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Association of myeloperoxidase -463G>A polymorphism with cervical cancer in Chinese Han women

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Summary

Objective: Myeloperoxidase (MPO) is an endogenous oxidant in neutrophilic and monocytic cytoplasmic granules. Several epidemiological studies indicate that MPO genetic polymorphisms are associated with cancer risk. Thus the present assessed an association between the MPO -463G>A polymorphism and cervical cancer risk in Chinese Han women. *Materials and Methods:* A hospital-based case-control study included 276 Chinese Han patients with cervical cancer and 284 age-, and ethnic-matched controls. The MPO -463G>A polymorphism was analyzed with PCR-restriction fragment length polymorphism (RFLP). Associations between the MPO polymorphism and cervical cancer risk were confirmed with χ^2 tests. *Results:* No significant differences occurred among genotype distributions of the MPO -463G>A polymorphism between patients and controls. In a subgroup analysis of cervical cancer patients, there was no association between the MPO polymorphism and cervical cancer histological types or grades or tumor stage. *Conclusions:* The MPO -463G>A polymorphism is not associated with a risk of cervical cancer in Chinese Han women.

Key words: Myeloperoxidase; Polymorphism; Cervical cancer; Chinese.

Introduction

Worldwide, cervical cancer is the fourth most common cause of cancer and cancer-related death for women [1]. Approximately 80% of cervical cancers occur in developing countries, and cervical cancer is currently an important public health issue in mainland China. According to the National Health and Family Planning Commission, 132,000 new cervical cancer cases are recorded in China annually, accounting for 28% of the world's total cancer burden [2]. Cervical cancer prematurely kills ~30,000 Chinese women annually.

The human papillomavirus (HPV) infection is necessary for the development of invasive cervical cancer [3]. Epidemiological investigations indicate that more than 80% of cervical cancer patients are infected with high-risk HPV types. Other risk factors, such as genetic susceptibilities, smoking, oral contraceptives, weakened immune systems, and sexual activity at a young age are involved [4-8]. New predictive factors are under investigation for improving diagnosis and prediction of cervical cancer. Studies of the association between genetic polymorphism and cervical cancer risk are of interest and polymorphisms in cytokine and sex hormone receptor genes, clusters of differentiation and miRNA genes, and genes that modulate reactive oxygen species (ROS) are associated with cervical cancer risk [4, 9-12].

Myeloperoxidase (MPO) is an endogenous oxidant in the cytoplasmic granules of neutrophils and monocytes. During neutrophil respiratory burst, MPO promotes oxidative stress during inflammation by producing hypochlorous acid (HOCl) and tyrosyl radical, which are cytotoxic and used by the neutrophil to kill bacteria and other pathogens [13]. However, HOCl may also act as a pro-carcinogen causing DNA damage as well as compromise the repair process [14]. There is evidence that cervical epithelial tissues, targets for HPV, are exposed to ROS [15]. MPO is an important mediator for ROS production, hence it is thought to be associated with cervical cancer risk. MPO locus polymorphisms may contribute to genetic susceptibility of cervical cancer.

Although studies have focused on the association of the MPO -463G>A polymorphism with cervical cancer risk, Chinese women have not been exclusively studied, so the present authors assessed this in a hospital-based case-control study to investigate any association of the MPO polymorphism with genetic risk of cervical cancer in this population.

Materials and Methods

The authors enrolled 276 patients with cervical cancer and 284 controls from Fuzhou General Hospital of Nanjing Military Command between January 2011 and December 2012. All subjects were genetically unrelated ethnic Han Chinese women, permanently residing in Fujian province in China. All cervical cancers were histologically confirmed based on the World Health Organization (WHO) diagnostic criteria. Patient data appear in Table 1. Similarly, aged controls had no history of cancer or genetic disease, and all were treated for benign gynecological diseases with

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no cytologically or histologically confirmed cervical intraepithelial neoplasia (CIN). No controls had a history of conization and/or hysterectomy. All subjects with known HIV, hepatitis B or C infection were excluded. EDTA-treated blood samples (five ml) from patients and controls were collected and all study protocols were approved by the Ethics Committee of Fuzhou General Hospital, Fuzhou, China (LL-1013/Dec 2010); each subject provided informed consent.

