack of power to detect true associations. A more recent Caucasian study on larger cohorts (Nurses Health Study: n=454 cases, n=1132 controls; Women's Health Study: n=137 cases, n=411 controls) provided further evidence for an increased risk of disease in GG carriers compared to TT carriers (OR 1.87, 95%CI 1.29-2.73 for the pooled analysis) [24].

This study is specifically interested in the relationship between endometrial cancer risk and polymorphisms in TP53 and MDM2. Additionally, we evaluated whether there was evidence for higher grade of endometrial carcinoma with polymorphisms in these genes in 191 endometrial cancer patients and 291 controls.

Materials and Methods

Study Population

This study initially consisted of 213 consecutively recruited women with histologically confirmed endometrial cancer who presented for treatment at the Hunter Centre for Gynaecological Cancer, John Hunter Hospital, Newcastle, New South Wales, Australia between the years 1992 and 2005. Women that had additionally been diagnosed with breast cancer were excluded from this study.

The final analysis included 191 endometrial cancer patients. Data on reproductive and environmental risk factors including ethnicity, body mass index (BMI), diabetes, high blood pressure (HBP), age of diagnosis of endometrial cancer, age of menarche, age of menopause, other personal cancer history, family cancer history (Family history of cancer was defined as cancer in the index patient plus one or more 1st or 2nd degree relatives diagnosed with cancer), parity, breastfeeding, oral contraceptive use, chemotherapy, radiotherapy, hormone replacement therapy (HRT), smoking and alcohol use was collected using self reported questionnaires. Information regarding recurrence, stage, grade and histology of endometrial cancer was collected from the medical records, see table 1.

The control population consisted of 291 women that were recruited between the years 2004 and 2005 for the Hunter Community Study. This study aims to identify genetic and environmental factors associated with ageing in a cohort of individuals obtained from the Hunter region, Newcastle, New South Wales, Australia. Any control that had a prior diagnosis of either breast or endometrial cancer was excluded from the study. Controls were matched to cases by sex and age.

All participants provided informed written consent prior to participation in this study. Ethics approval was obtained from the Human Research Ethics Committee, University of Newcastle and the Hunter Area Research Ethics Committee, Hunter New England Health Service, Newcastle, New South Wales, Australia.

DNA Isolation

Genomic DNA was extracted from 10ml EDTA blood using the “salting-out” method [25].

Molecular Analysis

Two polymorphisms, MDM2 SNP309 (rs2279744) and TP53 R72P (rs1042522) were genotyped using the 5’ nuclease assay (TaqMan®) and allelic discrimination was performed on an ABI PRISM 7500 Real-Time PCR System (PE Applied Biosystems, Foster City, CA). Assay-by-Design℠, a service offered by Applied Biosystems (PE Applied Biosystems), was used to design primers and probes. The methods for genotyping of the TP53 R72P and MDM2 SNP309 polymorphisms were previously described [26]. For MDM2 SNP309 genotyping the following primers and probes were used: Forward Primer: 5´-CGGGAGTTCAGGGTAAAGGT 3´, Reverse Primer 5´-ACAGGCACCTGCGATCATC-3´, Wild Type Reporter 5´-CTCCCGCGCCGAAG-3´–VIC, Mutant Reporter 5´-TCCCGCGCCGCAG-3´FAM (designed on the reverse strand). Briefly, the assay functioned under universal conditions with each reaction containing: 50ng DNA, 0.125µl 40x Assay Mix and 2.5µl TaqMan® Universal PCR master mix made up to 5µl with sterile water. The thermal cycling conditions were 50°C for 2 min, 95°C for 10 min, and 50 cycles of 92°C for 15 sec and 60°C for 1 min. Post PCR, the plate was scanned to allow discrimination between the different genotypes.

The TP53 16bp insertion in intron 3 (rs17878362) and the G>A polymorphism in intron 6 (rs1625895) were genotyped by PCR-based fragment and restriction fragment length polymorphism (RFLP) analyses. The primers used for genotyping these polymorphisms are previously described [27, 28]. Experimental conditions are may be made available on request. The genotyping results were confirmed by a second laboratory research assistant and 5% of the samples were re-genotyped with 100% concordance. Any sample where a genotype could not be accurately assessed was re-genotyped. If it failed a second time, it was discarded from the analysis. The overall call rates were in the range from 99.5-100%.

