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Associations differ by sex for catechol-*O*-methyltransferase genotypes and bladder cancer risk in South Egypt☆

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Abstract

Objectives—To examine associations between urinary bladder cancer risk and polymorphisms of the gene encoding the catechol estrogen-metabolizing enzyme, catechol-*O*-methyltransferase (COMT), among Egyptian women and men.

Materials and methods—We used questionnaire and genotype data from a case-control study in Egypt. This analysis focused on South Egypt cases with confirmed urothelial (UC) or squamous cell (SCC) carcinoma of the bladder, and controls frequency-matched on sex, 5-year age-group, and residence governorate. Real-time PCR on blood specimen DNA was used to determine COMT genotypes encoding for *Val/Val*, *Val/Met*, and *Met/Met*, the enzyme forms associated with high, intermediate, or low activity, respectively.

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Results—The study sample, which included 255 women and 666 men, consisted of 394 cases with histologically confirmed UC (225) or SCC (n=169), and 527 controls. The odds of having either type of bladder cancer was lower among men with genotypes encoding *Val/Met* or *Met/Met* than among those with the genotype encoding *Val/Val*, even after adjustment for other factors, such as smoking and schistosomiasis history [adjusted odds ratio (AOR): 0.64; 95% confidence interval (CI): 0.43, 0.96]; however, the association was statistically significant for SCC (AOR 0.57; 95% CI: 0.34, 0.96) but marginal for UC (AOR: 0.64; 95% CI: 0.39, 1.02). No significant associations were detected between bladder cancer risk and COMT genotypes among postmenopausal women.

Conclusions—These findings suggest that, even after controlling for established risk factors, the involvement of COMT genotypes in bladder cancer risk differs among men compared to women in South Egypt.

Keywords

Catechol-*O*-methyltransferase gene polymorphism; Urinary bladder cancer; Smoking; Schistosomiasis; Sex differences; Egypt

1. Introduction

The two main histological types of urinary bladder cancer include urothelial carcinoma and squamous cell carcinoma (UC and SCC, respectively). UC is associated with cigarette smoking and occupational exposures, occurs at older ages (>60 y), and accounts for >90% of malignancies in developed countries [1]. In contrast, SCC is associated with chronic bladder inflammation, occurs at younger ages (<50 y), and accounts for >75% of cases in areas endemic for *Schistosoma haematobium* infection [2, 3]. However, these well-established risk factors do not account for all bladder cancer and do not explain the disproportionate incidence found worldwide among men compared to women [4, 5].

In Egypt, bladder cancer is the most common malignancy among men, and the male-to-female ratio is estimated at 4:1 [1, 2, 6]. Despite reports of increasing numbers of male but not female smokers [4] and a shift in the country's most common bladder cancer type from SCC to UC after schistosomiasis eradication measures in the 1980s [1, 2], the expected drop in the incidence of this malignancy among women did not materialize. The persistence of the 4:1 pre-eradication male-to-female ratio indicates that schistosomiasis and smoking do not completely account for the excess among men in Egypt [1, 4], consistent with findings from studies addressing bladder cancer worldwide [4, 5].

The quest to understand the male excess incidence in bladder cancer has spurred investigations into the roles of other possible risk factors, including genetic and environmental exposures and their interactions. The gene encoding catechol-*O*-methyltransferase (COMT), a steroid hormone pathway enzyme, has been considered a candidate for bladder cancer because it mediates metabolism of endogenous and exogenous estrogens and other potentially carcinogenic compounds, such as those from tobacco smoke exposure and other agents that produce inflammation [7–16]. COMT reduces the formation of reactive oxygen species (ROS) by catalyzing their methylation and inactivation, thus reducing the potential for DNA damage and carcinogenesis [16].

Found on chromosome 22, the gene encoding the COMT enzyme has a single nucleotide polymorphism where a G→A change at codon158 leads to a valine→methionine substitution in the enzyme. The resulting *Val/Val*, *Val/Met*, and *Met/Met* variants have been characterized as high-, intermediate-, and low-activity enzymes, respectively [17]. Only three studies in the searchable literature have investigated associations between COMT

genotypes and bladder cancer risk; they focused primarily on men and the UC type of bladder cancer, with inconsistent findings [18–20]. To further explore the possible role of COMT, we examined associations between COMT genotypes and bladder cancer risk by sex, as well as by case type (UC vs.SCC), among case-control study participants in Egypt.

