Association study between catechol-O-methyltransferase polymorphisms and uterine leiomyomas in a Japanese population

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Summary

Purpose: To investigate a possible association between uterine leiomyomas and catechol-O-methyltransferase (COMT) polymorphisms in a Japanese population. *Methods:* We compared the allele frequencies and genotype distributions of the exon 4 *NlaIII* restriction site polymorphism (RSP), the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene in 250 leiomyoma cases and 182 controls using polymerase chain reaction-restriction fragment-length polymorphism analysis. *Results:* No significant differences in allele frequencies and genotype distributions of the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP were found between uterine leiomyoma cases and controls. Moreover, no associations were noted between these three polymorphisms in *COMT* genes and leiomyoma size or a family history of uterine leiomyomas. *Conclusion:* COMT gene polymorphisms are unlikely to be associated with an increased risk of uterine leiomyomas in a Japanese population.

Key words: Leiomyoma; Gene polymorphism; Polymerase chain reaction; Restriction fragment length polymorphism; Catechol-O-methyltransferase.

Introduction

Uterine leiomyomas are common benign neoplasms originating from smooth muscles in the myometrium, which frequently occur in premenopausal women [1]. This disease causes prolonged menstrual bleeding, dysmenorrhea, and reproductive dysfunction.

Accumulating evidence supports the concept that uterine leiomyomas are sex steroid hormone-dependent neoplasms. Leiomyomas increase with age during the premenopausal periods and typically regress and become asymptomatic with the onset of menopause [2, 3]. Tissue concentrations of estrogen are reported to be increased in leiomyoma tissues compared with those in the myometrium [4, 5]. Recent studies have demonstrated that the regulation of estrogen metabolizing enzymes may contribute to the pathogenesis of uterine leiomyomas [6].

Catechol-O-methyltransferase (COMT) is one of the enzymes that metabolize catechol estrogens [7]. This enzyme converts catechol estrogens, 2-hydroxyestradiol and 4-hydroxyestradiol, into inactive metabolites, 2-methoxy-estradiol and 4-methoxy-estradiol, respectively; 4-hydroxyestradiol activity was shown to be increased in human uterine leiomyomas compared with that in uterine myometrium [8]. This suggests that COMT may play an important role in the development and pathogenesis of uterine leiomyomas.

Polymorphisms in the *COMT* gene, especially the exon 4 *NlaIII* RSP (Val 108/158 Met polymorphism), have been studied as possible factors influencing susceptibility in several diseases. The Val/Val genotype in the exon 4 *NlaIII* RSP was reported to be associated with higher prevalence of systolic blood pressure compared with the Met/Met or Met/Val genotypes [9]. The Met/Met genotype was associated with increased risks of adenomyosis, sporadic breast cancer, and depressive disorders [10-12].

The exon 4 *NlaIII* RSP in the *COMT* (Val 108/158 Met polymorphism) was reported to be associated with an increased risk of uterine leiomyomas in African American, White, and Hispanic populations, suggesting that *COMT* gene polymorphisms may influence the establishment and development of this disease [13]. However, no additional studies that support such an association have been reported in other populations, and there is no information available regarding the P2 promoter *HindIII* RSP at -1217 and the exon 6 *BglI* RSP in the *COMT* gene.

In the present study, we investigated potential associations between uterine leiomyomas and the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene in a Japanese population.

Materials and Methods

Subjects

The Medical Ethics Review Committee of Kobe University Graduate School of Medicine approved the study design, and written informed consent was obtained from all participants. The case group consisted of 250 unrelated, ethnically Japanese women who were pathologically diagnosed as having uterine leiomyomas after hysterectomy or myomectomy. Women were excluded from the study if they had undergone GnRH agonist therapy preoperatively or an unexpected pathology was found (e.g., adenomyosis).

The control group consisted of 182 healthy women who were confirmed to have no uterine leiomyomas by ultrasonography. We also performed an analysis on patients with regard to a family history of uterine leiomyomas. We defined a positive family history as the presence of a leiomyoma in a first-degree relative. The cases ranged in age from 24 to 73 years with a mean age of 44.0, and controls ranged in age from 26 to 57 years with a mean age of 46.7.

