

Relationship between Single Nucleotide Polymorphisms in CYP1A1 and CYP1B1 Genes and Bone Mineral Density in Postmenopausal Japanese Women on Hormone Replacement Therapy

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Received December 12, 2006; accepted December 21, 2006

Summary. The objective of this study was to evaluate the relationships between single nucleotide polymorphism of estrogen metabolizing genes and lumbar bone mineral density (BMD) and to determine the effects of hormone replacement therapy (HRT). We conducted a case study at the Niigata University Medical and Dental Hospital. The patients were 124 Japanese women diagnosed with osteopenia or osteoporosis and taking HRT for 12 months. Seven single nucleotide polymorphism (SNPs) in the cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1) genes were characterized by a single nucleotide primer extension assay. The lumbar bone mineral density (BMD) were measured before and following 12 months of HRT. The genotyping of the 124 postmenopausal Japanese women revealed three out of seven SNPs to be quite common, with the minor allele frequency being at least 20%. A SNP in exon3 of CYP1B1 (L432V) was found to be significantly associated with the effect of HRT on BMD. In patients with the GG genotype of L432V, the responses to HRT in BMD markedly decreased. We demonstrated that a SNP in the CYP1B1 gene is associated with the magnitude of BMD in response to HRT. These results suggest that L432V polymorphism in the CYP1B1 gene could be used to predict the effect of HRT on lumbar BMD.

Key words— bone mineral density (BMD); CYP1A1, CYP1B1, hormone replacement therapy (HRT); single nucleotide polymorphism (SNP).

INTRODUCTION

Estrogen plays a significant role in bone metabolism, and its deficiency after menopause is the main reason for accelerated bone loss and the development of postmenopausal osteoporosis, both of which are preventable by estrogen administration. Postmenopausal hormone replacement therapy (HRT) is generally an effective treatment modality for the prevention of bone loss; however, individual variations exist.^{1,2,3} Some postmenopausal women respond strongly to HRT, while out of approximately 8% who are compliant with this therapy are nonetheless non-responders.⁴ This raises the possibility that some genetic determinants as well as gene-environment interactions might modulate the responses to HRT in individual patients.

Individual genetic variability of the estradiol metabolism has been described to be a significant contributor to disease susceptibility, with variations depending on ethnic background. Among others, the genetic variations of the genes encoding cytochrome P450 (CYP) enzymes are considered to play an important role in this standpoint.⁵ CYP enzymes play an important role in the production, bioavailability, and degradation of estradiol. A series of polymorphisms and mutations of the CYP enzyme complex have been identified. Cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1) catalyze the hydroxylation of estradiol, and several single nucleotide polymorphic sites of those

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Abbreviations— BMD, bone mineral density; HRT, hormone replacement therapy; SNP, single nucleotide polymorphism.

genes have been described.^{6,7)} Polymorphisms—especially single nucleotide polymorphisms (SNPs) in the exon with amino acid changes, lead to functionally relevant biochemical consequences for capable of influencing the responses to HRT.

In this study we attempted to clarify whether SNPs in the exons of the CYP1A1 and CYP1B1 genes affected the change in the bone mineral density (BMD) in postmenopausal Japanese women during HRT.

PATIENTS AND METHODS

Patients

The patients was comprised of 124 Japanese women, ranging from 40 to 64 years of age (49.8 ± 1.0 years, mean \pm SEM), who had been diagnosed with osteopenia or osteoporosis and had been taking HRT for 12 months. The diagnoses of osteopenia and osteoporosis were based on the criteria recommended by the Japanese Society of Bone and Mineral Research: an L2-L4 BMD of $< 80\%$ and $< 70\%$ of the young adults (20-44 years) mean, respectively. In all cases, more than six months had elapsed since the last menstrual bleeding, the serum estradiol level was lower than 20 pg/mL, and the serum follicle-stimulating hormone (FSH) level was more than 50 mIU/mL. The patients were not genetically related. HRT was administered in a sequential regimen consisting of 0.625 mg of conjugated equine estrogen (CEE) for 24 days (days 1 to 24) and 5 mg of medroxyprogesterone acetate (MPA) for 10 days (days 15 to 24), or in a continuous regimen consisting of 0.625 mg of CEE and 2.5 mg of MPA for 28 days.

Bone densitometry

BMD, was expressed as the mass per unit area (grams per square centimeter), was measured in the anterior-posterior plane of the lumbar spine (L2 to 4), using dual-energy X-ray absorptiometry (DXA) with a QDR-2000 analyzer (Hologic Inc, Waltham, MA, USA); absorptiometries were examined by the same observer. The average coefficients of variation (CV) of phantom measurements of bone mineral content (BMC), bone area (BA), and BMD during the study period were 1.1, 0.7, and 0.6%, respectively. In addition, for the control women, the CV of the *in vivo* precision of BMD between two measurements (mean interval: 2.6 ± 1.2 years) was 0.9%. There was no scanner drift observed during the study period. BMD change (Δ BMD) was expressed as the percentage of BMD change in comparison to the pretreatment baseline.