2. Materials and Methods

The source study uses a case-control design to recruit cases from the three major referral centers for bladder cancer patients in Egypt: (1) The National Cancer Institute (NCI) of Cairo University in Cairo, (2) The Minia Oncology Center in Minia, and (3) The South Egypt Cancer Institute in Assiut. Institutional Review Boards of these three recruitment sites; the University of Maryland, Baltimore; and Georgetown University approved the study. As previously described [21], trained interviewers approached patients referred to the departments of urology, radiation therapy, or surgery if they had been diagnosed with presumed urinary bladder cancer. Only patients who were >18 y of age, diagnosed within 1 y, and self-identified as well enough to complete a 20-min interview were eligible. After explaining the purposes and procedures of the study, the recruiters obtained informed consents from and enrolled only those patients who agreed to participate. Of the 1759 presumed cases approached between July 2006 and November 2008, 92.3% were eligible, and 95.3% agreed to participate.

Healthy controls were randomly selected from the general population to frequency-match the cumulative group of cases by sex, 5-year age-group, and governorate of residence [21]. The study recruiters obtained the Ministry of Health's approval, visited each of the villages selected within the governorates, and approached occupants of houses on each randomly chosen street. If none of the residents matched the required sex and age-groups, the recruitment team moved to the next house. In the home, the trained interviewer explained the purpose of the study and secured informed consent. On each prospective participant, the study physician who accompanied the recruitment team performed abdominal ultrasound, a procedure not commonly provided at the local health clinics although helpful in revealing abdominal anomalies. The study physician used the ultrasound results to exclude those with questionable tumor-like masses in the bladder, referring each individual with abnormal findings to the nearest governmental health unit for follow-up. Of the 1,919 eligible control subjects, 98.9% agreed to participate.

The study questionnaire was used to collect data on sociodemographic characteristics, exposure to environmental tobacco smoke (ETS) at home and elsewhere, and medical history, including history of schistosomiasis and its treatment, as well as other urinary tract infections (UTIs). Women were queried for reproductive history, including menstrual status and age at menopause.

No proxy interviews were conducted. Each recruiter administered the questionnaire face-to-face and obtained a venous blood sample from each participant.

DNA was extracted from buffy coats using the QIAamp DNA Mini Kits (QIAGEN, Valencia, CA, USA). For the COMT genotyping, real-time PCR was performed using the ABI PRISM 7900 Sequence Detection System 2.1 (Applied Biosystems, Foster City, CA). Reactions were carried out in 384-well plates, each containing 15- μ l total volume: 7.5 μ l of the TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA), 0.6 μ l each of each probe (COMT1-FAM TCGCTGGCGTGAAG and COMT1-VIC TTCTGCTGGCATGAAG), 0.675 μ l each of each primer (COMT1-F CCCAGCGGATGGTGGAT forward and COMT1-R CAGGCATGCACACCTTGTC reverse), and 4.95 μ l of dH₂O. Each plate included two negative controls using 7.5 μ l dH₂O.

and 7.5 µl of the TaqMan® Genotyping Master Mix. PCR conditions included Stage 1, consisting of 1 cycle at 95°C for 10 min, and Stage 2, consisting of 40 cycles at 95°C for 15 sec and at 60°C for 1 min. For quality control, genotyping of 10% of the samples from each plate was repeated, and if 100% results concordance was not observed, genotyping for all samples on the plate was repeated. Also, if >5% of samples from a plate yielded indeterminate results, genotyping of all samples on the plate was repeated.

One of the study's two pathologists from the NCI of Cairo University reviewed the histology for each case and classified the tumor as UC, SCC, adenocarcinoma, or undifferentiated carcinoma of the urinary bladder (90% of the reviewed cases); other tumors, including metastases, were excluded.

Because more than 80% of the cases, and thus the majority of the genotyped specimens, were from South Egypt, we limited this analysis to data from participants who reported South Egypt governorates of residence.