Genotyping

Genomic DNA was extracted from whole blood anti-coagulated with EDTA using the Wizard DNA purification kit (Promega, Madison, WI, USA). The exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene were determined using polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) analysis, as described previously [14]. Genotyping for the exon 4 *NlaIII* RSP was performed using the forward primer 5'-GCC CGC CTG CTG TCA CC-3' and the reverse primer 5'-CTG AGG GGC CTG GTG ATA GTG-3', followed by digestion with the restriction enzyme *NlaIII*. Genotyping for the P2 promoter *HindIII* RSP at -1217 was performed using a PCR fragment amplified by the forward primer 5'-CTC TGG CGG AAA GGA AT-3' and the reverse primer 5'-TCG GCA TCA AAA GGA GGA AAA AG-3', followed by digestion with the restriction enzyme *HindIII*. Genotyping for the exon 6 *BglI* RSP was performed using the forward primer 5'-TGC GGA AGG GGA CAG TGC TAC-3' and the reverse primer 5'-CCG GAG CCG CAG AAG GTC A-3', followed by digestion with the restriction enzyme *BglI*. The PCR conditions were as follows: PCR in a 20 µl reaction mixture containing 20 ng of genomic DNA, 10 pmol of each primer, 250 µM of dNTPs, and 1.0 unit of Taq gold DNA polymerase. The concentration of $MgCl_2$ was 1.5 mM for all of these three polymorphisms. The PCR was conducted with an ABI 9700 thermocycler (PE Applied Biosystem, Foster City, CA, USA) using the following thermal profiles: an initial denaturing cycle of 96°C for 12 min, 30 cycles of denaturing cycle at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, and a final cycle of 72°C for 10 min for the exon 4 *NlaIII* RSP; an initial denaturing cycle of 96°C for 12 min, 35 cycles of denaturing cycle at 94°C for 30 sec, annealing at 53°C for 30 sec, extension at 72°C for 35 sec, and a final cycle of 72°C for 10 min for the P2 promoter *HindIII* RSP at –1217; an initial denaturing cycle of 96°C for 12 min, 35 cycles of denaturing cycle at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 35 sec, and a final cycle of 72°C for 10 min for the exon 6 *BglI* RSP. Digestions with the appropriate restriction enzymes were performed at 37°C for 24 hours according to the manufacturer's instructions (New England Biolabs, Beverly, MA, USA). DNA fragments were subjected to electrophoresis in a 4% NuSieve 3:1 agarose gels for the exon 4 *NlaIII* RSP, and in a 2% agarose gels for the P2 promoter *HindIII* RSP at -1217 and the exon 6 *BglI* RSP. Gels were stained with 0.1 g/ml ethidium bromide and visualized by ultraviolet illumination.

Statistical analysis

Genotypic distributions were examined for significant departure from the Hardy-Weinberg equilibrium by a goodness of fit χ^2 test. The χ^2 test was used to examine the differences in the genotype proportions of the polymorphisms between uterine leiomyomas patients and controls. The Fisher correction was applied when appropriate. Odds ratio (OR) and 95% confidence interval (CI) were used to compare categorical variables. The cases were subdivided into groups with or without a family history of uterine leiomyomas, and the distribution of the genotypes in these groups were analysed separately. The independent Student's *t*-test was used to examine the relationship between the size of the largest leiomyoma and each genotype.

Results

The genotype distributions were confirmed to be in Hardy-Weinberg equilibrium in each group studied.

Typical gels are shown in Figure 1. The G allele of the exon 4 *NlaIII* RSP was not cleaved by *NlaIII* and had a single band of a fragment length of 114 bp (Figure 1A). The A allele was cleaved by *NlaIII* and yielded two small fragments of 96 bp and 18 bp (Figure 1A). The heterozygote had three bands of 114 bp, 96 bp, and 18 bp (Figure 1A).

The A allele of the P2 promoter *HindIII* RSP was not cleaved by *HindIII* and had a single band of a fragment length of 306 bp (Figure 1B). The G allele was cleaved by *HindIII*, yielding two fragments of 231 bp and 75 bp (Figure 1B). The heterozygote had three bands of 306 bp, 231 bp, and 75 bp (Figure 1B).

The C deletion allele of the exon 6 *BglI* RSP was not cleaved by *BglI* and had a single band of a fragment length of 277 bp (Figure 1C). The C insertion allele was cleaved by *BglI* and yielded two fragments of 196 bp and 82 bp (Figure 1C). The heterozygote had three bands of 277 bp, 196 bp, and 82 bp (Figure 1C).

