Research Article

Epistatic Interaction of CYP1A1 and COMT Polymorphisms in Cervical Cancer

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There is a clear association between the excessive and cumulative exposure to estrogens and the development of cancer in hormone-sensitive tissues, such as the cervix. We studied the association of CYP1A1 M1 (rs4646903) and COMT (rs4680) polymorphisms in 130 cervical cancer cases (c-cancer) and 179 controls. The CYP1A1 TT genotype was associated with a lower risk for c-cancer (OR = 0.39, \( p = 0.002 \)). The allele C of CYP1A1 was for c-cancer (OR=2.29, \( p = 0.002 \)). Women with COMT LL genotype had a higher risk of developing c-cancer (OR = 4.83, \( p < 0.001 \)). For the interaction of the CYP1A1 & COMT, we observed that TC&HL genotypes had a greater risk for c-cancer (OR = 6.07, \( p = 0.006 \)) and TT&HL genotypes had a protection effect (OR = 0.24, \( p < 0.001 \)). The CYP1A1 TT and COMT LL genotypes had higher estradiol levels in c-cancer (\( p < 0.001 \) and \( p = 0.037 \), resp.). C-cancer is associated with less production of 2-methoxy-estradiol resultant of functional polymorphisms of CYP1A1 and COMT, separately. CYP1A1 and COMT work in a metabolic sequence and their interaction could lead to an alternative pathway of estrogen metabolism with production of 16-OH-estrone that is more proliferative.

1. Introduction

Cervical cancer is the fourth most common cause of cancer in women worldwide [1]. The association between human papillomavirus (HPV) infection and the development of cervical cancer has been established [2]. These viruses have a sexual transmission and can cause cancer in the cervix, anus, vulva, vagina, penis, and oropharyngeal cancers [3, 4]. Cervical cancer arises via a series of four steps: HPV transmission, viral persistence, progression of a clone of persistently infected cells to precancer, and invasion [5]. Backward steps may occur, namely, clearance of HPV infection. Regression of precancer to normality may also occur but is less frequent [5]. Cervical cancer develops slowly; it is often established over a decade after initial infection with high-risk HPV and only arises in those women whose infections do not resolve spontaneously. Infection by high-risk HPV is necessary but not sufficient for progression to cancer [6]. Mutations in cellular genes and chromosomal rearrangements induced by genomic instabilities are important contributing events [2, 3, 7]. There is a clear association between the excessive and cumulative exposure to estrogens and the development of cancer in hormone-sensitive tissues [7]. The carcinogenesis associated with estrogens may be the result of multiple overlapping mechanisms. One major pathway is the extensively studied hormonal pathway, by which estrogen stimulates cell proliferation through nuclear estrogen receptor-mediated signaling, thus resulting in an increased risk of genomic
mutations during DNA replication. However, estrogen can also lead to cancer through its metabolism, producing reactive species of oxygen (ROS) and metabolites that form DNA adducts [8–10]. Two pathways can metabolize estrogen: the 16α-hydroxyestrone pathway and the formation of catechol-estrogens [11]. The pathway that leads to the formation of catechol-estrogens starts with the hydroxylation of estrogens, reaction mediated by the enzymes CYPIA1 and CYPBI1, which yields 2-hydroxy-estrogen (2-OH-CE) and 4-hydroxy-estrogen (4-OH-CE), respectively [8, 12]. Catechol-estrogens may suffer oxidation, forming semiquinones and quinones, in a process that leads to the formation of ROS and DNA adducts, that may damage the DNA, resulting in mutations [8, 10, 13, 14].

However, catechols can be methylated by COMT resulting in metabolites that do not yield quinones or ROS. In fact, the methylation of 2-hydroxy-estrogen leads to the formation of 2-methoxy-estrogen, which has been shown to have antiapoptotic and antiangiogenic properties [10]. Estrogen receptors may also influence cervical cancer by binding to an ERE (estrogen responsive element) in the viral genome, leading to an increased expression of oncogenic HPV genes of E6 and E7 [15, 16]. Therefore, CYPIA1 and COMT are important enzymes in the metabolism of estrogen. The role of CYPIA1 as a key enzyme involved in the metabolic activation of polycyclic aromatic hydrocarbons is very well established. The gene for this enzyme is located on the long arm of chromosome 15 (15q22–q24) [17, 18].