The main variable of interest was the COMT genotype, used as a dichotomous [(1) *Met/Met* or *Met/Val* vs. (2) *Val/Val*] or trichotomous [(1) *Met/Met*, (2) *Val/Met*, or (3) *Val/Val*] variable. The variables collected from the questionnaire were used as continuous or categorical in the exploratory analyses, and some were regrouped base on their distributions.

Statistical methods consisted of univariate, bivariate, and stratified analyses, followed by unconditional logistic regression modeling. We carried out analyses for men and women separately. Age and any variables with which bladder cancer risk was significantly associated were included in the unconditional logistic model and also tested for potential interactions with the main variable of interest. Unadjusted (UORs) and adjusted odds ratios (AORs), as well as 95% confidence intervals (CIs), were estimated for the associations between the COMT genotype and bladder cancer risk. Additionally, the Hardy-Weinberg equilibrium test was used to compare observed and expected COMT genotype frequencies among controls, female controls, and male controls, and the Cochran-Armitage test was used to evaluate the statistical significance of trends observed in the UORs estimating the association between COMT genotypes and bladder cancer risk using the trichotomous COMT variable. All of the analyses were performed with SAS version 9.1.

3. Results

The study sample for this analysis consisted of the controls and the SCC and UC cases who had been COMT-genotyped by March 2009 (n=921: 394 histologically confirmed cases, 169 SCC, 225 UC; 527 controls).

Table 1 shows the distribution of the key variables among cases and controls by gender. Equal proportions of female and male cases and controls were COMT heterozygotes. None of the female controls reported having ever smoked cigarettes, and only 3 (~3%) of the female cases indicated that they currently smoked cigarettes or shisha (also known as waterpipe). More than twice as many male (62.0%) as female (22.5%) cases reported having had schistosomiasis. Among women, 46 were still menstruating, and the remaining were postmenopausal for 11.3 y on average. Among the cases, the male:female ratio was 2.8 for SCC and 5.2 for UC.

After exclusion of 17 participants with indeterminate COMT genotypes, the sample size for the statistical analysis was 904. Among controls the observed COMT genotype distributions were not significantly different from the Hardy-Weinberg expected frequencies (X^2 p-value for controls: overall, 0.36; females, 0.74; males, 0.38).

Among the women, the COMT genotype was not statistically significantly associated with odds of having bladder cancer (UOR: 0.70, 95% CI: 0.38, 1.28). When only postmenopausal women were included in the analysis, similar results were observed (UOR: 0.90, 95% CI: 0.47, 1.74) (Table 2). Among premenopausal women, decreased odds of having SCC were marginally significantly associated with the *Met/Met* or *Met/Val* COMT genotype category (UOR: 0.13, 95% CI: 0.02, 0.77), but the small subgroup size (n=46) prohibited further meaningful analysis.

Among the men (Table 2), the *Met/Met* or *Val/Met* COMT genotype category was significantly associated with reduced odds of having bladder cancer (UOR: 0.63, 95% CI: 0.44, 0.91). To assess the potential gene-dose effect, the Cochran-Armitage test was performed with COMT as a trichotomous variable (*Val/Val*, *Val/Met*, *Met/Met*), where *Val/Val* remained the reference group. The results indicated a significant trend (2-sided p-value=0.004) for the unadjusted associations between decreasing bladder cancer risk and the number of *Met* alleles (Table 2). Neither history of schistosomiasis nor history of other UTIs were significantly associated with the COMT genotype.

In the binary logistic regression model the *Val/Met* or *Met/Met* genotype category remained significantly associated with reduced bladder cancer risk in men (AOR: 0.64, 95% CI: 0.43, 0.96), even after adjustment for all significant covariates (Table 3). There were no significant interaction terms.

Using the trichotomous COMT variable in the model the AOR trend for *Val/Met* (0.69, 95% CI: 0.44, 1.06) and *Met/Met* (0.57, 95% CI: 0.35, 0.93) was consistent with the trend observed with the Cochran-Armitage test for the unadjusted estimates, and the model yielded nearly the same adjusted associations for the covariates as found with the dichotomous COMT (Table 3).