Genotype distributions and allele frequencies of the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene in leiomyomas cases and the controls are shown in Tables 1, 2, and 3. The cutting allele frequencies of the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP, and the exon 6 *BglI* RSP were 32.2%, 31.6%, and 43.0% in the cases, and 30.2%, 28.9%, and 42.6% in the controls, respectively (Tables 1, 2, 3). No significant differences were found in either the allele frequencies or genotype distributions of the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in *COMT* gene between the cases and controls (Tables 1, 2, 3).

The allele frequency of the exon 4 *NlaIII* RSP in our cases were comparable with those in previous reports using a Japanese population (Table 4) [12, 15]. However, there has been no previous study that reported the allele frequencies of the P2 promoter *HindIII* RSP at -1217 and the exon 6 *BglI* RSP using a Japanese population. We investigated whether family history of uterine leiomy-
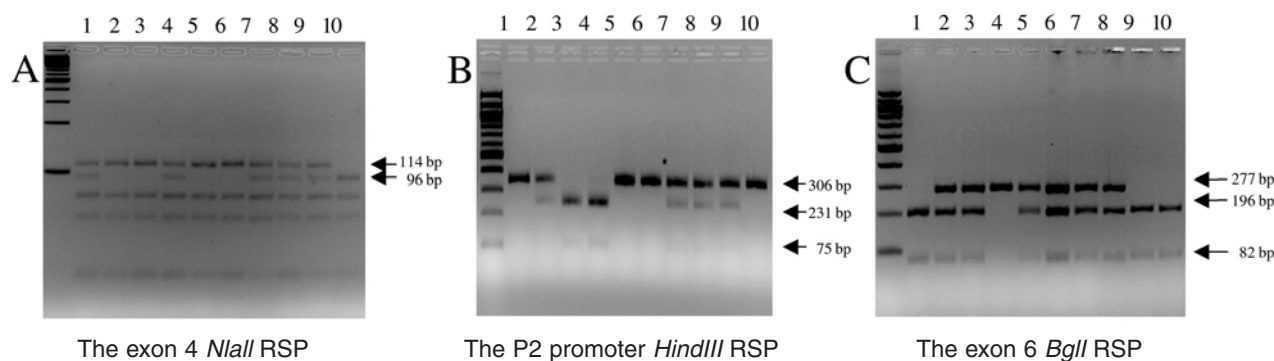
Figure 1. — The exon 4 *NlaIII* RSP by PCR-RFLP (A). Lane 10 confirming 100 bp molecular wt marker is the best standpoint-/standard to prove the accuracy of RFLP analysis. Lane 2, 3, 5, and 6 are G/G. Lane 1, 4, 7, 8, and 9 are G/A. Lane 10 is A/A. The P2 promoter *HindIII* RSP at -1217 by PCR-RFLP (B). Lane 10 confirming 100 bp molecular wt marker is the best standpoint-/standard to prove the accuracy of RFLP analysis. Lane 1, 5, 6, and 10 are A/A. Lane 2, 7, 8, and 10 are A/G. Lane 3 and 4 are G/G. The exon 6 *BglI* RSP by PCR-RFLP (C). Lane 10 confirming 100 bp molecular wt marker is the best standpoint-/standard to prove the accuracy of RFLP analysis. Lane 1 is delC/delC. Lane 2, 3, 5, 6, 7, and 8 are delC/insC. Lane 1, 9 and 10 are insC/insC.
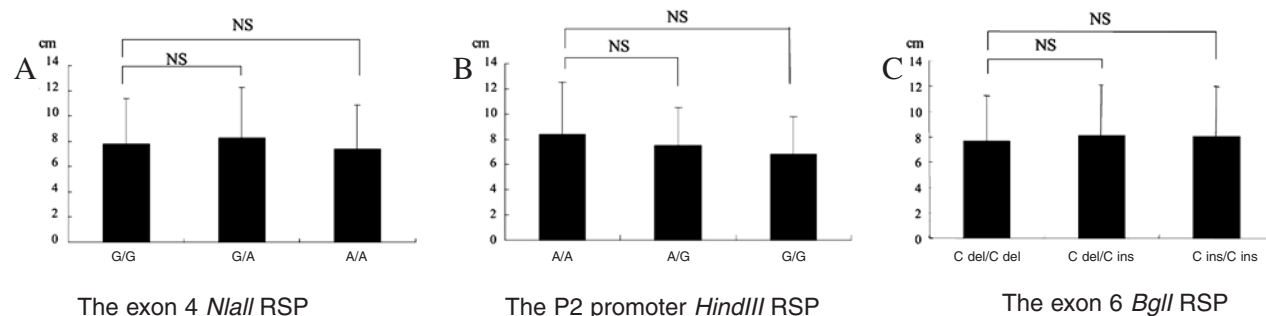

Figure 2. — The exon 4 *NlaIII* RSP and leiomyoma sizes (A). The P2 promoter *HindIII* RSP at -1217 and leiomyoma sizes (B). The exon 6 *BglI* RSP and leiomyoma sizes (C). Data on leiomyoma size and polymorphic status were collected as described in the results. Bars represent mean ± SEM.

