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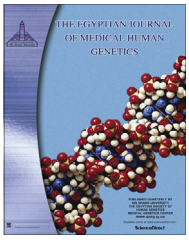

ORIGINAL ARTICLE

Association of glutathione-S-transferase P1 (GSTP1)-313 *A* > *G* gene polymorphism and susceptibility to endometrial hyperplasia among Egyptian women

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KEYWORDS

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Abstract *Background:* Endometrial hyperplasia (EH) occurs when the endometrium, the lining of the uterus, becomes too thick, causing abnormal uterine bleeding. In some cases, it can lead to endometrial carcinoma if untreated. Glutathione-S-transferases (GSTs) enzymes have a role in the metabolism of a lot of disease-causing carcinogens and mutagens that are present in environments of human. *GSTP*1–313 *A* > *G* gene polymorphism was associated with significantly high risk of endometrial carcinoma in some reports. The aim of this work is to assess the association of this polymorphism with the susceptibility of EH among a sample of Egyptian women.

Subjects and methods: This study included 84 unrelated EH Egyptian women who were compared to 134 healthy controls from the same locality. For all subjects, DNA was genotyped for *GSTP*1–313 *A* > *G* (*Ile105Val*) polymorphism using the PCR–RFLP technique.

Results: The frequency of *GSTP*1–313 *AG* and *GG* genotypes was noted to be significantly higher among most of the cases with EH compared to controls (79.8% *vs.* 42.0%, OR = 8.63, 95% CI = 4.53–16.46, $p < 0.001$). Also the frequency of the *GSTP*1–313 *G* allele was significantly higher among most of the cases compared to controls (24.9% *vs.* 16.8%, OR = 3.72, 95% CI = 2.39–5.79, $p < 0.001$). Furthermore, there was a significant increase of endometrial thickness in the EH women compared to controls ($p < 0.001$).

Conclusions: *GSTP*1–313 *G* allele carriage was associated with susceptibility of EH among a sample of Egyptian women.

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1. Introduction

Endometrial hyperplasia (EH) is defined as an excessive proliferation of the cells of endometrium. It does not represent a health risk to women, however, it is a precursor lesion of most endometrial carcinoma. It is always detected following the investigation of women with symptoms of abnormal uterine bleeding [1,2]. EH has been classified into 3 main types: (1) simple hyperplasia that is characterized by less amount of endometrial glandular crowding with low risk of hyperplasia progress to carcinoma; (2) complex hyperplasia that is characterized by major amount of endometrial glandular crowding with medium risk of progression; and (3) atypical hyperplasia that is comprised of endometrium with complex glandular crowding and the risk of endometrial carcinoma progression is greatest [3,4].

The endometrium, a target tissue of sex-steroid hormones, consists of two main structural components, the specialized endometrial stroma and the endometrial glands [5]. Endometrial hyperplasia typically occurs when unopposed estrogen (i.e., in the absence of progesterone) stimulates abnormal proliferation of endometrial glands. Estrogen drives endometrial proliferation during the menstrual cycle, and chronic excess estrogen activity is a well established risk factor for endometrial hyperplasia [5,6]. The etiology of endometrial hyperplasia is unclear, although a multifactorial origin from a combination of immunologic, genetic and environmental factors are considered to be the most plausible [5–7]. Recently, multiple lines of evidence from genetic origin suggest that EH has a substantial hereditary component [8–10]. Moreover, there is an increased risk of EH by conditions accompanied with intermittent or the absence of ovulation. After menopause when ovulation has ceased, EH is very common in women who have conditions that cause the levels of circulating estrogen to increase such as in estrogen replacement therapy or obesity [6,7]. Decreased progesterone activity is a risk factor for endometrial hyperplasia. Obesity contributes to decreased progesterone activity due to obesity-associated anovulation [7].

