CYPIA1 Ile462Val Polymorphism as a Risk Factor in Cervical Cancer Development in the Polish Population

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Abstract
Background and objective There are inconsistent data of the cytochrome P450 1A1 (CYPIA1) Ile462Val (rs1048943) single nuclear polymorphism (SNP) as a genetic susceptibility factor for cervical cancer in various populations. Moreover, little is known about the interaction of this SNP with other risk factors, including contraceptive use, postmenopausal status, parity, and tobacco smoking.

Methods Polymerase chain reaction-restriction fragment length polymorphism was used to study the prevalence of the CYPIA1 Ile462Val SNP in women with cervical cancer (n = 456) and controls (n = 495).

Results Logistic regression analysis adjusting for age, parity, oral contraceptive use, tobacco smoking, and menopausal status demonstrated that the CYPIA1 Ile/Val polymorphism was not associated with an increased risk of cervical cancer in all patients. The adjusted odds ratio (OR) for patients with the Ile/Val genotype vs. Ile/Ile genotype was 1.539 (95 % confidence interval [CI] 0.932–2.541, p = 0.091). However, an increase in cervical cancer risk was seen among patients with a positive history of tobacco smoking and parity. The adjusted OR for positive history of tobacco smoking with the Ile/Val vs. Ile/Ile genotypes was 2.978 (95 % CI 1.382–6.418, p = 0.0052). The adjusted OR for parity with the Ile/Val vs. Ile/Ile genotype was 1.739 (95 % CI 1.006–3.009, p = 0.0472).

Conclusion Our genetic study suggests that the CYPIA1 Ile462Val SNP may be a risk factor for cervical cancer among patients with a positive history of tobacco smoking and parity.

1 Introduction
Cervical cancer is the third most common type of cancer in women worldwide [1]. This cancer develops slowly; starting from a precancerous dysplasia designated cervical intraepithelial neoplasia (CIN) that may further develop to invasive cervical carcinoma [2, 3]. Almost all cervical cancers are caused by the human papilloma virus (HPV), which is considered the primary etiologic factor of this cancer [3, 4]. Some oncogenic HPV oncoproteins, including E6 and E7, cause a defect in the host’s innate and adaptive immunity and change the apoptotic pathway, leading to immortalization of normal cervical epithelial cells [2, 4]. Apart from HPV, multiparity, oral contraceptive use, tobacco smoking, and environmental insults have been recognized as secondary risk factors [5].

Cytochrome P450 1A1 (CYPIA1) belongs to the CYP family and is involved in the metabolism of endogenous molecules and xenobiotics [6]. The actions of CYPIA1 include aryl hydrocarbon hydroxylase activity, which is the first step in the metabolism of tobacco polycyclic aromatic hydrocarbons, leading to their carcinogenic activation [7].
CYP1A1 also participates in the metabolism of estrogen [8]. Therefore, the activity of CYP1A1 may influence cells via at least two distinct pathways, i.e., smoking and estrogen, on cervical carcinogenesis [5].

Two single nucleotide polymorphisms (SNPs) in the CYP1A1 gene have been studied as risk factors of cervical carcinogenesis: T3801C (rs4646903) and A2455G (rs1048943) [9]. The T3801C SNP is located in the 3′ untranslated region and A2455G corresponds to the substitution Ile462Val in exon 7. These two SNPs have been suggested as being in linkage disequilibrium (LD) in Caucasian populations [9]. The role of both of these SNPs in CYP1A1 as risk factors in cervical cancer development in different ethnicities is still disputable [10–13]. Moreover, little is known about the interaction of these SNPs with the other known risk factors of cervical cancer. We evaluated the CYP1A1 Ile462Val genotype and allele frequencies in patients with cervical cancer (n = 456) and controls (n = 495) in the Polish population, stratified based on contraceptive use, postmenopausal status, parity, and tobacco smoking.

2 Patients and Methods

2.1 Patients and Controls

The patients included 456 women with histologically determined cervical carcinoma according to the International Federation of Gynecology and Obstetrics. All women were enrolled between April 2007 and December 2012 at the Department of Radiotherapy, Greater Poland Cancer Center in Poznan, Poland (Table 1). The control group included 285 unrelated healthy female volunteers who were matched by age to the patients. The controls were enrolled during medical examination at the University Hospital, Clinic of Gynecological Surgery at Poznan University of Medical Science (Table 1). Data about parity, oral contraceptive use, tobacco smoking, and menopausal status were obtained during the clinical interview. Patients and controls were Caucasian, enrolled from the Wielkopolska (Greater Poland) area of Poland. Patients and controls provided written informed consent. The study was approved by the Local Ethical Committee of Poznan University of Medical Sciences.