We further examined the association between the COMT genotype and each type of bladder cancer among men, using separate logistic regression models for SCC and UC cases (Table 3). After adjusting for covariates, the *Val/Met* or *Met/Met* genotype category was significantly associated with reduced SCC odds (AOR: 0.57, 95% CI: 0.34, 0.96), but only marginally with UC (AOR: 0.64, 95% CI: 0.39, 1.02).

4. Discussion

We found that the low- or intermediate-activity (*Val/Met* or *Met/Met*) COMT genotype category was associated with decreased odds of having either type of bladder cancer among men in South Egypt, after adjustment for known risk factors, including smoking, schistosomiasis, and other UTI history; the association was significant for the SCC but marginal for the UC type. No significant associations were observed among women between COMT genotypes and either type of bladder cancer risk.

Our study is the first to investigate the association between COMT genotypes and SCC risk. The only three previous studies to examine such associations have focused on the UC type. Two, conducted in France and Italy with samples limited to male sex and the UC type, detected no significant associations [18, 19]. Using a sample of nonsmokers exposed to air pollution and ETS, the third investigation found a significant association between increased bladder cancer risk, the COMT *Val/Met* variant, and three other genes involved in metabolism and DNA repair; it included women and men, but the sample size was relatively small (n=124) [20]. Given that our finding of an association between the *Val/Met* or *Met/Met* genotype category and decreased odds of having bladder cancer among men pertains to the SCC type, it is not necessarily inconsistent with the report from The Netherlands where the UC type predominates.

COMT is characterized as an important enzyme that catabolizes estradiol metabolites into inactive compounds no longer able to bind to estrogen receptors and exert their effects on cell DNA [9, 15, 17, 22–24]. Therefore, we hypothesized that a COMT genotype encoding the high-activity enzyme form (*Val/Val*) would be associated with decreased odds of having bladder cancer. Our results did not support this hypothesis; on the contrary, we found an association between the COMT genotype category encoding the intermediate- or low-activity enzyme (*Val/Met* or *Met/Met*) and reduced odds of having bladder cancer, and only among men.

The mechanism behind the protective effect of the low-activity COMT genotype on bladder cancer risk among men is not clear. However, one may speculate that a polymorphism affecting the activity of COMT may directly or indirectly modulate the amount of estrogen available to bind to its receptors, and estrogens are protective against bladder cancer. Indeed, increased serum estradiol levels were reported in middle-aged men with the low-activity COMT genotype [25], and estrogens have been noted to protect against bladder cancer by other investigators [26] and by us recently [21]. Furthermore, estrogen and its metabolites were reported to act as prooxidants and antioxidants, i.e., to adversely affect, as well as to protect [27], depending on the cell type, timing of the exposure, and the interstitial milieu [10].

Another possible explanation for the association of the low- or intermediate-activity (*Val/Met* or *Met/Met*) COMT genotypes with decreased risk of bladder malignancy among men, which is stronger for the SCC than for the UC type, could involve the lower-activity enzyme's ability to reduce the burden of carcinogenic exposures resulting from inflammation and recognized as risk factors for SCC, but not other carcinogens present in tobacco smoke. In addition, low COMT activity might not be able to compensate for the anti-estrogenic effect of smoking, an established risk factor for UC [28].

In an alternative scenario, the higher-activity COMT enzyme could lead to increased formation of intermediates with potentially stronger carcinogenic effects than the original substrate, analogous to the mechanism demonstrated to occur with enzymes encoded by glutathione S-transferase theta gene variants [29–32]. COMT with lower activity would produce lower levels of such intermediates, and subsequently, a lower risk of malignant transformation.

The effect of the local cellular environment on enzyme activity may also contribute to explaining the lower risk of bladder cancer observed among men but not women. Unlike middle-aged men, among whom those with the low-activity COMT genotype were shown to have higher levels of estradiol than those with the high-activity COMT genotype [25], postmenopausal women with the low- activity COMT genotype do not have high levels of estradiol [33]. Consistent with the latter, our results show no significant association between COMT genotypes and odds of having either type of bladder cancer type among postmenopausal women, but a significant association among the subgroup of premenopausal women (UOR: 0.13, 95% CI: 0.02, 0.77). However, the small subgroup size (n=46) precluded further analyses. A larger study of young women with bladder cancers, although needed, will be difficult to conduct, considering that this malignancy occurs primarily among the elderly.