Glutathione-S-transferases (GSTs) are key enzymes of phase II related to the metabolism of numerous genotoxic compounds. Glutathione-S-transferases M1 (GSTM1) and T1 (GSTT1), the important subtypes of GSTs enzymes, are reportedly involved in detoxification of estrogen and reactive oxygen species, which are considered to play a key role in the occurrence of various endocrine-related cancers [11,12]. Another member of the glutathione-S-transferase (GST) family, GSTP1, which is located at 11q13, has a role in the detoxification of electrophilic compounds by glutathione conjugation [11]. It has been found that two genetic polymorphisms in exon 5 and exon 6, lead to amino acid substitutions. However, only the transition in exon 5 was linked to activity of enzymes since this is located within the region coding for the enzyme's active site. The genetic change in exon 5 at the site -313, results in polymorphism at codon 105, where an adenosine-to-guanidine ($A > G$) transition causes an Ile-to-Val substitution [13]. Furthermore, many studies reported that GSTP1 has a role in biotransformation of many pollutants of environment [14]. This suggests that GSTP1 may be the most important GST in the pathogenesis of endometrial hyperplasia. For this reason, several studies have been conducted to evaluate the association of *GSTP*1–313 $A > G$ polymorphism with endometrial carcinoma risk [15–22], but there were no previous results evaluating the association between this polymorphism and endometrial hyperplasia. Therefore, the aim of our study is to derive a more precise estimation of the association between *GSTP*1–313 $A > G$ (*Ile105Val*) polymorphism and EH susceptibility among Egyptian women.

2. Subjects and methods

This work is a case controlled study involving 84 women represented by a history of simple endometrial hyperplasia (SEH) [3,4] with an age mean ± SD of 48.2 ± 3.1 years, in addition to a control sample of 134 healthy unrelated women with an age mean ± SD of 47.2 ± 3.3 years. They were recruited from the outpatient clinic of Obstetrics and Gynecology Department, Faculty of Medicine, Mansoura University, Egypt. All the patients were asked to complete a questionnaire regarding general characteristics including age, age at menarche, gravidity and parity. Body mass index (BMI) was calculated as weights (kg) divided by the heights (m^2). Exclusion criteria included women who were documented to have uterine fibroid, endometrial carcinoma, cancer ovary, cancer colon, as well as those who received any hormonal therapy during the last 3 months prior to the study.

For all participants, genomic DNA from a peripheral blood was extracted and purified (Gentra Systems, USA). Genotyping of the *GSTP1 Ile105Val* gene polymorphism at exon 5 was carried out via the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP), using the forward (5′-ACC CCA GGG CTC TAT GGG AA-3′) and reverse (5′-TGA GGG CAC AAG AAG CCC CT-3′) primers, which were used to generate a segment of 176 bps of the *GSTP1* gene [23]. The PCR amplification was carried out using Veriti® Thermal Cycler (Applied Biosystems) in a volume containing about 50 ng genomic DNA template, 200 μM of each dNTP, 200 ng of each primer, 1.5 mM MgCl2, 1× PCR buffer (50 mM KCl, 10 mM Tris–HCl, pH 8.3), and 1 U Taq DNA polymerase. This amplification was carried out with an initial denaturation step at 95 °C for 10 min, this step was followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s and a final extension at 72 °C for 10 min. The amplified products were digested for 2 h at 37 °C with 2 U *Alw26I* (Thermo Fisher Scientific, Catalog number: FD0034). The product of digestion was applied on a 2% agarose gel that was stained with ethidium bromide. Electrophoresis of the digested PCR products showed individuals homozygous (ile/ile) for the *GSTP1 ile105val* polymorphism as one band of 176 bp. Heterozygous (ile/val) for the polymorphism showed three bands of 176, 91 and 85. Homozygotes (val/val) showed two bands of 91 and 85 bp.