DNA isolation and genotyping

Peripheral blood samples were collected after obtaining informed consent from each patient. Genomic DNA was extracted from the peripheral blood leukocytes using a DNA purification Kit (QIAamp DNA Blood Mini kit, QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. All PCRs were performed on a Perkin Elmer GeneAmp 9700 system and the presence of amplicons was checked on agarose gel. A single nucleotide primer extension assay was carried out to analyze SNP using a SNaPshot Kit (Applied Biosystems, Foster City, CA, USA). The extended primers were analyzed on an ABI 3100 device (Applied Biosystems). The primer sequences for the PCRs and primer extension reactions are available in the JSNP database. Initial denaturation was performed at 95°C for two min, followed by 35 cycles each, consisting of denaturation at 95°C for 30 sec, annealing at 60°C, and extension at 72°C for one min, followed by final extension at 72°C for eight min. This study was approved by the Niigata University Human Investigation Committee.

Statistical analysis

Differences in the baseline characteristics, the absolute BMD value among genotypes, were tested using an analysis of covariance (ANCOVA) with age and BMI as covariates. To evaluate the relationships between CYP polymorphisms and the changes during HRT, we used a linear regression analysis and ANOVA with Fisher's protected least significant difference (PLSD) test.

All data are expressed as the mean \pm SEM. Differences of $P < 0.05$ were considered to indicate a statistical significance. All data management and statistical computations were performed with StatView 4.0 (Abacus Concepts, Berkeley, CA, USA) or SPSS 10.0 software program (SPSS Inc., Chicago, IL, USA).

RESULTS

In this study, we characterized 7 SNPs — 3 SNPs in the CYP1A1 gene and 4 SNPs in the CYP1B1 genes — from a total of 248 chromosomes from 124 postmenopausal Japanese women. Fig. 1 indicates the location of each SNP analyzed in this study. All SNPs exist within the exon, in association with in amino acid substitution.

Although the genotypic distribution of I462V in the CYP1A1 gene was in the Hardy-Weinberg equilibrium, those of A48G and L432V in the CYP1B1 gene were observed to deviate from this equilibrium. The frequencies of the variant SNP alleles ranged from 19 to 23 %. There were no variant alleles in 4 SNPs — G45D

Table 1. Genotype and allele frequencies of 7 SNPs of the CYP gene in Japanese patients

Gene	Polymorphism	Genotype	Homo (major)	Hetero	Homo (minor)	Current study	Allele frequency (major allele)			dbSNP
							Sasaki M et al.	* ⁶⁾ in dbSNP	JSNP-ID	
CYP1A1	G45D	G/A	124 (100%)	0 (0%)	0 (0%)	1.00	—	—	ssj0003953	rs4646422
	I462V	A/G	78 (63%)	42 (34%)	4 (3%)	0.80	—	0.902	ssj0007951	rs1048943
	A463G	C/G	124 (100%)	0 (0%)	0 (0%)	1.00	—	—	IMS-JST026484	rs2278970
CYP1B1	R48G	C/G	80 (76%)	10 (10%)	15 (14%)	0.81	0.68	0.653	ssj0007955	rs10012
	A119S	G/T	124 (100%)	0 (0%)	0 (0%)	1.00	0.85	0.648	ssj0007956	rs1056827
	L432V	C/G	78 (63%)	36 (29%)	10 (8%)	0.77	0.82	0.592	IMS-JST085313	rs1056836
	N453S	A/G	124 (100%)	0 (0%)	0 (0%)	1.00	1.00	0.889	—	rs1800440

* , reference number.