Genomic DNA was isolated from 500 µL of whole human blood using a genomic DNA isolation kit according to the manufacturer's instructions and samples were stored with EDTA at -20°C.

PCR-RLFP was used to measure the MPO -463G>A polymorphism as described previously. Briefly, genomic DNA was amplified in a 50 µL reaction mixture using primers 5′-GGTATAGGCACACAATGGTGAG-3′ and 5′-GCAATGGTTCAAGCGATTCTTC-3′ (initial denaturation for five minutes at 94°C, 30 cycles of 0.5 minutes at 94 °C, 0.5 minutes at 58°C, and 0.5 minutes at 72°C, followed by a final elongation for five minutes at 72°C). PCR master mix was used. The PCR-amplified product was 350 bp long and digested with *Aci* I restriction enzyme at 37°C for 16 hours according to the manufacturer's instruction. Digestion products were separated with 3% agarose gel at 100 V for 60 minutes. Three possible genotypes were defined by three distinct bands: GG (169, 120 and 61 bp fragments), AG (289, 169, 120, and 61 bp fragments), and AA (289 and 61 bp fragments). Ten random samples were selected for sequencing to confirm results of restriction digestion, and data confirmed 100% coincidence.

A surface plasmon resonance (SPR)-based W2600 system was used to genotype HPV after extracting and subjecting the DNA to PCR amplification (20620591). The system could detect 24 known HPV genotypes, including 16 high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 81) and 8 low-risk types (HPV 6, 11, 40, 42, 43, 44, 54, 70). The SPR instrument and the PCR and SPR kits were obtained and processed according to the manufacturer's instructions.

Hardy-Weinberg equilibrium was assessed with a χ^2-test comparing observed and expected genotype frequencies. Differences in frequencies of the MPO -463G>A polymorphism between patients with cervical cancer and controls were investigated using χ^2 tests. The association between the MPO polymorphism and the risk of cervical cancer was estimated by *p*-values and odds ratios (OR) [95 % confidence interval (95 % CI)] ($p < 0.05$ was considered statistically significant). All statistical analyses were carried out using SPSS 12.0.

Results

Patient data appear in Table 1. There were no significant differences in age distribution among patients and controls ($p = 0.703$). High-risk HPV infection data was a significant risk factor for cervical cancer (Table 1). Chief cancer types were squamous cell carcinomas (87.3%) and adenocarcinomas (10.5%). According to FIGO 2013 cervical staging and histologic grading, Stages I, II, and III+ IV accounted for 29.3%, 27.9%, and 42.8%, and Grade 1, 2 and 3 tumors were 39.5%, 24.6%, and 35.9% of those studied, respectively.

The MPO -463G>A polymorphism was analyzed with PCR-RFLP in 276 patients with cervical cancer and 284 controls. Allele and genotype frequencies appear in Table 2 and the genotype distributions were in line with Hardy-Weinberg equilibria. No significant differences appeared in genotype distributions between patients and controls. MPO GA and AA genotype associations appear in Table 3 and were not statistically significant. In a subgroup analysis of cervical cancer patients, no association appeared between the MPO -463G>A polymorphism and histological types or grades and tumor stage (Table 4). No statistically significant differences occurred in the genotype distribution between patients with younger (≤ 48 years-of-age) and older (> 48 years-of-age) age onset cervical cancer ($p = 0.134$).

Table 1. — *Characteristics of cervical cancer patients and controls.*

Characteristics	Patients (%) (n=276)	Controls (%) (n=284)	*p*-values	OR [95% CI]
Age in years (mean ± SD)	48.1±8.4	47.6±7.9	0.703	
HPV high-risk infection				
Negative	19 (6.9)	243 (85.6)	< 0.001	1
Positive	257 (93.1)	41 (14.4)		6.45 [4.851-8.577]

Table 2. — *Genotypes and allele frequencies of the MPO-463G>A polymorphism in cervical cancer patients and controls.*

	GG (%)	GA (%)	AA (%)	G allele (%)	A allele (%)	HW
Patients	161 (58.3)	103 (37.3)	12 (4.3)	425 (77.0)	127 (23.0)	0.375
Controls	149 (52.4)	120 (42.3)	15 (5.3)	418 (73.6)	150 (26.4)	0.142

