A Functional Polymorphism in Catechol-O-Methyltransferase Gene Explains Increased Risk of Uterine Leiomyomas in Black Americans

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"Old Red"
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ABSTRACT

Introduction: Uterine leiomyomas (fibroids) are the most common tumors in premenopausal women. Numerous clinical and biochemical observations suggest that initiation/promotion of leiomyomas is associated with estrogenic influence. The catechol-O-methyltransferase (COMT) enzyme is essential in the estrogen metabolism pathway. Our aim was to investigate whether polymorphisms in the COMT gene are associated with an increased risk of uterine leiomyomas.

Methods: We identified 186 women with surgically and histologically confirmed uterine leiomyomas and 142 matched controls with normal uteri. We analyzed the functional polymorphism Val(158)Met in exon III of the COMT gene using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). The valine-to-methionine substitution is associated with a fourfold decrease in enzymatic activity.

Results: The COMT Val/Val genotype was associated with significantly increased risk of uterine leiomyoma in all ethnic groups (black, white, and Hispanics). Overall, women with COMT Val/Val genotype were 2.5 times (confidence limits 1.017–6.151) more likely to have uterine leiomyomas than other genotypes. The overall prevalence of Val/Val genotype was significantly higher in black women (47%) than in whites (19%) or Hispanics (30%) ($P = .003$). Val/Val myometrial cell lines exhibited a significantly enhanced response to estrogen in proliferation and ERE-Luciferase (Luc) reporter assays compared with their Met/Met counterparts. Myometrial specimens from Val/Val women demonstrated distinct gene expression of several E2-regulated genes when compared with matched Met/Met tissues including (1) increased expression of COX2, Cyclin D1, PR-A, PR-B, and Bcl2; and (2) decreased expression of Bax—a profile consistent with higher estrogenic milieu. COMT-specific inhibitors arrested growth and attenuated ERE-Luc reporter tranactivation in leiomyoma cells. This effect was mediated, at least in part, by activation of apoptosis and inhibition of genes that regulate cell cycles.

Conclusions: These results suggest that the COMT Val/Val genotype might be one of the genetic risk factors for uterine leiomyoma development. The higher prevalence of this genotype in blacks might explain the increased occurrence of this tumor among black women.

INTRODUCTION

Uterine leiomyoma (ULM) arise from the smooth muscle cells of the myometrium and are the most common tumor of the female reproductive tract. It is widely accepted that symptomatic ULM are more common in black women than in white women. Over the past 120 years, a higher incidence of ULM in dark-skinned races has been reported. The molecular basis of this ethnic variation is unknown. Catechol-O-methyltransferase (COMT) is a ubiquitous enzyme that catalyses the S-adenosyl-L-methionine dependent methyl conjugation of the hydroxylgroups of catechol estrogens. Therefore, regulation of COMT activity may indirectly modulate the biological effects of estrogen and play an etiologic role in leiomyoma formation. A common genetic polymorphism, G-to-A transition at codon 158 that results in a valine-to-methionine substitution, is associated with thermal instability and a fourfold decrease in enzymatic activity. The genotypes designated in relation to the predicated enzymatic activity of the protein are high (COMT Val/Val), intermediate (COMT Val/Met), and low (COMT Met/Met).

AIM

To understand the molecular basis of why uterine leiomyoma is more common in black women than in whites women.

MATERIALS & METHODS

Patients

Subjects were classified as either black or white based on (1) their statement of race at hospital admission for surgery and (2) agreement of statement with skin color. We studied 186 women with histologically documented uterine leiomyomas and 142 matched controls with histologically documented leiomyoma-free normal uteri. The subjects were a well-balanced representation from the three ethnic groups: 46% white, 31% black, and 23% Hispanic.

Genotyping

DNA was extracted from peripheral blood samples and human myometrium (snap frozen in liquid nitrogen), as described before. The following primers were used: 5′-CTC ATC ACC ATC GAG ATC AA-3′ (forward) and 5′-CCA GGT CTG ACA ACC GGT CA-3′ (reverse). Cell culture, proliferation assays, luciferase reporter assays, Western blot analysis, COMT inhibitor experiments, and statistical methods were performed using standard techniques.