Table 1. — *The exon 4* NlaIII *RSP genotypes and alleles in leiomyoma cases and controls.*

	Genotype				Allele		
	G/G n (%)	G/A n (%)	A/A n (%)	*P* value versus controls	G n (%)	A n (%)	*P* value versus controls
Leiomyoma (n = 250)	121 (46.4)	97 (38.8)	32 (12.8)	p = 0.84	339 (67.8)	161 (32.2)	p = 0.54; OR = 0.91 95% CI 0.68-1.22
Leiomyoma with FH (n = 99)	53 (53.5)	36 (36.4)	10 (10.1)	p = 0.87	142 (71.7)	56 (28.3)	p = 0.87; OR = 1.10 95% CI 0.75-1.61
Leiomyoma without FH (n = 60)	28 (46.7)	25 (41.7)	7 (11.6)	p = 0.75	81 (67.5)	39 (32.5)	p = 0.75; OR = 0.90 95% CI 0.58-1.40
Controls (n = 182)	93 (51.1)	68 (37.4)	21 (11.5)		254 (69.8)	110 (30.2)	

FH: family history.

Table 2. — *The P2 promoter* HindIII *RSP genotypes and alleles in leiomyoma cases and controls.*

	Genotype				Allele		
	A/A n (%)	A/G n (%)	G/G n (%)	*P* value versus controls	G n (%)	A n (%)	*P* value versus controls
Leiomyoma (n = 250)	117 (46.8)	108 (43.2)	25 (10.0)	p = 0.77	342 (68.4)	158 (31.6)	p = 0.48; OR = 0.90 95% CI 0.67-1.20
Leiomyoma with FH (n = 99)	49 (49.5)	39 (39.4)	11 (11.1)	p = 0.74	137 (69.2)	61 (30.8)	p = 0.83; OR = 1.04 95% CI 0.71-1.53
Leiomyoma without FH (n = 60)	25 (41.7)	30 (50.0)	5 (8.33)	p = 0.67	80 (66.7)	40 (33.3)	p = 0.35; OR = 0.81 95% CI 0.52-1.26
Controls (n = 182)	91 (50.0)	77 (42.3)	14 (7.69)		259 (71.1)	105 (28.9)	

FH: family history.

Table 3. — *The exon 6* BglI *RSP genotypes and alleles in leiomyoma cases and controls.*

	Genotype				Allele		
	delC/delC n (%)	delC/insC n (%)	insC/insC n (%)	*P* value versus controls	delC n (%)	insC n (%)	*P* value versus controls
Leiomyoma (n = 250)	84 (33.6)	117 (46.8)	49 (19.6)	p = 0.97	285 (57.0)	215 (43.0)	p = 0.90; OR = 0.98 95% CI 0.68-1.22
Leiomyoma with FH (n = 99)	35 (35.4)	43 (43.4)	21 (21.2)	p = 0.53	113 (57.1)	85 (42.9)	p = 0.94; OR = 0.99 95% CI 0.69-1.40
Leiomyoma without FH (n = 60)	17 (28.3)	34 (56.7)	9 (15.0)	p = 0.41	68 (56.7)	52 (43.3)	p = 0.89; OR = 0.97 95% CI 0.64-1.47
Controls (n = 182)	61 (33.5)	87 (47.8)	34 (18.7)		209 (57.4)	155 (42.6)	

FH: family history.