2.1. Ethical approval and informed consent

This study was started after getting an approval from the university scientific and ethics committees. In addition, an informed consent was obtained from all participants before their enrollment into the study. The work has been carried out in accordance with the code of ethics of the world medical

association (declaration of Helsinki) for experiments in humans results.

2.2. Statistical analysis

Data were analyzed and processed using the Statistical Package of Social Science (SPSS, version 17.0). The frequencies of studied allelic and genotypic variants among cases were compared to the controls using Fisher's exact test and odds ratio (OR) with the 95% confidence interval (95% CI). Quantitative traits were compared using the Student *t*-test while nominal traits were compared using the Chi square test. Conformity with the Hardy Weinberg law of genetic equilibrium (HWE) was tested comparing the observed versus the expected genotype frequencies in control and cases. A minimum level of statistical significance was obtained at a p level of <0.05.

3. Results

The clinical and demographic data of the EH women are shown in Table 1. It was noted that there was no significant difference of age, age at menarche, gravidity, parity and BMI between EH women and controls. However, there was a significant increase of endometrial thickness in the EH women compared with controls ($p < 0.001$). The genotypic and allelic frequencies of the *GST*91–313 $A > G$ gene SNP in EH women and controls are shown in Table 2. Hardy–Weinberg equilibrium (HWE) relied no significant difference ($p = 0.63$) between the observed and frequencies that were expected of *GSTP*1–313 $A > G$ genotypes of the controls that may be supporting the selection of controls. However, HWE showed a significant deviation of the observed frequencies of cases from the expected ones ($p < 0.001$), probably due to the high frequency of heterozygote on the expense of homozygote genotypes. This is attributed to the small sample size rather than the existence of heterozygote advantage or heterosis (Table 2).

Testing for the dominant model of inheritance ($AG + GG$ *vs.* AA) showed that cases had a significantly higher frequency of $AG + GG$ genotypes compared to controls (79.8% *vs.* 42.0%, OR = 8.63, 95% CI = 4.53–16.46, $P < 0.001$). Testing for Heterozygote model (AG *vs.* AA), cases showed a significantly higher frequency of AG genotype compared to controls (73.8% *vs.* 29.1%, OR = 8.60, 95% CI = 4.47–16.55, $P < 0.001$). Testing for the recessive model (GG *vs.* $AA + AG$), cases showed no significant difference of the GG homozygous genotype compared to the controls (6.0% *vs.* 2.3%, OR = 2.76, 95% CI = 0.64–11.88, $p = 0.26$). Regarding the allelic frequencies, cases showed a significantly higher frequency of the *GST*91–313 G allele compared to controls (24.9% *vs.* 16.8%, OR = 3.72; 95% CI = 2.39–5.79, $P < 0.001$) (Table 2).

Table 1 The main demographic and clinical characteristics of patients of endometrial hyperplasia compared to controls.

Variable*	Cases $n = 84$	Controls $n = 134$	p
Age, years, M ± SD	48.2 ± 3.1	47.2 ± 3.3	0.35
Age at menarche, years, M ± SD	12.8 ± 6.2	13.6 ± 4.8	0.76
Gravidity, M ± SD	6.2 ± 1.6	5.8 ± 2.2	0.32
Parity, M ± SD	4.4 ± 1.2	3.9 ± 0.85	0.53
BMI, (kg/m^2)	28.9 ± 2.3	27.6 ± 1.8	0.82
Endometrial thickness (ET), mm	15.8 ± 1.9	12.2 ± 1.4	<0.001**

* BMI, Body mass index.
** $p < 0.001$ = highly significant.

4. Discussion

Several hormonal, immunologic and environmental factors have a role in the endometrial hyperplasia development [9]. The GST gene is one of the important genes which has a role in regulate immune, metabolism, endocrine and are closely

Table 2 The genotype and allele frequencies of GSTP1–313 $A > G$ in the EH patients and control groups.