2. Materials and Methods

2.1. Subjects. This investigation was conducted as part of a case-control study of genetic polymorphisms in cervical cancer. Written informed consent was obtained from all participants. The 130 participating women had cervical cancer with ages ranging from 19 to 77 years (mean: 42.75 ± 13.18 years) and only 35.1% had smoking habits.

At the first visit, women in the study group were observed after evaluation of diagnostic tests, in order to make the correct clinical stage cancer (Protocol Gynecology Oncology Service). Women referenced by precancerous lesions were examined by colposcopy for identification of these lesions and then the cytology/histology was performed. All original histological examinations (preneoplastic lesions and cancer) were consigned by the Pathology Service of Virology Laboratory of the Portuguese Institute of Oncology.

2.2. Genomic DNA Isolation. Whole blood samples from patients and controls were stored with EDTA at −20°C. The genomic DNA was isolated through a nonenzymatic method adapted from Lahiri and Numberger (1991) method [22].

2.3. Genotyping of CYPIA1 M1 (rs4646903). The CYPIA1 genotypes were determined by the polymerase chain reaction (PCR) technique; the polymorphic region was amplified in a 50 μL reaction mixture: 10 mM of each primer (forward: 5'-CCTTCTTGCTGGACCCCAT-3' and reverse: 5'-GGAAGTCCAATACTGGCACC-3'), 200 ng of genomic DNA, and 0.2 mM of PCR nucleotide Mix Thermo Scientific DreamTaq Green containing 10 mM dNTPs, 1.5 mM MgCl₂, and 1 U Taq polymerase. PCR conditions involved an initial denaturation of DNA at 94°C for 2 min (hot start), followed by 35 cycles of amplification at 94°C for 30 sec (melting), 58°C for 30 sec (annealing), and 72°C (extension) for 75 sec. The amplified fragments of 899 bp were then digested by the restriction endonuclease MspI at 37°C for 16 h according to the manufacturer's recommendations. The digestion products were analyzed by electrophoresis in 3% agarose gel stained with ethidium bromide (10μg/mL) for 60 minutes, with 80 volts. With this process we are able to differentiate genotypes: the TT genotype gives rise to one single band of 899 bp; the CC genotype appears as two bands, one with 693 bp and the other with 206 bp; the TC genotype has all three bands.

2.4. Genotyping of COMT (rs4680). Using the PCR technique, the polymorphic region was amplified in a 50 μL reaction mixture: 10 mM of each primer (forward: 5'-GGCTCA-TCACCATGCAGATCA-3' and reverse: 5'-CCAGGTCTCTTGCAACGGGTCA-3'), 200 ng of genomic DNA, and 0.2 mM of PCR nucleotide Mix Thermo Scientific DreamTaq Green containing 10 mM dNTPs, 1.5 mM MgCl₂, and 1 U Taq polymerase. PCR conditions involved an initial denaturation of DNA at 94°C for 2 min (hot start), followed by 35 cycles of amplification at 94°C for 45 sec (melting), 60°C for 45 sec (annealing), and 72°C (extension) for 60 sec. After the 35 cycles, there is a final extension phase for 7 minutes at 72°C. The amplified fragments were then digested by the restriction endonuclease NlaIII at 37°C for 18 hours and 20 min at 65°C according to the manufacturer's recommendations.
The digestion products were analyzed by electrophoresis in 3% agarose gel stained with ethidium bromide (10 μg/mL) for 90 minutes, with 85 volts. With this process we are able to differentiate genotypes: the HH genotype gives rise to two bands, one of 111 bp and the other of 89 bp; the LL genotype appears as one single band, with 71 bp; the HL genotype has all three bands.

2.5. Estradiol. The levels of estradiol were determined by ELISA kit (R&D Systems) according to manufacturer’s instructions.

2.6. Statistical Analysis. Observed genotype frequencies were tested for deviation from Hardy-Weinberg equilibrium (HWE) with the Chi-square goodness-of-fit test ($\chi^2$). This test was also used to evaluate the significant differences between the two populations, in order to know if the odds ratio (OR) test was justifiable. OR for cervical cancer risk and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to each polymorphism, to analyze its risk factor and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to each polymorphism, to analyze its risk factor and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to each polymorphism, to analyze its risk factor and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to each polymorphism, to analyze its risk factor and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to each polymorphism, to analyze its risk factor and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to each polymorphism, to analyze its risk factor and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to each polymorphism, to analyze its risk factor and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis.