Statistical Analysis

Power calculations were performed using Quanto (Version 1.2.3, May 2007, http://hydra.usc.edu/GxE). The calculations performed showed that the number of cases and controls (ratio 1:1.52) in our cohort was large enough to detect a significant, $p<0.05$, 2-fold increased risk (OR>2.0), with 80% power, assuming a dominant genetic model, with a minor allele frequency of 6.5%. The minor allele frequencies for the TP53 R72P, intron 3 16bp insertion and intron 6 G>A polymorphism are 35.2%, 6.5% and 6.5%, respectively. There is no minor allele frequency information available for MDM2 SNP309. Therefore, our study has a large enough sample size to statistically demonstrate that significant OR values over 2.0 provide a statistically robust result. For each polymorphism, Hardy-Weinberg Equilibrium (HWE) was calculated in the control groups to check for compliance using the Institute for Human Genetics, statistics website, http://ihg.gsf.de/ihg/polymorphisms.html (Munich, Germany). To determine differences in genotype frequencies and environmental and reproductive risk factors between the cases and controls, chi-squared (χ^2) statistics and odds ratios (ORs) and 95% confidence intervals (CI) were calculated using unconditional logistic regression. Multivariate unconditional logistic regression was performed to determine if any risk factors altered the significance of the genotype frequency results. The risk factors taken into account were: age (continuous), BMI ($<25kg/m^2$ and $\geq 25kg/m^2$), diabetes (yes/no), HBP (yes/no), HRT (yes/no), personal history of cancer (yes/no), smoking (ever/never) and alcohol consumption (ever/never). Other risk factors such as age of menopause were not included in the analysis since this information was not available for the controls.

The genotype frequencies of all polymorphisms were compared in the case group stratified for the environmental and reproductive risk factors by using chi-squared (χ^2) analysis and ORs and 95% CI were calculated using unconditional logistic regression. For the environmental and reproductive risk factors that were significantly different between the subgroups, risk of apoptotic ability was calculated by combining the polymorphisms. If an individual was carrying no variant allele, the person was classified as low risk of reduced apoptotic ability, whereas one variant allele was evaluated as medium risk and two or more variant alleles as high risk.

T-tests were used to determine differences in the age of diagnosis of endometrial cancer by genotype. Kaplan Meier survival analysis was used to plot the cumulative survival versus the patient's age of diagnosis of endometrial cancer. By comparing the Kaplan-Meier survival curves for each genotype, we tested if there were differences in the age of diagnosis of endometrial cancer by genotype. The Wilcoxon's test was used to determine the significance of observations from early ages of diagnosis, log-rank test, which gives more weight to later ages and Tarone-Ware test, which is an intermediate of the two other tests were used to examine the homogeneity of the survival curves. The polymorphisms that showed a statistically significant difference between the genotypes and the age of diagnosis of endometrial cancer for all three statistical tests were further examined by a multivariate Cox regression model where a number of specific risk factors were incorporated into the analysis.

Haplotypes were estimated using SIMHAP [29]. Linkage disequilibrium (LD) was tested applying Lewontin's D' statistic using the pwld function in STATA. Associations of single haplotypes and combinations of haplotypes with endometrial cancer risk were performed using SIMHAP.

The significance levels of all tests were set at $p<0.05$ and were two-sided. All statistical analysis was performed with SIMHAP (Laboratory for Genetic Epidemiology, Western Australian Institute for Medical Research, Australia), Intercooled STATA 8.2 (Stata Corp., College Station, TX, USA), SPSS Version 15 (SPSS Inc. Chicago, IL, USA) and GraphPad Instat version 3.06 (GraphPad Software, San Diego, CA, USA).

Results

Cases and controls were different with respect to potential endometrial cancer risk factors, including HBP, diabetes, HRT, alcohol consumption, personal history of any cancer, personal history of ovarian cancer, cervical cancer and other cancers. The characteristics of the cases and controls are shown in table 2.

The distributions of the genotypes of all three TP53 polymorphisms and MDM2 SNP309 among the controls did not deviate from HWE. The three TP53 polymorphisms were in high LD (D' values; R72P + intron 3 insertion 16bp = 0.83, R72P + intron 6 G>A polymorphism = 0.96 and intron 3 insertion 16bp + intron 6 G>A polymorphism = 0.96).

The genotype frequencies were compared between the cases and controls for the three TP53 polymorphisms and MDM2 SNP309: No significant differences were observed (see table 3).