Table 4. — *Comparison of the exon 4* NlaIII *RSP, the P2 promoter* HindIII *RSP at -1217, and the exon 6* BglI *RSP alleles and genotypes in this study and previously published studies.*

	Genotype			Allele			
	G/G n (%)	G/A n (%)	A/A n (%)	G n (%)	A n (%)	population	
the exon 4 *HlaIII* RSP	93 (51.1)	68 (37.4)	21 (11.5)	254 (69.8)	110 (30.2)	Japanese	Our control data
	81 (54.0)	56 (37.3)	13 (0.87)	218 (72.7)	82 (27.3)	Japanese	Ohmori *et al.* [12]
	58 (43.0)	59 (43.7)	18 (11.1)	175 (64.8)	95 (35.2)	Japanese	Ohara *et al.* [15]
	23 (25.0)	47 (51.1)	22 (23.9)	93 (90.5)	91 (49.5)	Caucasian	Fitz *et al.* [18]
	24 (15.1)	76 (47.8)	59 (37.1)	124 (39.0)	194 (61.0)	Caucasian	Worda *et al.* [19]

	Genotype			Allele			
	A/A n (%)	A/G n (%)	G/G n (%)	A n (%)	G n (%)	population	
the P2 promoter *HindIII* RSP	91 (50.0)	77 (42.3)	14 (7.69)	259 (71.2)	105 (28.9)	Japanese	Our control data
				547 (58.6)	387 (41.4)	Caucasian	Funke *et al.* [20]

	Genotype			Allele			
	delC/delC n (%)	delC/insC n (%)	insC/insC n (%)	delC n (%)	insC n (%)	population	
the exon 6 *BglI* RSP	61 (33.5)	87 (47.8)	34 (18.7)	209 (57.4)	155 (42.6)	Japanese	Our control data
	18 (18.2)	54 (54.5)	27 (27.3)	90 (45.5)	108 (54.5)	Chinese	Chen *et al.* [16]

omas may affect allele frequencies and genotype distributions of the *COMT* gene polymorphisms. However, no significant differences were observed in either the allele frequencies or the genotype distributions of the the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene between women with or without a family history of uterine leiomyomas (Tables 1, 2, 3)

Lastly, we investigated the relationship between leiomyoma size and these three genotypes (Figure 2). The exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene did not affect the size of the leiomyomas (Figures 2A, 2B, 2C).

Discussion

In the present study, we investigated possible associations between the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene and uterine leiomyomas in a Japanese population using PCR-RFLP analysis. We could not find any associations in the allele frequencies and genotype distributions of these three polymorphisms in the *COMT* gene with uterine leiomyomas, irrespective of a family history and leiomyoma size. This is the first study to demonstrate no associations between uterine leiomyomas and the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene.

Previously, two authors investigated the association between the exon 4 *NlaIII* RSP and uterine leiomyomas in different populations [13, 16] with inconsistent results. Al-Hendy *et al.* investigated a possible association between the exon 4 *NlaIII* RSP in the *COMT* gene and uterine leiomyomas in African American, White, and Hispanic populations using PCR-RFLP analysis [13]. The G allele of the exon 4 *NlaIII* RSP was found to be significantly more frequent in uterine leiomyoma cases (63.7%) than in controls (45.1%). They demonstrated a positive association between this polymorphism and uterine leiomyomas in African American, White, and Hispanic populations, suggesting that the G/G genotype of the exon 4 *NlaIII* RSP was 2.5 times more likely to develop uterine leiomyomas than other genotypes (p < 0.001; 95% CI 1.017-6.151). In contrast, Denschlag *et al.* investigated the same association in a Caucasian population by performing pyrosequencing, but they could not find any association between the exon 4 *NlaIII* RSP and uterine leiomyomas [17]. Our results coincided with the data of Denschlag *et al.*, who showed no significant differences in allele frequency and genotype distribution for the exon 4 *NlaIII* RSP (p = 0.3 and p = 0.6, respectively) between 128 cases and 139 controls [17].

There are several possible explanations for the discrepancy between our results and those of Al-Hendy *et al*. Firstly, one explanation is the relatively small sample sizes in the previous study. In the present study, we included 250 uterine leiomyoma cases and 182 controls, whereas Al-Hendy *et al*. used 81 cases and 22 controls in an African American population, 59 cases and 92 controls in a White population, and 46 cases and 28 controls in a Hispanic population. Although they evaluated totally 186 uterine leiomyoma cases and 142 controls in the admixture of the ethnic groups including African, White, and Hispanic women, the small sample size of each ethnic group may limit the power of their study to conclude the positive association of the exon 4 *NlaIII* RSP with the development of uterine leiomyomas.

Secondly, the discrepancy may be due to the differences between the ethical populations examined because the exon 4 *NlaIII* RSP A allele frequencies vary among ethnic groups. Indeed, the frequency of the A allele is less frequent in a Japanese population (27-35%) [12, 15] than in a Caucasian population (50-61%) [18, 19]. In our study, the subjects examined had a homogeneous genetic background of Japanese origin alone. In complex traits, susceptibility differences between populations may be as dependent, if not more dependent, on the relative frequencies of polymorphisms conferring susceptibility as on environmental factors. Thus, the exon 4 *NlaIII* RSP might be associated with uterine leiomyomas in African American, White, and Hispanic populations, but not in those from other ethnic groups such as a Japanese population.