RESULTS

Val/Val variant is associated with an increased risk of leiomyoma in all ethnic groups.

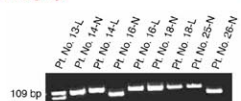


Figure 1. COMT DNA Genotyping. The Val and Met alleles were discriminated by digesting the polymerase chain reaction (PCR) product (109 bp) with 2 units of NlaIII (New England BioLabs, Beverly, Mass) at 37°C overnight, followed by 4.5% agarose gel electrophoresis. The Val/Val homozygotes (86 and 23 base pairs), Met/Met homozygotes (68 and 18 base pairs), and Val/Met heterozygotes (86, 68, 23, and 18 base pairs) were visualized by ethidium bromide staining.

Table 1. Distribution of Catechol-O-Methyltransferase (COMT) Polymorphism in Women With or Without Uterine Leiomyoma in Different Ethnic Groups*

Genotype	No. of Black Patients with Leiomyoma (%)	No. of Black Controls Without Leiomyoma (%)	No. of White Patients With Leiomyoma (%)	No. of White Controls Without Leiomyoma (%)	No. of Hispanic Patients with Leiomyoma (%)	No. of Hispanic Controls Without Leiomyoma (%)
Val/Val	42 (52)	6 (27)	15 (25)	14 (15)	16 (35)	6 (21)
Val/Met	36 (44)	14 (64)	29 (50)	44 (48)	26 (56)	18 (64)
Met/Met	3 (4)	2 (9)	15 (25)	34 (37)	4 (9)	4 (15)

*The Val/Val genotype was associated with significantly ($P < .001$) increased risk of uterine leiomyoma in all ethnic groups.
COMT: catechol-O-methyltransferase.

Table 2. The Association of Diagnosis With Catechol-O-Methyltransferase (COMT) and Ethnicity Using Logistic Model

Comparison	Estimated Odds Ratio	95% Wald Confidence Limits	Significance
Black vs white	5.307	2.477	11.369 S
Hispanic vs white	2.127	1.052	4.299 S
Val/Val vs Met/Met	2.501	1.017	6.151 NS
Val/Met vs Met/Met	1.193	0.569	2.502 S

S, statistically significant; NS, not statistically significant.

Distribution of COMT variants in different ethnic groups

Table 3. Distribution of Catechol-O-Methyltransferase (COMT) Polymorphism Among all Women from Different Ethnic Groups*

Genotype or Allele	Black (%)	White (%)	Hispanic (%)
Val/Val	48 (47)	29 (19)	22 (30)
Val/Met	50 (49)	73 (48)	44 (59)
Met/Met	5 (5)	49 (33)	8 (11)
Val allele	146 (71)	131 (43)	88 (59)
Met allele	60 (29)	171 (57)	60 (41)

*The distribution of the Val/Val genotype, as well as the Val allele, was significantly higher in black women compared with the two other ethnic groups ($P = .003$).

Val/Val human primary myometrial cells grow faster than Met/Met.

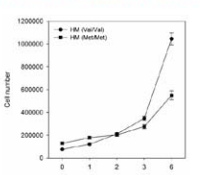


Figure 2. Effect of catechol-O-methyltransferase (COMT) polymorphism on E2-induced proliferation of primary human myometrial cells. Four human myometrial (HM) cell lines with COMT Val/Val genotype (HM137, HM140, HM154, and HM197) grew significantly faster than four COMT Met/Met HM cell lines (HM97, HM157, HM186, and HM280) ($P = .001$). The results are shown at an estrogen concentration of 10 nM, and similar results were obtained at 1 μM, 100 nM, 10 nM, and 100 pM. Each value is the mean ± standard error of the mean of the corresponding cells in triplicate wells in two independent experiments.

Val/Val myometrial cells support higher E2-induced ERE-reporter transactivation.

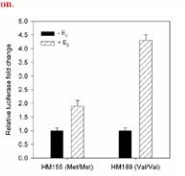


Figure 3. The catechol-O-methyltransferase (COMT) Val/Val genotype mediates higher E2-induced ERE-reporter transactivation in human myometrial cells. Several human myometrial (HM) cell lines with either COMT Val/Val or Met/Met genotype were transfected with Ad-ERE-tk-Luciferase reporter. Cells were stimulated with various concentrations of estradiol and analyzed 24 hours later. The difference between the two COMT genotypes was statistically significant ($P = .001$). The results are shown at an estrogen concentration of 1 μM, and similar results were obtained at 100 nM, 10 nM, 1 nM, and 100 pM. Each value is the mean ± standard error of the mean of triplicate wells in two independent experiments.