3. Results

We performed the determination of CYPIA1 (rs4646903) and COMT (rs4680) polymorphisms in 130 women with cervical cancer and 180 healthy women. The control group had significantly higher ages than patients ($p = 0.001$), being the majority with age higher than 40 years old ($p = 0.029$). Actually, most of women from control group were postmenopausal (≥50 years old) (69.4%), contrarily to cervical cancer women (age < 49 years old, 72.8%) ($p < 0.001$) (data not shown).

For estradiol levels, we observed that women with cervical cancer had higher levels (Table 3), $p < 0.001$.

### Table 1: Distributions of CYPIA1 M1 (rs4646903) polymorphism in cervical cancer cases and controls.

| CYPIA1 | Controls | Cervical cancer | $P$ | OR [95% CI] | OR crude$^a$ | OR adjusted$^a$ | $p^*$ | $p^*$ |
|--------|----------|-----------------|-----|------------|-------------|--------------|-------|-------|
| rs4646903 |          |                 |     |            |             |              |       |       |
| TT     | 99 (79.8) | 64 (61.0)       |     | 0.39 [0.22–0.71] | 0.66 [0.27–1.66] | **0.002** | 0.381 |
| TC     | 23 (18.5) | 36 (34.3)       | $0.006$ | 2.29 [1.25–4.20] | 1.25 [0.48–3.27] | **0.007** | 0.655 |
| CC     | 2 (1.6)   | 5 (4.8)         |     | 3.05 [0.58–16.06] | 3.16 [0.33–30.22] | 0.188 | 0.317 |
| T      | 221 (89.0) | 164 (78.0)     | $0.002$ | 0.44 [0.26–0.73] | 0.63 [0.29–1.39] | **0.002** | 0.255 |
| C      | 27 (11.0) | 46 (22.0)       |     | 2.29 [1.37–3.85] | 1.58 [0.72–3.48] | **0.002** | 0.255 |

$^a$The values for the genotypes and respective allele frequencies represent absolute frequencies (relative frequencies, %). $p$: $\chi^2$ test values; OR: odds ratio; $p^*$: values for OR crude; $p^*$: values adjusted for age (regression binary logistic); values statistically significant for $p < 0.05$.

3.1. CYPIA1 M1 (rs4646903). Allele and genotype frequencies in cervical cancer and healthy women for CYPIA1 M1 polymorphism are shown in Table 1. The genotype distributions in control group were in HWE equilibrium ($p > 0.05$). We observed significant differences between cervical cancer patients and healthy women ($p = 0.006$). The CYPIA1 TT genotype was more frequent in control group (79.8%) (Table 1). On one hand, individuals carrying the TC genotype were 2.29-fold at a higher risk for developing cervical cancer compared with those having homozygous association of CYPIA1 CC & TT genotypes ($OR = 2.29, 95\% CI = 1.25–4.20$, $p = 0.006$). On the other hand, the association of CYPIA1 TC & CC genotypes represents almost 3-fold risk for patients ($OR = 2.54, 95\% CI = 1.41–4.57$, $p = 0.002$) (data not shown).

Indeed, we observed significant differences in distribution of CYPIA1 allelic frequencies ($p = 0.002$), where the allele C is a risk for cervical cancer (Table 1). However, all these results lose statistical significance when adjusted for age ($p > 0.05$) (Table 1).

We also evaluate the levels of estradiol in relation to the studied polymorphisms (Table 3). According with the following model as CYPIA1 TC & CC and CYPIA1 TT genotypes separately, we observed significant higher values of estradiol in cervical cancer ($p > 0.001$) (Table 3). Although not statistically significant, the values of estradiol in patients were slightly higher in CYPIA1 TT genotype (data not shown) (Table 3).