This analysis focused on all four polymorphisms in combination with known environmental/reproductive confounders reported for the cases. When analysed separately, the variant genotypes for MDM2 SNP309 and TP53 R72P were significantly associated with a higher grade of cancer (p=0.002 and p=0.032, respectively) compared to the wild-type genotype. However, the variant genotypes for the TP53 intron 3 16bp insertion and the intron 6 G>A polymorphism were not significantly associated with a high grade of cancer (p=0.067 and p=0.260, respectively), see table 4. To assess reduced apoptotic ability in association with grade of cancer, MDM2 SNP309 and TP53 R72P were combined. Additionally, since the three TP53 polymorphisms are in high linkage disequilibrium, they were also included in a combined analysis with MDM2 SNP309. For the combination of MDM2 SNP309 and TP53 R72P, the results revealed that patients with medium or high risk genotype combinations compared to the low risk genotype had an increased risk of being diagnosed with a higher grade of endometrial carcinoma (data not shown). The greatest effect was seen for the combination of all four polymorphisms, see table 5.

The three TP53 SNPs, R72P, intron 3 16bp insertion and intron 6 G>A were analysed separately and revealed that the variant genotypes were associated with a higher frequency of family members with breast cancer (p=0.001, p=0.018 and p=0.044, respectively), see

table 6. For the combination of the three TP53 variants, patients with medium or high risk genotypes had a greater frequency of family members with breast cancer in comparison to those with the low risk genotypes (see table 7). When MDM2 SNP309 was combined into this analysis, the results were no longer statistically significant (data not shown).

Kaplan-Meier survival analysis and T-tests were used to evaluate the influence of the TP53 and MDM2 polymorphisms on the age of diagnosis of endometrial cancer. No significant differences were observed (data not shown).

Haplotype frequencies were estimated for the three TP53 polymorphisms and MDM2 SNP309. No significant differences were found for the combination of the three TP53 polymorphisms or the incorporation of MDM2 SNP309 with the three TP53 polymorphisms (data not shown).

Discussion

Studies involving the elucidation of genetic variants in cancer have provided a greater understanding of individual disease-risk differences. This study has examined the association of reduced apoptotic ability as a result of a combination of polymorphisms in TP53 and MDM2 with endometrial cancer risk.

Five reports have been previously published for the TP53 R72P polymorphism and endometrial cancer risk. One study found an increased risk of endometrial cancer with the Arg/Arg genotype compared to the Arg/Pro and Pro/Pro genotypes [17] however another study reported the opposite relationship. They found an increased risk of endometrial cancer development and the Pro allele [18]. However, three studies reported no link between the R72P polymorphism and endometrial cancer [19-21]. Our results are in concordance with the latter three studies since there was no association of these polymorphisms and endometrial cancer risk. The two studies that reported a significant association between the R72P polymorphism in TP53 and endometrial cancer were Asian whereas this study population and the reports by Peller et al. (1999) and Esteller et al. (1997) are Caucasian. It is highly likely that the discrepant results could be due to variation in genotype frequencies of different ethnicities. In addition, the size of the cohort in these two studies was much smaller than our study and their positive associations could be due to type I statistical error.

In regards to the MDM2 polymorphism, SNP309, two previous reports revealed that the homozygous variant genotype (GG) was associated with an increased risk of developing endometrial cancer in comparison to those women with the homozygous wild type (TT) or heterozygous (TG) genotypes [23, 24]. The results reported herein do not support the findings of these studies. There are several explanations for the conflict in findings between these three studies. Endometrial cancer is highly likely to be dependent on environmental influences and major differences between the three studies are the adjustments made for non-genetic risk factors.

Analysing the grade of endometrial cancer and the polymorphisms in TP53 R72P and MDM2 SNP309 separately, showed an association between the variant genotypes and endometrial cancer risk. A relationship between TP53 intron 3 insertion 16bp and intron 6

G>A polymorphism was not observed. Since the three TP53 polymorphisms were in high linkage disequilibrium, they were combined with MDM2 SNP309 to assess their association with endometrial cancer grade. Firstly, the combination of TP53 R72P and MDM2 SNP 309 alone showed an increased risk of developing high grades of endometrial cancer with the reduced apoptotic ability genotypes. Furthermore, the combination of all four polymorphisms revealed an even stronger relationship between the variant genotypes and the grade of endometrial cancer. Women with one or more variant genotypes (medium or high risk) had a greater likelihood of having a higher grade (grade 2 or 3) endometrial cancer compared to those with wild-type genotypes (low risk).