2.2 Genotyping

DNA was obtained from peripheral leukocytes employing a salting-out procedure. Identification of the CYP1A1 Ile462Val (rs1048943) polymorphic variant was performed by polymerase chain reaction-restriction fragment length polymorphism. The CYP1A1 Ile462Val DNA fragment was amplified employing the primers 5′ ACCCATCTG AGTTCCCTACC 3′ and 5′ TCCACCTTACCGCCGAGT 3′. The PCR-amplified fragments of CYP1A1 that were 199 bp in length were isolated and digested with endonuclease HpyCH4III (5′ ACNGT 3′) from the New England BioLabs (Ipswich, USA). The CYP1A1 Val allele was cleaved into 116-bp and 83-bp fragments, whereas the CYP1A1 Ile

| Characteristic | Patients (n = 456) | Controls (n = 495) |
|---------------|-------------------|-------------------|
| Mean age *(years) ± SD | 52.4 ± 9.8 | 51.9 ± 10.2 |
| Tumor stage, n (%) | | |
| IA | 61 (13.4) | |
| IB | 63 (13.8) | |
| IIA | 59 (12.9) | |
| IIB | 55 (12.1) | |
| IIIA | 148 (32.5) | |
| IIIB | 53 (11.6) | |
| IVA | 9 (2.0) | |
| IVB | 8 (1.7) | |
| Histologic grade, n (%) | | |
| G1 | 87 (19.1) | |
| G2 | 145 (31.8) | |
| G3 | 98 (21.5) | |
| Gx | 126 (27.6) | |
| Histologic type, n (%) | | |
| Squamous cell carcinoma | 384 (84.2) | |
| Adenocarcinoma | 56 (12.3) | |
| Other | 16 (3.5) | |
| Parity, n (%) | | |
| Never | 51 (11.2) | 56 (11.31) |
| Ever | 405 (88.8) | 439 (88.6) |
| Oral contraceptive pill use, n (%) | | |
| Never | 248 (54.4) | 278 (56.2) |
| Ever | 208 (45.6) | 217 (43.8) |
| Tobacco smoking, n (%) | | |
| Never | 295 (64.7) | 331 (66.9) |
| Ever | 161 (35.3) | 164 (33.1) |
| Menopausal status, n (%) | | |
| Premenopausal | 159 (34.9) | 190 (38.4) |
| Postmenopausal | 297 (65.1) | 305 (61.6) |
| HPV genotypes*, n (%) | | |
| 16 and 18 | 311 (68.2) | |
| 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 | 352 (77.2) | |

* Age at first diagnosis

b HPV genotypes were determined by Cobas® HPV Test Roche Molecular Systems, Inc. (Alameda, CA, USA)

HPV human papilloma virus, SD standard deviation

△Adis
allele remained uncut. Presence of the CYP1A1 Ile462Val polymorphism was verified by commercial sequencing analysis of 10% randomly selected samples.

2.3 Statistical Analysis

The differences in genotypic and allelic distribution between patients and controls and their genotype deviation from the Hardy–Weinberg (HW) equilibrium were evaluated by the Chi-squared test. Moreover, the odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. Unconditional logistic regression analysis was used to adjust for the effect of confounders such as age, parity, oral contraceptive use, tobacco smoking, and menopausal status. A p-value of <0.05 was considered statistically significant.

3 Results

3.1 Distribution of the CYP1A1 Ile462Val Polymorphism in Women with Cervical Cancer

Prevalence of the CYP1A1 Ile462Val genotypes did not display significant differences from the HW equilibrium between patients and controls. The distribution and adjusted analyses of the CYP1A1 Ile462Val genotypes in cases and controls are presented in Table 2. The CYP1A1 Ile/Val heterozygous genotype frequency was 1.5-fold greater in women with cervical cancer compared with controls and was 0.09 and 0.06, respectively. There were no individuals, either patients or controls, with the homozygous CYP1A1 Val/Val genotype. The CYP1A1 Val allele frequency was slightly increased in patients with a positive history of tobacco smoking and parity. In patients with a positive history of tobacco smoking, the adjusted OR for the Ile/Val vs. Ile/Ile genotype was 2.978 (95% CI 1.382–6.418, p = 0.0052). The adjusted OR for parity in patients with the Ile/Val vs. Ile/Ile genotype was 1.739 (95% CI 1.006–3.009, p = 0.0472). However, no significant association was seen between CYP1A1 Ile462Val and patients with a positive history of oral contraceptive use and menopausal women (Table 3; Fig. 1). Moreover, we did not find an association between CYP1A1 Ile462Val and tumor stage, histologic grade, and type (Table 4; Fig. 2).