Table 2. Baseline characteristics according to the CYP genotypes

Variables	Genotype of I462V (CYP1A1)			Genotype of R48G (CYP1B1)			Genotype of L432V (CYP1B1)			p-value		
	AA	GA	GG	CC	CG	GG	CC	GG	GC			
n	78 (63%)	42 (34%)	4 (3%)	—	80 (76%)	10 (10%)	15 (14%)	—	78 (76%)	36 (29%)	10 (8%)	—
Age (years)	50.1 ± 0.8	49.2 ± 0.8	51.3 ± 1.8	0.73	49.2 ± 0.6	49.9 ± 1.6	50.1 ± 1.3	0.33	50.6 ± 0.8	49.0 ± 0.9	47.8 ± 1.4	0.74
Age at menopause (years)	47.4 ± 0.6	47.7 ± 0.6	49.0 ± 1.9	0.89	47.4 ± 0.6	45.6 ± 2.3	48.2 ± 0.6	0.39	47.8 ± 0.6	47.2 ± 0.6	46.6 ± 1.9	0.39
Height (cm)	154.9 ± 0.6	151.6 ± 2.4	157.8 ± 7.6	0.19	154.6 ± 0.7	154.0 ± 1.6	153.0 ± 0.9	0.65	153.6 ± 0.9	156.1 ± 1.2	158.5 ± 1.1	0.66
Weight (kg)	52.5 ± 0.7	51.7 ± 1.0	51.0 ± 6.0	0.76	51.7 ± 0.7	54.1 ± 2.0	53.4 ± 1.7	0.61	52.0 ± 0.7	52.2 ± 1.2	53.2 ± 2.5	0.40
BMI (kg/m ²)	21.9 ± 0.26	25.3 ± 3.5	20.3 ± 0.9	0.38	21.6 ± 0.3	22.8 ± 0.6	22.8 ± 0.7	0.21	23.7 ± 1.9	21.7 ± 0.4	22.1 ± 0.7	0.74
L2-4 BMD (g/cm ³)	0.76 ± 0.02	0.76 ± 0.02	0.79 ± 0.08	0.96	0.76 ± 0.02	0.84 ± 0.07	0.79 ± 0.05	0.28	0.77 ± 0.02	0.76 ± 0.02	0.78 ± 0.07	0.23

Data are presented as mean ± S.E.

Table 3. Correlation between changes in the lumbar BMD and the genotype of the CYP genes

Polymorphism	n*	Changes in the lumbar BMD [#]	
		Correlation coefficient : r	p-value
I462V (CYP1A1)	124	0.006	0.944
R48G (CYP1B1)	115	0.019	0.849
L432V (CYP1B1)	124	0.306	0.001

*, Number of genotyped patients; #, Changes at 12 months after hormone replacement therapy (HRT).

Table 4. Changes in the BMD after HRT according to the genotype of L432V in the CYP1B1 gene

Genotype		CC	CG	GG	p-value
Number of patients		78	36	10	
BMD	% change	3.4 ± 0.55	1.80 ± 1.01	-3.65 ± 2.35	0.001
	Absolute value	0.782 ± 0.017	0.773 ± 0.022	0.747 ± 0.069	0.775

Data are presented as mean ± S.E.

and A463G (CYP1A1), A119S and N453S (CYP1B1) — in the population analyzed in this study (Table 1).

No significant differences were observed in the baseline lumbar BMD with any genotypes tested in this study. No significant differences were observed in any of the other baseline characteristics including age, age at menopause, height, body weight, and BMI with any genotypes (Table 2).

To test whether these three exon SNPs might be involved in the response to HRT, we conducted a linear regression of the correlation between the percentage of change in the lumbar BMD after HRT. L432V in the CYP1B1 gene demonstrated significant associations with lumbar BMD after 12 months of HRT. Neither I462V (CYP1A1) nor R48G (CYP1B1) demonstrated a significant association with the lumbar BMD (Table 3).

The mean changes in the BMD of all patients at 12 months of treatments was 2.3 ± 0.5%. Although the absolute value of the BMD did not show any significantly difference among the different genotype groups, the patients with the homo (variant) genotype (GG) of L432V showed significantly a lower BMD change (-3.7 ± 2.4%) than those with the hetero (CG; 1.8 ± 1.0%) and homo (wild type) (CC; 3.4 ± 0.6%) genotypes (Table 4).

DISCUSSION

In this study, we characterized a total of 7 SNPs within exons in the CYP1A1 and CYP1B1 genes

of 124 postmenopausal Japanese women diagnosed with osteopenia or osteoporosis and thus showed an association between a L432V polymorphism in the CYP1B1 gene and the rate of change in lumbar spine BMD during HRT. HRT was associated with a negative change in the BMD in those with the GG (variant homo) genotype of L432V. The responses to HRT markedly decreased in patients with the GG genotype in comparison to those with GC and CC genotypes.

Variations in the estrogen metabolizing genes, such as CYP1A1, CYP1B1, CYP17, CYP19, and Catechol-*O*-methyltransferase (COMT) genes, have been reported in association with the susceptibility of women to breast cancer, and such variations were also found to influence the clinical course.^{8,9)} Furthermore, the SNPs of these genes have been evaluated in patients with a variety of conditions, such as the age at menarche and natural menopause,¹⁰⁾ breast density,¹¹⁾ and plasma estrogen levels.^{12,13)}

Prior studies showed that polymorphisms and mutations in various CYP genes caused changes in the function of proteins. Individuals who had mutations in CYP2A6 and CYP2D6 have been reported to have a very low catalytic activity for these enzymes.^{14,15)} On the other hand, polymorphisms in CYP1A1 and CYP1B1 have been reported to be related to enhanced protein activation.^{16,17)}