The results relating to the TP53 R72P polymorphism and the grade of endometrial cancer are in concordance with Saffari et al. (2005) [22]. The seven patients harbouring the R72P polymorphism in Saffari's study had a lower overall rate of survival in comparison to those women without this polymorphism. Our study adds further support to the hypothesis that reduced apoptotic ability is associated with a higher grade of endometrial cancer and consequently lower survival rates. The combination of the four polymorphisms in MDM2 and TP53 appear to be related to higher grade of endometrial cancer. MDM2 is the key negative regulator of TP53 and dysfunction of these genes has been associated with an increased rate of accumulation of genetic errors thereby enhancing the progression of disease.

Interestingly, endometrial cancer patients harbouring the variant genotypes of one of the three TP53 polymorphisms, R72P, 16bp insertion in intron 3 and the G>A polymorphism in intron 6 were found to have a greater likelihood of having a first and/or second degree relative affected with breast cancer. Combining these polymorphisms showed that endometrial cancer cases with one or more variant TP53 genotypes compared to the wild-type genotypes had a highly significant increased frequency of family members with breast cancer. Previous studies that have examined the relationship between these three polymorphisms and breast cancer have shown inconsistent findings. The most recent and largest study of TP53 R72P and MDM2 SNP309 did not reveal any association of these polymorphisms alone or in combination with breast cancer risk [30]. Relatively few studies have examined cancer families where the dominant phenotypes are endometrial and breast

cancer. It remains to be determined if this association represents a real entity or is just a coincidental finding.

In conclusion, to verify the findings reported herein, they should be replicated in an independent data set. The results of this study provide evidence for the association of reduced apoptotic ability due to polymorphisms in TP53 and MDM2 with a higher grade of endometrial cancer, which is associated with lower survival rates. There also appears to be a specific association between the risk of endometrial cancer and breast cancer in patients harbouring polymorphisms in TP53.

Acknowledgements

The authors would like to thank the participants of this study and to acknowledge the funding contributions from the University of Newcastle, the NBN Children's Cancer Research Group, Ms Jennie Thomas and the Hunter Medical Research Institute.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Article Precis

This study provides evidence of an association between polymorphisms in MDM2 and TP53 combined and higher grades of endometrial cancer.

Table 1: Recurrence, Histology, Grade and Stage of Endometrial Cancer Cases.

Recurrence	**Yes (%)**	**No (%)**	**Unknown**		
	13 (6.8)	178 (93.2)	0 (0.0)		
Histology	**Type I - adenocarcinomas (%)**	**Type II - other carcinomas (%)**	**Unknown**		
	131 (85.1)	23 (14.9)	37 (19.4)		
Grade	**1 (%)**	**2 (%)**	**3 (%)**	**Unknown**	
	40 (22.3)	52 (29.1)	87 (48.6)	12 (6.3)	
Stage	**I - A,B,C (%)**	**II - A,B (%)**	**III - A,B,C (%)**	**IV - A,B (%)**	**Unknown**
	121 (79.6)	18 (11.8)	11 (7.2)	2 (1.3)	39 (20.4)

Table 2: Comparison of Environmental and Reproductive Risk Factors between Cases and Controls.

Risk Factor	Group	Cases n (%)	Controls n (%)	OR	95% CI	P value
BMI (<25kg/m^2 and >=25kg/m^2)[A]	<25kg/m^2	34 (19.1)	72 (24.7)	0.718	0.454-1.136	p=0.157
	>=25kg/m^2	144 (80.9)	219 (75.3)			
High Blood Pressure (yes/no)	yes	107 (56.0)	114 (39.2)	1.978	1.366 – 2.864	p<0.001
	no	84 (44.0)	177 (60.8)			
Diabetes (yes/no)	yes	44 (23.0)	31 (10.7)	2.51	1.519 – 4.148	p<0.001
	no	147 (77.0)	260 (89.3)			
Hormone Replacement Therapy (yes/no)	yes	47 (24.6)	40 (13.7)	2.048	1.282 – 3.273	p=0.003
	no	144 (75.4)	251 (86.3)			
Smoking (ever/never)	ever	52 (27.2)	68 (23.4)	1.227	0.807 – 1.865	p=0.338
	never	139 (72.8)	223 (76.6)			
Alcohol consumption (ever/never)	ever	92 (48.2)	228 (78.4)	0.257	0.172 – 0.382	p<0.001
	never	99 (51.8)	63 (21.6)			
Personal History of Any Cancer (yes/no)	yes	51 (26.7)	28 (9.6)	3.422	2.066 – 5.667	p<0.001
	no	140 (73.3)	263 (90.4)			
History of Ovarian or Cervical Cancer (yes/no)	yes	15 (7.9)	3 (1.0)	8.182	2.335 – 28.663	p=0.001
	no	176 (92.1)	288 (99.0)			
Ovarian Cancer (yes/no)	yes	7 (3.7)	1 (0.3)	11.033	1.346 – 90.403	p=0.025
	no	184 (96.3)	290 (99.7)			
Cervical Cancer (yes/no)	yes	8 (4.2)	2 (0.7)	6.317	1.327 – 30.077	p=0.021
	no	183 (95.8)	289 (99.3)			
History of Skin Cancer (yes/no)	yes	20 (10.5)	19 (6.5)	1.674	0.869 – 3.228	p=0.124
	no	171 (89.5)	272 (93.5)			
History of Bowel Cancer (yes/no)	yes	10 (5.2)	8 (2.7)	1.954	0.757 – 5.045	p=0.166
	no	181 (94.8)	283 (97.3)			
History of Other Cancer (yes/no)	yes	10 (5.2)	4 (1.4)	3.964	1.255 – 12.828	p=0.022
	no	181 (94.8)	287 (98.6)			