3.2. COMT (rs4680). Table 2 represents the distribution of COMT genotypes and allelic frequencies between cervical cancer patients and healthy women. Using $\chi^2$ test, we established that the control group was in HWE ($p > 0.05$). We observed statistically significant differences in genotype and allelic frequencies ($p = 0.003$ and $p = 0.015$, resp.). The COMT HH and HL genotypes were more frequent in control group and COMT LL genotype in patients (Table 2). The allele H was more frequent in controls contrary to cervical cancer patients ($p = 0.015$, 70% and 40%, resp.). Indeed, when adjusted for age, the allele H was protector for cervical cancer ($OR = 0.34, 95\% CI = 0.21–0.56$, $p < 0.001$), where the allele L is a risk, which could represent up to 3 times more risk ($OR = 2.93, 95\% CI = 1.80–4.78$, $p < 0.001$).
whether profiles of To investigate 3.3. Interaction of CYP1A1 (rs4646903) and COMT (rs4680) (datanotshown). 

representupto5timesmorerisk(OR=4.83,95%CI=2.08–11.20, 𝑝<0.001).

Because the enzymes COMT and CYP1A1 are closely related and work in metabolic sequence in the metabolism of estrogens, it is important to examine if the functional polymorphisms of these enzymes in association change the risk to develop cervical cancer in the carriers. In Table 4 it is shown the different associations of CYP1A1&COMT genotypes between patients and healthy women. From all possible associations, we identified two models that best fit as protection or risk in cervical cancer. The highest risk for cervical cancer was for the association of CYP1A1&COMT TC&HL (OR = 6.07, 95% CI = 1.67–22.09, 𝑝= 0.006), without adjusting for age. We also observed that the CYP1A1&COMT TC&HH might increase the risk for cervical cancer, though with a wide confidence interval (OR = 13.67, 95% CI = 1.72–109.39, 𝑝= 0.014) (data not shown). In the other side, for protection we identified the CYP1A1&COMT TT&HL as a best model, even when adjusted for age (OR = 0.18, 95% CI = 0.06–0.53, 𝑝= 0.002).

Finally, when we adjusted these models for estradiol levels, we observed that the CYP1A1&COMT TT&HL genotypes maintain their protection role for cervical (OR = 1.32; 95% CI = 1.12–1.55, 𝑝= 0.001).

For adjusted OR, the COMT HH genotype seems to be protector for cervical cancer (OR = 0.31, 95% CI = 0.15–0.65, 𝑝= 0.002), but the COMT polymorphism protection role was more statistically significant when associated the COMT HH&HL genotypes (OR = 0.21, 95% CI = 0.09–0.48, 𝑝< 0.001) (data not shown). When evaluating the risk for cervical cancer associated with the COMT genotypes, we observed that the best model was the COMT LL genotype, which could represent up to 5 times more risk (OR = 4.83, 95% CI = 2.08–11.20, 𝑝< 0.001).

For the levels of free estradiol, we found higher values for patients compared to healthy women, when considering the COMT HH&HL and COMT LL genotypes, separately (Table 3). Although not statistically significant, we observed that cervical patients had higher values of estradiol in COMT LL genotype (data not shown) (Table 3). When adjusted for estradiol's levels, we did not find statistically significant differences in COMT polymorphism in our sample (data not shown).

3.3. Interaction of CYP1A1 (rs4646903) and COMT (rs4680) Polymorphisms in an Epistatic Relation. To investigate whether profiles of CYP1A1 and COMT genotypes might be associated with the risk of cervical cancer, we examined the effect combinations of genotypes.

Because the enzymes COMT and CYP1A1 are closely related and work in metabolic sequence in the metabolism of estrogens, it is important to examine if the functional polymorphisms of these enzymes in association change the risk to develop cervical cancer. In Table 3 it is shown the different associations of CYP1A1&COMT genotypes between patients and healthy women. From all possible associations, we identified two models that best fit as protection or risk in cervical cancer. The highest risk for cervical cancer was for the association of CYP1A1&COMT TC&HL (OR = 6.07, 95% CI = 1.67–22.09, 𝑝= 0.006), without adjusting for age. We also observed that the CYP1A1&COMT TC&HH might increase the risk for cervical cancer, though with a wide confidence interval (OR = 13.67, 95% CI = 1.72–109.39, 𝑝= 0.014) (data not shown). In the other side, for protection we identified the CYP1A1&COMT TT&HL as a best model, even when adjusted for age (OR = 0.18, 95% CI = 0.06–0.53, 𝑝= 0.002).