	EH patients n (%) 84	Controls n (%) 134
Genotypes		
AA (ile/ile)	17 (20.2)	92 (68.6)
AG (ile/val)	62 (73.8)	39 (29.1)
GG (val/val)	5 (6.0)	3 (2.3)
Alleles		
A (ile)	96 (57.1)	223 (83.2)
G (val)	72 (24.9)	45 (16.8)
HWE	$\chi^2 = 21.6$, $p < 0.001$**	$\chi^2 = 0.23$, $p = 0.63$

Statistics		OR (95% CI)	p
Recessive	GG vs. AA + AG	2.76 (0.64–11.88)	0.26
Dominant	AG + GG vs. AA	8.63 (4.53–16.46)	<0.001**
Heterozygote model	AG vs. AA	8.60 (4.47–16.55)	<0.001**
Overdominant	AG vs. AA + GG	6.86 (3.72–12.67)	<0.001**
Allele	G vs. A	3.72 (2.39–5.79)	<0.001**

OR, odds ratio; CI, confidence intervals; HWE, Hardy–Weinberg equilibrium.
** $p < 0.001$ = highly significant.

related to the susceptibility with endometrial carcinoma [24]. GSTP1 is the most important member of GST family which contributes to the detoxification of electrophilic compounds by glutathione conjugation [11]. Furthermore, GSTP1 is a key substance in the biotransformation and inactivation of certain environmental pollutant. Due to enzymatic activity of *GSTP*1–313 *A* > *G* polymorphism, GSTP1 may confer susceptibility to several cancers [23,25].

Multiple studies have been focused on *GSTP*1–313 *A* > *G* polymorphism and the risk of various cancers, including lung, breast, colorectal, bladder, pancreatic, thyroid and prostate cancer [25]. Some reports showed that *GSTP*1–313 *A* > *G* polymorphism was associated with the risk of prostate, bladder and gastric cancer [26–28]. On the contrast, this polymorphism was not associated with susceptibility to colorectal cancer, hepatocellular carcinoma or breast cancer [29–31]. Knowing these facts, this study was planned to investigate the association between *GSTP*1–313 *A* > *G* polymorphism and endometrial hyperplasia. To our knowledge, this is the first report of a probable association between this polymorphism and Egyptian EH women.

High *GSTP*1–313 *G allele carriage* (*AG* + *GG* genotypes) was significantly noted among Egyptian women with EH coping with the dominant model of inheritance.

Several studies have been performed to assess the association of *GSTP*1–313 *A* > *G* polymorphism with endometriosis risk, but the results remain inconclusive. One study done among Turkish women, found that *GSTP*1–313 *A* > *G* polymorphism might contribute to the risk of endometriosis with significantly decreased risk for GSTP1 val/val and increased risk for GSTP1 ile/ile [21]. Nonetheless, several other studies have found contradictory results in the form of no association between *GSTP*1–313 *A* > *G* and endometriosis risk in some other populations like that done among Chinese [15], Japanese [16], Italian [17], Indian [20] and Korean [22] subjects. A recent meta-analysis study showed that *GSTP*1–313 *A* > *G* polymorphism was not associated with endometriosis risk in the overall population (*A vs. G*: OR = 1.02, *P* = 0.511) [24]. These contradictory results might be attributed to different ethnic origins, environmental difference in addition to problems related to research methodologies like the adequacy of sample size and proper diagnostic methods. Therefore, authors do document some limitations of the study in the form of its relatively small sample size in addition to the lack of data concerning the *GSTP1* gene expression in studied cases. In this respect, we recommend undertaking a wider scale study of a large sample size including different disease phenotypes; investigating their genetic *GSTP1* polymorphisms along with its expression state in the blood and at the site of inflammation.

In conclusion, our data support the association of the *GSTP1 G* allele with susceptibility of EH among Egyptian women.

Conflict of interest

The authors declare that they have no conflict of interest related to this work. There is no financial and personal relationship with other people or organizations that could inappropriately influence this work.

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