Furthermore, interactions of other exposures, such as dietary, environmental, and genetic factors, with the cellular hormonal milieu could potentially account for the observed association between the COMT genotype encoding lower-activity enzyme and decreased risk of SCC among men. For example, in a study that reported interactions between cruciferous vegetable intake and genotypes for *GSTM1* and *NAT2* enzymes, the odds of

having bladder cancer were the lowest among those who had the highest cruciferous vegetable intake and either the *GSTM1*-null or the *NAT2* (slow acetylator) genotype, both candidate genes that were previously associated with increased risk [34–37]. Manuguerra et al. [20] present additional evidence of significant genetic and environmental interaction between COMT and other DNA metabolic and repair pathway gene variants. Another recent study [38] demonstrates the presence of an estradiol-related molecule in *S. haematobium* worm extracts that could potentially compete with estrogens for COMT binding, up- or down-regulate the activity of the enzymes encoded by the COMT gene variants, and increase or decrease formation of and exposure to carcinogenic intermediates.

Additional factors, including occupational exposures and other genetic susceptibility loci, could modify the effects of the COMT genotype. We cannot rule out the possibility of linkage disequilibrium between the COMT polymorphism and a true but unknown susceptibility locus.

Notwithstanding such possibilities, our results suggest that, even after controlling for other major, established risk factors, the involvement of COMT genotypes in bladder cancer risk differs among men compared to women in South Egypt.

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Table 1

Sociodemographic characteristics, catechol-*O*-methyltransferase genotypes, medical history, and tobacco smoke exposure of bladder cancer controls and cases in South Egypt

Variable	Women n (%)		Men n (%)	
	Controls N = 175	Cases N = 80	Controls N = 352	Cases N = 314
SOCIODEMOGRAPHIC CHARACTERISTICS				
Mean age $\pm$ standard deviation (y)	56.3 $\pm$ 10.7	55.4 $\pm$ 11.2	54.3 $\pm$ 12.4	58.1 $\pm$ 11.0
Age category (y)				
<40	10 (5.7)	5 (6.2)	32 (9.1)	11 (3.5)
40 to 45	15 (8.6)	11 (13.7)	64 (18.2)	33 (10.5)
>45 to 50	32 (18.3)	14 (17.5)	53 (15.1)	36 (11.5)
>50 to 55	33 (18.9)	17 (21.2)	59 (16.8)	53 (16.9)
>55 to 60	34 (19.4)	9 (11.2)	41 (11.6)	55 (17.5)
>60 to 65	21 (12.0)	12 (15.0)	28 (7.9)	37 (11.8)
>65	30 (17.1)	12 (15.0)	75 (21.3)	89 (28.3)
Marital status				
Married	100 (57.1)	52 (65.0)	338 (96.0)	302 (96.2)
Widowed	72 (41.1)	26 (32.5)	12 (3.4)	8 (2.5)
Divorced or separated	0	2 (2.5)	0	1
Not married	3 (1.7)	0	2 (0.6)	3 (1.0)
Education				
None	151 (86.3)	75 (93.7)	198 (56.3)	222 (70.7)
Literacy classes or primary school	22 (12.6)	5 (6.2)	82 (23.3)	77 (24.5)
Preparatory, high, or technical school	2 (1.1)	0 (1.2)	61 (17.3)	15 (4.7)
College or university	0	0	11 (3.1)	0
CATECHOL-O-METHYLTRANSFERASE GENOTYPE (enzyme activity)				
<i>Val/Val</i> (high)	38 (21.7)	23 (28.8)	72 (20.5)	91 (29.0)
<i>Val/Met</i> (intermediate)	82 (46.8)	36 (45.0)	163 (46.3)	144 (45.9)
<i>Met/Met</i> (low)	50 (28.6)	20 (25.0)	110 (31.2)	75 (23.9)
Indeterminate	5 (2.9)	1 (1.2)	7 (2.0)	4 (1.3)
MEDICAL HISTORY				
Schistosomiasis history				
No	139 (79.4)	50 (71.4)	165 (46.9)	98 (33.4)
Yes	22 (12.6)	20 (22.3)	168 (47.7)	195 (66.5)
Unknown	14 (8.0)	10 (11.8)	19 (5.4)	21 (6.6)
Urinary tract infection other than schistosomiasis				
No	154 (88.0)	54 (67.5)	301 (85.5)	181 (57.6)