Finally, when we adjusted these models for estradiol levels, we observed that the CYP1A1&COMT TT&HL genotypes maintain their protection role for cervical (OR = 1.32; 95% CI = 1.12–1.55, 𝑝= 0.001).

4. Discussion

This is the first study in Portugal that exposes the interaction of CYP1A1 and COMT polymorphisms in cervical cancer.

In the current study, it was shown that women with cervical cancer have the genotypes of CYP1A1 M1 TC&C, which represented almost 3-fold risk when associated to CYP1A1 CC genotype, in a dominant model (Table 1). Indeed, the allele C of CYP1A1 features associated to risk for cervical cancer, which contributes to a higher activity expression of
the enzyme. So, according to other authors, the carriers of these genotypes may have a higher concentration of catechol-estrogens, when compared to the genotype TT that represents a lower inducible enzyme activity [18, 20].

Also in relation to the metabolism of estrogens, the COMT appears to be an important enzyme on metabolisms of phase two (II) [23]. In our study, the allele L of COMT Gi58A gives rise to a lesser enzymatic activity, being a risk factor, since it is associated with a higher production of 4-OH-CE, a catechol-estrogen, that may enter redox cycles to form quinones that may lead to DNA lesions. The catechol oxidation to quinones is a competitive reaction with its methylation; the diminished activity of COMT will lead to oxidation to quinones that may lead to DNA lesions. The catechol is affected by age, contrarily to what was observed for COMT polymorphism, due to the negative feedback that the methylated products of COMT have on CYP1A1's activity and CYP1B1's activity. This inhibition probably leads to a shift in the pathways of detoxification of estrogens to the 16α-hydroxylation pathway, catalyzed by CYP3A4. This pathway presents proliferative effects on cells, which could contribute to an increase in the risk for cervical cancer [26, 27].

In our study, we also observed that the CYP1A1 polymorphism seems to be somehow affected by age, compared to what was observed for COMT polymorphism, due to the more dependency on hormone induction of CYP1A1 by aryl hydrocarbon receptor (AhR) [28]. Thus, the increased susceptibility induced by the studied polymorphism for cervical cancer might be associated with a greater immune depression associated with AhR [29].

Knowing that estradiol stimulates the growth of HPV-positive cervical cancer cells [15] and as described by other authors, the levels of free estradiol are higher when the activity of COMT were low (COMT LL genotype) [30], contrary to CYP1A1. Noteworthy, most of cervical cancer women were perimenopausal, which is consistent with higher levels of estradiol, ultimately, within COMT polymorphism, affecting the formation of 2-methoxyestradiol and immune system [31].

Actually these results may have a plausible explanation since these enzymes work in metabolic sequence in the metabolism of estrogens.

5. Conclusion

The study of the COMT and CYP1A1 polymorphisms individually resulted in predictable results according to the published works. The lower activity of COMT represents a higher risk for cervical cancer and so does the highest activity of CYP1A1. However, when we studied the interaction of these polymorphisms, our results were apparently contradictory. We observed that the genotypes for CYP1A1 and COMT for intermediate activity represented the highest risk for developing cervical cancer of that observed individually for COMT genotype that was protector. The COMT polymorphism possibly represents a more important role for protection than that of risk for cervical cancer. The other possible model
for risk, namely, the CYPIA1&COMT TC&HH genotypes, may be due to a series of facts: either the methylation of COMT’s promoter that causes hypoxpression of the enzyme or the fact that COMT has a highest affinity for 2-OH-CE, leaving 4-OH-CE free to enter redox cycles and to suffer oxidation to more mutagenic quinones. It can also be due to the negative feedback that methylated products of COMT have on CYPIA1 and CYP1B1; this inhibition probably leads to a shift in the estrogen metabolizing pathways, causing proliferative effects on cells, which could contribute to an increase in the risk for cervical cancer. Therefore, there are still studies to be done in this area to truly understand the role of estrogens and key enzymes in its metabolism in cervical cancer.

These results can help to define an individualized tumor prevention and therapy based in characteristics of the host and to contribute to the understanding of the oncogenesis behind cervical cancer.

Conflict of Interests
The authors declare that they have no conflict of interests.

Authors’ Contribution
Andreia Matos and Cindy Castelão contributed equally to the paper.

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