4 Discussion

The CYP1A1 Ile462Val polymorphism has been shown to be a risk factor for many types of cancer and hematopoietic malignancies [14–21]. To date, the CYP1A1 Ile462Val SNP has been shown to be a risk factor in the development of pharyngeal, prostate, lung, oral, ovary, bladder, and colorectal cancers [14–20]. In addition to these findings, the CYP1A1 Ile462Val SNP has been suggested to be a low-penetrant risk factor for acute leukemia [21]. There are also studies suggesting that the CYP1A1 Ile462Val SNP may be a risk factor for cervical cancer development [11, 12]. However, we did not observe, in our study, the

| Genotype  | Patients (frequency) | Controls (frequency) | OR (95% CI) | p-Valuea | Adjusted OR (95% CI)b | p-Valuea |
|-----------|---------------------|---------------------|------------|----------|----------------------|----------|
| Ile/Ile   | 415 (0.91)          | 466 (0.94)          | Referent   | –        | Referent             |          |
| Ile/Val   | 41 (0.09)           | 29 (0.06)           | 1.588 (0.9689–2.416) | 0.0646  | 1.539 (0.932–2.541)  | 0.091    |
| Val/Val   | 0 (0.00)            | 0 (0.00)            |            |          |                      |          |
| MAF       | 0.04                | 0.03                |            |          |                      |          |

a Chi-square analysis
b ORs were adjusted by age, parity, oral contraceptive use, tobacco smoking, and menopausal status
CI confidence interval, OR odds ratio

△ Adis
CYP1A1 Ile462Val polymorphism to be a risk factor of cervical cancer in our patient group. Our results are similar to those of Gutman et al. [10], who did not find an increased risk for cervical cancer in the Jewish Israeli population. Moreover, there was no significant association between the CYP1A1 Ile462Val SNP and cervical cancer in the Japanese population [13]. In contrast, the study conducted by Taskiran et al. [11] in the Turkish population demonstrated the CYP1A1 Val gene variant as a significant risk factor of CIN 1 and 2 and for cervical adenocarcinoma and squamous cell carcinoma. Joseph et al. [12] observed a significant contribution of the CYP1A1 Ile462Val SNP to cervical cancer, when adjusted for HPV status in Indian populations. Moreover, the CYP1A1 Ile462Val polymorphism has been inconsistently shown to be a risk factor of cervical cancer in the Chinese population [22–27]. In addition to these findings, two recently conducted meta-analyses have indicated that the Val gene variant may be a risk factor in cervical cancer development in Caucasian but not Asian populations [28, 29].

The use of tobacco has been recognized as a factor that can increase the risk of numerous carcinomas, including lung, larynx, mouth, esophagus, kidneys, urinary tract, cervix, and others [30]. In our study, we found that the CYP1A1 Ile462Val SNP increased the risk of cervical cancer in women with a positive history of tobacco smoking. Gutman et al. [10] demonstrated that tobacco smoking is an independent risk factor for cervical cancer. The human CYP1A1 enzyme is present in many epithelial tissues and conducts oxidative reactions involved in the bioactivation of tobacco’s aromatic hydrocarbons, aromatic amines, and nitrosamines to carcinogens [7, 31]. These substances interact with DNA leading to the formation of DNA adducts, which make the carrier prone to carcinogenesis [7, 31]. It has been demonstrated that tobacco consumers bearing the CYP1A1 462Val variant possess more polycyclic aromatic hydrocarbon-DNA adducts in