BMI not known for 13 cases.

Table 3: Associations of TP53 and MDM2 Polymorphisms with Endometrial Cancer Risk.

Genes and Polymorphisms	Genotype	Cases n (%)	Controls n (%)	χ^2	OR (95% CI) and p value	
MDM2 SNP309	TT	78 (40.8)	128 (44.0)		1.00 (reference)	
	TG	84 (44.0)	126 (43.3)	p=0.673	1.09 (0.74-1.62)	p=0.66
MAF: G (no data available)	GG	29 (15.2)	37 (12.7)		1.29 (0.73-2.26)	p=0.38
	Any G	113 (59.2)	163 (56.0)	p=0.494*	1.14 (0.79-1.65)	p=0.49
TP53 R72P	GG	101 (52.9)	166 (57.2)		1.00 (reference)	
	GC	75 (39.3)	107 (36.9)	p=0.035	1.15 (0.78-1.69)	p=0.47
MAF: (C) 0.352	CC	15 (7.9)	17 (5.9)		1.45 (0.69-3.03)	p=0.32
	Any C	90 (47.2)	124 (42.8)	p=0.346*	1.19 (0.83-1.72)	p=0.35
TP53 intron 3 ins 16bp	0/0	146 (76.8)	216 (74.2)		1.00 (reference)	
	0/16	40 (21.1)	70 (24.1)	p=0.723	0.85 (0.54-1.32)	p=0.46
MAF: (16) 0.065	16/16	4 (2.1)	5 (1.7)		1.18 (0.31-4.48)	p=0.80
	Any 16	44 (23.2)	75 (25.8)	p=0.516*	0.87 (0.57-1.33)	p=0.52
TP53 G>A SNP Intron 6 (MspI)	GG	148 (77.5)	225 (77.6)		1.00 (reference)	
	GA	40 (20.9)	64 (22.1)	p=0.342	0.95 (0.61-1.48)	p=0.82
MAF: (A) 0.065	AA	3 (1.6)	1 (0.3)		4.56 (0.47-44.26)	p=0.19
	Any A	43 (22.5)	65 (22.4)	p=0.980*	1.01 (0.65-1.56)	p=0.98

The genotype frequencies for each SNP are similar to other studies on Caucasians.

MAF: Minor Allele Frequency as determined by www.ncbi.nlm.nih.gov Entrez SNP website (PDR90 population).

* p value: Wild type genotype compared to combination of heterozygous and homozygous variant genotypes.

The odds ratios were adjusted for age, BMI, HBP, diabetes, HRT, personal history of cancer, smoking and alcohol use.

Table 4: MDM2 SNP309, TP53 R72P, intron 3 insertion 16bp and intron 6 G>A polymorphism and Grade of Endometrial Cancer