Val/Val myometrium has a higher expression of E2-regulated genes than Met/Met myometrium.

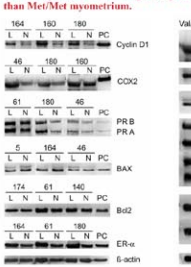


Figure 4. Differential protein expression of cyclinD1, COX2, PR-A, PR-B, BAX, Bcl2, and ERα genes in human normal myometrium versus adjacent leiomyoma. ß-actin is the internal loading control. PC = positive control.

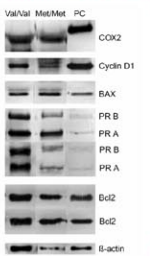


Figure 5. Effect of catechol-O-methyltransferase (COMT) polymorphism on cell cycle, apoptosis, and steroid receptor protein expression in normal human myometrium. ß-actin is the internal loading control. PC = positive control.

COMT inhibitors and 2-hydroxy estradiol arrest leiomyoma and myometrial cell growth.

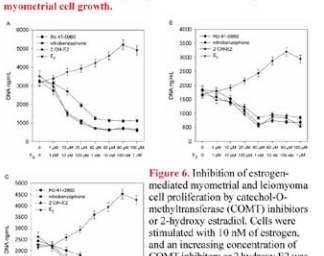


Figure 6. Inhibition of estrogen-mediated myometrial and leiomyoma cell proliferation by catechol-O-methyltransferase (COMT) inhibitors or 2-hydroxy estradiol. Cells were stimulated with 10 nM of estrogen, and an increasing concentration of COMT inhibitors or 2 hydroxy E2 was added. Cell numbers were examined by measuring DNA contents using the Hoechest method 72 hours (ELT3 rat leiomyoma cell line [6a]) or 96 hours (human myometrial cells, HM) later. HM154 has COMT Val/Val genotype (6b), and HM155 has COMT Met/Met (6c).

COMT inhibitors decrease ERE-Luc reporter transactivation in human myometrial and rat leiomyoma cells.

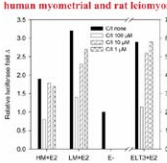


Figure 7. Catechol-O-methyltransferase (COMT) inhibitors reduce E2-induced ERE-Luc Reporter transactivation in myometrial and leiomyoma cells. Human myometrial (HM) and leiomyoma (LM) cell lines and rat ELT3 leiomyoma cell lines were transfected with Ad-ERE-tk-Luciferase reporter. After the addition of various doses of COMT inhibitors, cells were stimulated with estradiol (10 nM) and analyzed 24 hours later. The inhibitory effect of 100 to 150 μM of COMT inhibitor was statistically significant ($P = .002$). Each value is the mean ± standard error of the mean of triplicate wells in two independent experiments. C/I = COMT inhibitor.

COMT inhibitors modulate the expression of apoptosis and cell cycle genes in human myometrial cells.

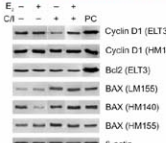
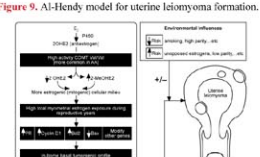


Figure 8. Effect of catechol-O-methyltransferase (COMT) inhibitors on cell cycle (cyclinD1) and apoptosis (Bcl2 and Bax) protein expression in normal human myometrium cells (HM) and rat ELT3 leiomyoma cell lines. ß-actin is the internal loading control. PC = positive control. C/I = COMT inhibitor. E2 = estradiol.

Figure 9. Al-Hendy model for uterine leiomyoma formation.



CONCLUSIONS

- The COMT Val/Val genotype may be one of the genetic risk factors for uterine leiomyoma development.
- The higher prevalence of this genotype in blacks might, at least in part, explain the increased occurrence of this tumor among black women.