Variable	Women n (%)		Men n (%)	
	Controls N = 175	Cases N = 80	Controls N = 352	Cases N = 314
Yes	21 (12.0)	26 (32.5)	51 (14.5)	133 (42.4)
TOBACCO SMOKE EXPOSURE				
Cigarette and shisha smoking				
Never either	173 (100.0)	77 (96.2)	71 (20.2)	33 (31.7)
Shisha only (former or current)	2 (1.1)	2 (2.5)	45 (12.8)	42 (13.4)
Cigarettes only (former or current)	0	1 (1.2)	207 (59.0)	172 (54.8)
Both (former or current)	0	0	28 (8.0)	67 (21.3)
Environmental tobacco smoke (among never smokers only)				
None	66 (38.1)	25 (32.9)	21 (29.6)	10 (30.3)
Either inside or outside of the home	98 (56.6)	40 (52.6)	42 (59.1)	16 (48.5)
Both inside and outside of the home	9 (5.2)	11 (14.5)	8 (11.3)	7 (21.2)

Table 2

Unadjusted associations between bladder cancer and COMT genotypes among men and women from South Egypt

COMT genotype (enzyme activity)	Men (all cases)	Women (all cases)	Postmenopausal women (all cases)
	UOR (95% CI)	UOR (95% CI)	UOR (95% CI)
<i>Val/Val</i> (high)	REF	REF	REF
<i>Val/Met</i> (intermediate)	0.69 (0.47, 1.01)	1.38 (0.71, 2.63)	1.06 (0.53, 2.16)
<i>Met/Met</i> (low)	0.53 (0.35, 0.81)	1.51 (0.73, 3.17)	1.19 (0.54, 2.62)
p-value for trend	0.004	0.27	0.75
<i>Val/Val</i> (high)	REF	REF	REF
<i>Val/Met</i> or <i>Met/Met</i> (intermediate or low)	0.63 (0.44, 0.91)	0.70 (0.38, 1.28)	0.90 (0.47, 1.74)

Abbreviations: UOR: unadjusted odds ratio; CI: confidence interval; AOR: adjusted odds ratio; COMT: catechol-*O*-methyltransferase.

Table 3

Associations between COMT genotypes and odds of having different types of bladder cancer among men in South Egypt, using unconditional logistic regression models *

Independent predictor	All cases	SCC	UC
	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)
COMT genotype (enzyme activity)			
Val/Val (high)	REF	REF	REF
Val/Met or Met/Met (intermediate or low)	0.64 (0.43, 0.96)	0.57 (0.34, 0.96)	0.64 (0.39, 1.02)
Shisha and cigarette smoking			
Never either	REF	REF	REF
Shisha only (former or current)	1.59 (0.81, 3.12)	1.52 (0.65, 3.59)	2.07 (0.93, 4.64)
Cigarettes only (former or current)	1.56 (0.94, 2.60)	1.36 (0.69, 2.68)	1.90 (1.03, 3.53)
Both (former or current)	4.46 (2.27, 8.77)	3.79 (1.57, 9.16)	5.54 (2.55, 12.07)
Schistosomiasis history			
No	REF	REF	REF
Yes	2.11 (1.46, 3.05)	1.86 (1.14, 3.04)	2.69 (1.74, 4.14)

* Abbreviations: SCC: squamous cell carcinoma; UC: urothelial (cell) carcinoma; CI: confidence interval; AOR: adjusted odds ratio; COMT: catechol-*O*-methyltransferase.

* Note: In addition to the above variables, each model included age (y) as a continuous variable, education, and urinary tract infection other than schistosomiasis.