Polymorphism	Genotype	Grade (n=179) n (%)			p value
		1	2	3	
MDM2 SNP 309	**TT**	13 (7.3)	11 (6.2)	48 (26.8)	**p=0.002**
	TG	20 (11.2)	30 (16.8)	28 (15.6)	
	GG	7 (3.9)	11 (6.2)	11 (6.2)	
TP53 R72P	**GG**	21 (11.7)	20 (11.2)	54 (30.2)	**p=0.032**
	GC	16 (8.9)	30 (16.8)	26 (14.5)	
	CC	3 (1.7)	2 (1.1)	7 (3.9)	
TP53 intron 3 insertion 16bp	**0/0**	29 (16.3)	39 (21.9)	71 (39.9)	p=0.067
	0/16	11 (6.2)	13 (7.3)	11 (6.2)	
	16/16	0 (0.0)	0 (0.0)	4 (2.2)	
TP53 intron 6 G>A SNP	**GG**	31 (17.3)	39 (21.8)	71 (39.7)	p=0.260
	GA	9 (5.0)	13 (7.3)	13 (7.3)	
	AA	0 (0.0)	0 (0.0)	3 (1.7)	

Table 5: Combined Genotypes for MDM2 SNP309, TP53 R72P, intron 3 insertion 16bp and intron 6 G>A polymorphism and Grade of Endometrial Carcinoma

Genes and Polymorphisms	Genotype Combinations	Grade (n=179, missing n=12) n (%)				Risk group comparisons	X^2 (Grade 1 versus 2 versus 3)	OR (95% CI) and p value (Grade 1 versus Grade 2+3)	
		1	2	3	2+3				
Combined MDM2 SNP309 T>G, TP53 R72P G>C, intron 3 16bp insertion 0/16 and intron 6 G>A SNP (MspI)	Low Risk (TT+GG+00+GG)	27 (75.0)	4 (11.1)	5 (13.9)	9 (25.0)	Low versus Medium	p=0.096	**2.70 (1.08-6.75)**	**p=0.031**
	Medium Risk (TG+GG+00+GG, TT+GC+00+GG TT+GG+0/16+GG, TT+GG+00+GA)	30 (52.6)	13 (22.8)	14 (24.6)	27 (47.4)	Low versus High	**p<0.001**	**5.60 (2.33-13.44)**	**p<0.001**
	High Risk*	30 (34.9)	35 (40.7)	21 (24.4)	56 (65.1)	Medium versus High	**p=0.054**	**2.07 (1.05-4.11)**	**p=0.035**
	Any Risk Allele	60 (42.3)	48 (33.8)	35 (23.9)	83 (57.7)	Low versus Any Risk Allele	**p=0.002**	**4.15 (1.82-9.46)**	**p=0.0003**

* High risk genotypes include all other combinations that have two or more variant alleles

Table 6: TP53 R72P, intron 3 insertion 16bp and intron 6 G>A polymorphism and Family History of Breast Cancer in Endometrial Cancer Patients

Polymorphism	Genotype	Family History of Breast Cancer		p value
		Yes n (%)	No n (%)	
TP53 R72P	**GG**	14 (7.3)	87 (45.6)	**p=0.001**
	GC	22 (11.5)	53 (27.7)	
	CC	8 (4.2)	7 (3.7)	
TP53 intron 3 insertion 16bp	**0/0**	29 (15.3)	117 (61.6)	**p=0.018**
	0/16	12 (6.3)	28 (14.7)	
	16/16	3 (1.6)	1 (0.5)	
TP53 intron 6 G>A SNP	**GG**	29 (15.2)	119 (62.3)	**p=0.044**
	GA	13 (6.8)	27 (14.1)	
	AA	2 (1.1)	1 (0.5)	

Table 7: Combined Genotypes for TP53 R72P, 16bp insertion in intron 3 and G>A SNP in intron 6 (MspI) and Family History of Breast Cancer

Genes and Polymorphisms	Genotype Combinations	FH Breast Cancer (n=191)		Risk group comparisons	OR (95% CI) and p value	
		Yes n (%)	No n (%)			
Combined TP53 R72P G>C, intron 3 insertion 16bp 0/16 and intron 6 G>A SNP (MspI)	Low Risk GG+00+GG	14 (14.4)	83 (85.6)	Low versus Medium	**3.16 (1.38-7.25)**	**p=0.034**
	Medium Risk (GC+00+GG, GG+0/16+GG, GG+00+GA)	14 (29.2)	34 (70.8)	Low versus High	**2.44 (1.05-5.66)**	**p=0.005**
	High Risk*	16 (34.8)	30 (65.2)	Medium versus High	1.29 (0.54-3.09)	p=0.559
	Any Risk Allele	30 (31.9)	64 (68.1)	Low versus Any Risk Allele	**2.78 (1.36-5.67)**	**p=0.004**

* High risk genotypes include all other combinations that have two or more variant alleles

Note: The association of the three TP53 polymorphisms and family history of breast cancer is no longer significant when MDM2 SNP309 is added to the combined analysis.