Table 3. — *Analysis of the MPO -463G/A polymorphism in cervical cancer risk.*

Genotype	Patients	Controls	OR	95% CI
GG	161	149	1	
GA	103	120	1.143	0.935-1.398
AA	12	15	1.554	0.773-3.125

Discussion

In this hospital-based, case-control study, the authors assessed any association between the MPO -463G>A polymorphism and cervical cancer risk in ethnic Han Chinese women and found no apparent ones. MPO is a XPO subfamily member of peroxidases and produces HOCl from hydrogen peroxide and chloride anion during neutrophil respiratory burst. Furthermore, it oxidizes tyrosine to tyrosyl radical using hydrogen peroxide as an oxidizing agent.

Table 4. — *Distribution of the MPO-463G>A polymorphism in cervical cancer patients.*

Characteristics		GG (%)	GA (%)	AA (%)	*p*-values
Histological types					
- Squamous cell carcinoma	241	140 (58.1)	92 (38.2)	9 (3.7)	0.689
- Adenocarcinoma	29	17 (58.6)	9 (31.0)	3 (10.3)	
- Adenosquamous cell carcinoma	5	3 (60.0)	2 (40.0)	0 (0.0)	
- Other	1	1 (100.0)	0 (0.0)	0 (0.0)	
FIGO Stages					
I	81	36	31	5	0.368
II	77	43	29	3	
III and IV	118	82	43	4	
Histologic grading					
1	109	66	39	4	0.868
2	68	39	23	6	
3	99	56	41	2	

HOCl and tyrosyl radical are cytotoxic and are exploited to eliminate bacteria and other pathogens [16]. However, HOCl may oxidatively damage bystander cells and host DNA which may drive the inflamed epithelia to malignancy at inflammatory sites [17].

The MPO -463G>A polymorphism was shown to be related to reduced MPO mRNA and protein and the loss of a SP1 transcription binding site. Several epidemiological studies focused on an association between the MPO promoter polymorphism and cancer risk indicate that the MPO -463G>A polymorphism is associated with reduced susceptibility to diverse cancers including lung and breast cancer and non-Hodgkin's lymphoma [18-20]. However, an association between the MPO -463G>A polymorphism and cervical cancer risk is unknown, and conflicting results have been reported. Most studies suggest that the MPO-463G>A polymorphism may not be key to developing cervical cancer [21, 22], and this is in agreement with the present data. One study, however, did associate this polymorphism with cancer risk [23]. Such conflicting findings may be explained by the dual nature of MPO function. MPO may initiate tumor development by causing cervical cell and DNA damage and it may be able to protect the host from cervical cancer by helping to eliminate tumor cells. Increased MPO expression in plasma and cervical cancer tissues has been noted [24, 25], and decreased MPO activity in peripheral blood neutrophils was observed in another report [26]. Therefore, contradictory data about MPO in cervical carcinogenesis may depend on the phase of tumor development. The present authors found no association with the MPO -463G>A polymorphism and tumor stage. Recently, Natter *et al.* reported that the MPO -463 G>A polymorphism was not associated with a risk of developing CIN in a relatively large number of Caucasian women [27]. Therefore, other factors may contribute to MPO's genetic effects on cervical cancer risk.

The -463 type G polymorphism of the MPO gene can increase transcriptional activation but other biological factors may also affect MPO expression *in vivo*. Local immune status of cervical cancer, such as oxidative stress and pathogenic infection, may recruit neutrophils and increase MPO expression [25-28]. Estrogens may upregulate MPO via an estrogen-dependent myeloid response and ethnic differences contribute to the frequency of the MPO -463G>A polymorphism and its effects on cancer risk [29, 30]. Thus, the association of MPO polymorphisms with MPO expression should be confirmed and genetic and related factors should be considered.

In conclusion, there was no significant association between the MPO -463G>A polymorphism and a risk of cervical cancer in ethnic Han Chinese women. Larger well-designed multicenter studies are suggested to verify the present findings, and these studies should include infections, endocrinological aspects, and ethnic factors.

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