| High-risk exposure | Genotype | Patients | Controls | Adjusted OR (95 % CI)\(^a\) | \(p\)-Value\(^b\) |
|--------------------|----------|----------|----------|----------------------------|-----------------|
|                    | Ile/Ile  | Ile/Val  | Ile/Ile  | Ile/Val                    |                 |
| Parity             |          |          |          |                            |                 |
| Ever               | 370      | 35       | 416      | 23                         | 1.739 (1.006–3.009) | 0.0472         |
| Never              | 45       | 6        | 50       | 6                          | 1.596 (0.441–5.773) | 0.4709         |
| Oral contraceptive use |          |          |          |                            |                 |
| Ever               | 187      | 21       | 205      | 12                         | 1.923 (0.918–4.030) | 0.0821         |
| Never              | 228      | 20       | 261      | 17                         | 1.370 (0.697–2.692) | 0.3597         |
| Smoking            |          |          |          |                            |                 |
| Ever               | 134      | 27       | 154      | 10                         | 2.978 (1.382–6.418) | 0.0052         |
| Never              | 281      | 14       | 312      | 19                         | 1.338 (0.575–3.113) | 0.4983         |
| Menopausal status  |          |          |          |                            |                 |
| Premenopausal      | 149      | 10       | 178      | 12                         | 0.939 (0.391–2.257) | 0.8885         |
| Postmenopausal     | 266      | 31       | 288      | 17                         | 1.857 (0.997–3.460) | 0.0507         |

\(^a\) (Ile/Val vs. Ile/Ile)
\(^b\) Chi square analysis
All \(p\)-values were adjusted by age. Significant results are highlighted in bold font

CI confidence interval, OR odds ratio

Fig. 1 Adjusted odds ratio (OR) plot for genotyping frequencies of the CYP1A1 Ile462Val polymorphism for patients stratified based on parity, oral contraceptive use, tobacco smoking, and menopausal status. Each OR value is represented by the corresponding black dot with arms representing 95 % confidence intervals (95 % CI)
white blood cells as compared with smokers without that variant [32]. Although the CYP1A1 Ile462Val substitution situated in the heme-binding region of the enzyme did not change the enzyme’s activity in vitro, this substitution is accompanied by a twofold increase in microsomal enzyme activity [33, 34]. The CYP1A1 Ile462Val SNP has been proposed to be in complete LD with the CYP1A1 T3801C (rs4646903) polymorphism, which experimentally exhibited up-regulation of enzymatic activity [9].

Moreover, we found a borderline risk for cervical cancer in women with the Ile462Val SNP that had a positive history of parity, which is in agreement with other studies demonstrating parity as a risk factor for cervical cancer [35]. It has been suggested that high parity might increase the risk of cervical carcinoma owing to the many years of transformation of the exocervical zone, leading this area to become more susceptible to direct exposure to HPV and carcinogens [35].

| Genotype  | Ile/Ile | Ile/Val | OR (95 % CI) | p-Value |
|-----------|---------|---------|--------------|---------|
| Controls  | 466     | 29      |              |         |
| Patients  |         |         |              |         |
| Clinical stage | |         |              |         |
| IA        | 55      | 6       | 1.753 (0.697–4.410) | 0.2275  |
| IB        | 57      | 6       | 1.691 (0.673–4.250) | 0.2584  |
| IIA       | 53      | 6       | 1.819 (0.722–4.583) | 0.1982  |
| IIB       | 49      | 6       | 1.968 (0.779–4.973) | 0.1455  |
| IIIA      | 139     | 9       | 1.040 (0.481–2.251) | 0.9200  |
| IIIB      | 48      | 5       | 1.674 (0.619–4.526) | 0.3051  |
| IVA       | 7       | 2       | 4.591 (0.912–23.107) | 0.1006c |
| IVB       | 7       | 1       | 2.296 (0.273–19.300) | 0.3907c |

Histologic grade

- G1: 80 / 7, OR 1.406 (0.596–3.319), p = 0.4347
- G2: 133 / 12, OR 1.450 (0.720–2.920), p = 0.2958
- G3: 91 / 7, OR 1.236 (0.525–2.908), p = 0.6267

Histologic type

- Squamous cell carcinoma: 353 / 31, OR 1.407 (0.832–2.379), p = 0.2003
- Adenocarcinoma: 48 / 6, OR 2.009 (0.794–5.081), p = 0.1336
Our genetic evaluation is the first to demonstrate that the CYP1A1 Ile462Val polymorphism can be a risk factor for cervical cancer in women with a positive history of tobacco smoking and parity, therefore this study should be replicated in other independent cohorts.

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