Both the CYP1A1 and CYP1B1 loci appear to play a prominent role within the genes involved in the estrogen metabolism. CYP1A1 catalyzes the C2-, C6-, and C15- α hydroxylation whereas CYP1B1 catalyzes the C4-

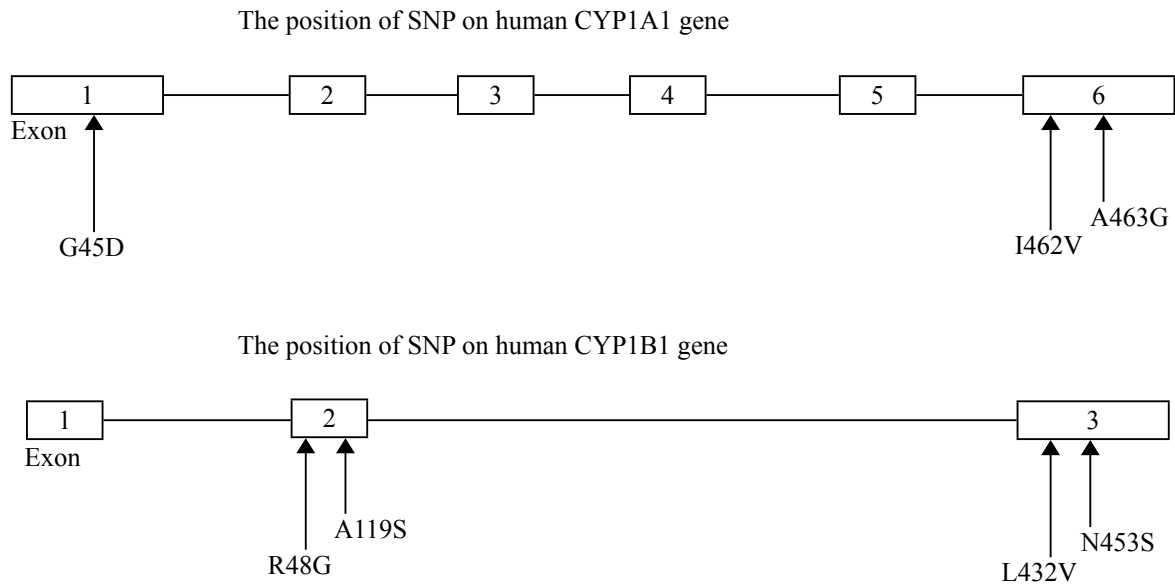


Fig. 1. The position of each SNP in the CYP1A1 and CYP1B1 genes. SNP, single nucleotide; CYP1A1, cytochrome P450 1A1; CYP1B1, cytochrome P450 1B1.

hydroxylation of estradiol. Various polymorphic sites of the CYP1A1 and CYP1B1 genes have been described either on introns or on exons. An association between SNPs in the CYP1A1 and CYP1B1 genes and the risks of breast cancer,¹⁸⁾ endometrial cancer,¹⁹⁾ and prostate cancer²⁰⁾ has been reported. I462V polymorphism in the CYP1A1 gene and L432V polymorphism in the CYP1B1 gene have also exhibited an association with the incidence of breast carcinoma. A119S and L432V polymorphisms in the CYP1B1 gene are also associated with prostatic and endometrial carcinogenesis.

In this study, patients with a homozygous variant allele of L432V showed significantly poor responses in lumbar BMD to HRT. The genotype frequency distributions of L432V in the CYP1B1 gene were found to deviate from the Hardy-Weinberg equilibrium because of a variant homozygote excess. This variant in the CYP1B1 gene may thus be an important candidate for SNP predisposing to develop either postmenopausal osteopenia or osteoporosis although the baseline BMD did not show any significant difference between the different genotypes in this study. The catalytic activities of variant enzymes, especially the nucleotide changes in exon2 (A119S polymorphism) and exon3 (L432V polymorphism) of the CYP1B1 gene, have been reported to be 2-4 times higher than those of wild-type enzymes.^{17,21,22,23)} A significant decrease in the estradiol levels in postmenopausal women with the L432V variant homo genotype has been also reported.¹³⁾ Therefore, the L432V variant which corresponds to the hyperactivity of CYP1B1 accelerates the estradiol metabolism and may

affect the response to HRT regarding the lumbar BMD.

In summary, our genetic analyses of the CYP1A1 and CYP1B1 variations and correlations between these features regarding the effect of HRT on the lumbar spine BMD, these findings suggest that L432V SNP in the CYP1B1 gene could act as a marker of the drug response. An analysis of the CYP1B1 gene SNPs could prove effective in appropriately selecting HRT for the management of either osteopenia in postmenopausal women. The number of the L432V variants in this study was limited. Additional studies are therefore necessary to clarify the precise mechanisms by which the CYP1B1 gene polymorphisms modulate the responsiveness of BMD to HRT.

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