Thirdly, the discrepancy between our results and those of Al-Hendy *et al*. might be explained by the differences in the genotyping methods used. Both studies used the same PCR-RFLP method, but the sequences of the primers for PCR were different.

A significant association has been shown between the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene and various diseases, to date. Birgit *et al*. reported that the A allele of the P2 promoter *HindIII* RSP was significantly more frequent in cases with schizophrenia, bipolar disorder, and major depressive disorders [20]. A allele of the P2 promoter *HindIII* RSP was also reported to be associated with low COMT activity in lymphocytes [21]. On the other hand, the exon 6 *BglI* RSP was a deletion/insertion polymorphism immediately 3' to the stop codon, thus the sequence variation at the 3' end could have an effect on RNA stability or translational efficiency [22]. Maria *et al*. and Chia-Hsiang *et al*. investigated the association between the exon 6 *BglI* RSP and schizophrenia, but neither of the two studies found any significant differences in allele frequencies and genotype distributions between schizophrenic patients and controls [16, 22]. These two polymorphisms in the *COMT* gene have not been studied in relation to uterine leiomyomas. In the present study, we investigated for the first time the possible associations between uterine leiomyomas and the P2 promoter *HindIII* RSP at -1217 and the exon 6 *BglI* RSP in *COMT* gene in a Japanese population. However, we could not identify any positive associations of these *COMT* gene polymorphisms with uterine leiomyomas.

COMT is present in a membrane bound form (M-COMT) and a soluble form (S-COMT), and plays a critical role in the metabolism of estrogen. COMT converts catechol estrogens, 2- and 4-hydroxyestradiol, into inactive metabolites, 2-methoxy- and 4-methoxy-estradiol; 4-hydroxyestradiol is similar to estradiol in its ability to bind to and activate the estrogen receptor and is hormonally active for stimulating uterine growth [23]. A high activity of 4-hydroxyestradiol was demonstrated in human uterine leiomyomas compared with uterine myometrium [8]. On the other hand, 2-methoxyestrasiol exerts an antiproliferative effect on human leiomyoma cells by inhibiting DNA and collagen synthesis, and induces G_2/M cell cycle arrest and apoptotic cell death [24]. Taken together, low COMT activity might up-regulate 4-hydroxyestradiol levels and down-regulate 2-methoxyestradiol levels, thereby inducing cell proliferation and down-regulation of DNA and collagen synthesis, and inhibiting apoptotic cell death in human leiomyomas.

COMT is genetically polymorphic caused by autosomal codominate alleles resulting in nearly a fourfold difference in enzyme activity [25]. A polymorphism of a G-to-A transition at codon 158 of S-COMT gene results in a valine-to-methionine substitution [26]. Homozygosity for 158Met leads to a 3-to 4-fold reduction in enzymatic activity, compared with homozygosity for 158Val [26]. Met/Met genotype was associated with an increased risk of adenomyosis (p = 0.006; OR = 3.2; 95% CI 1.3-7.8) [10]. Met/Met genotype was associated with an increased risk of sporadic breast cancer in premenopausal Turkish women (p = 0.005; OR = 2.28; 95% CI 1.27-4.12) [11]. Moreover, the presence of A allele (Met) was significantly associated with depressive disorders (p = 0.012; OR = 2.19; 95% CI 1.19-4.03) [12].

Considering these data, we hypothesized that the Met allele of the exon 4 *NlaIII* RSP and the A allele of the P2 promoter *HindIII* RSP that were reported to be associated with low enzyme activity might have an increased risk for the development of uterine leiomyomas. However, we could not find any positive associations between the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene and uterine leiomyomas.

In conclusion, we could not demonstrate any associations between the exon 4 *NlaIII* RSP, the P2 promoter *HindIII* RSP at -1217, and the exon 6 *BglI* RSP in the *COMT* gene and uterine leiomyomas in a Japanese population. Further studies will be needed to elucidate the relation of estrogen-metabolizing gene polymorphisms with uterine leiomyomas, since our results can not rule out the involvement of other estrogen-metabolizing single nucleotide polymorphisms in the development of uterine leiomyomas.

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