Val158Met Polymorphism in *Catechol-O-methyltransferase* Gene Associated with Risk Factors for Breast Cancer[1]

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Abstract

Extensive mammographic density is heritable, strongly associated with increased breast cancer risk, and is influenced by sex hormone exposure. In a cross-sectional study of 181 pre- and 171 postmenopausal women without breast cancer, we examined the relationship of a functional polymorphism in *catechol-O-methyltransferase* (*COMT*; VAL→MET) to mammographic density and other risk factors for breast cancer. We hypothesized that individuals who inherited the low-activity form of COMT (*COMT*2* allele) would have higher levels of breast density, presumably because of reduced inactivation/detoxification of catecholestrogens. Subjects were recruited across five categories of breast density. Risk factor information, anthropometric measures, and blood samples were obtained; sex hormone and growth factor levels were measured, and *COMT* genotypes determined. Mammograms were digitized and measured using a computer-assisted method. After adjustment for age and ethnicity, among pre- but not postmenopausal subjects, each low-activity *COMT*2* allele was associated with lower levels of percentage breast density. The statistical significance of this association was lost after further adjustment for serum growth factors [growth hormone, insulin-like growth factor-1 (IGF-1), and insulin-like growth factor binding protein-3 (IGFBP-3)], hormones [follicle-stimulating hormone (FSH) and progesterone], and body size (body mass index and waist:hip ratio). The low-activity *COMT*2* allele was also associated, after adjustment for age and ethnicity in premenopausal women, with lower serum levels of IGF-1, higher levels of FSH and progesterone, and with a larger waist:hip ratio, body mass index, and subscapular skinfold. After adjustment for body size, the associations of genotype with IGFBP-3 and FSH were no longer significant. These findings indicate that *COMT* genotype is associated with several risk factors for breast cancer and suggest that the low-activity *COMT*2* allele is associated with a reduced risk of breast cancer among premenopausal women.

Introduction

Variations among women in the radiological appearance, or mammogram pattern, of the breast reflect differences in tissue composition. Fat is radiologically lucent and appears dark on a mammogram, whereas stroma and epithelial tissue are radiologically dense and appear light on a mammogram, which we refer to here as "mammographic density." Wolfe (1, 2) first described an association between a qualitative classification of dense mammographic patterns and increased breast cancer risk, which has since been confirmed by several other studies. Studies that have used quantitative methods to classify mammographic density have in general found larger gradients in risk with 4–6-fold differences in risk between women with the most extensive categories of density compared with those with little or no density (3, 4).

Mammographic density is influenced by several factors associated with variations in exposure to endogenous hormones. Increasing age, greater parity, and greater body weight are associated with less extensive density (3). Density decreases at menopause (5) and is increased by hormone replacement therapy (6).

A recently completed classical twin study (7) has estimated in two populations, in Australia and North America, the extent to which genetic factors account for the large proportion of unexplained variance in mammographic density. After adjusting for age and measured covariates, heritability was 60% (95% CI,[3] 54–66%) from Australian twins, 67% (95% CI, 59–75%) from North American twins, and 63% (95% CI, 59–67%) from the studies combined. No specific genes have yet been identified, although the factors known to influence density suggest that genes concerned with hormone metabolism may be involved.

A polymorphism in the gene that codes for COMT has recently garnered much attention with respect to breast cancer risk because COMT is principally responsible for both the

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[3] The abbreviations used are: CI, confidence interval; COMT, catechol-*O*-methyltransferase; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; BMI, body mass index; WHR, waist:hip ratio. SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone.

inactivation and detoxification of catecholestrogens, which can cause oxidative damage (8, 9). The product of COMT, 2-methoxyestradiol, may also have a protective role against estrogen-induced carcinogenesis by inhibiting angiogenesis and cell proliferation both *in vitro* and *in vivo* (10).

Two variants of COMT exist, soluble and membrane bound, which are encoded by a single gene located at chromosome 22q11 using alternative promoters (11). In all of the tissues examined thus far, COMT activity has been mostly found in cytosol. A single base pair change (G→A) produces an amino acid change (Val→Met) at codon 108 of soluble COMT and codon 158 of membrane-bound COMT that reduces enzyme activity by 3–4-fold (12). The *COMT*1* allele encodes for the high-activity enzyme, and the *COMT*2* allele encodes for the thermolabile low-activity enzyme.

The purpose of this study was to determine, with a cross-sectional design, whether the Val→Met polymorphism in *COMT* is related to variations in mammographic density. We hypothesized that individuals who inherit the low-activity form of COMT will have higher levels of breast density, presumably because of reduced inactivation and/or detoxification of catecholestrogens, and decreased formation of antitumorigenic 2-methoxyestradiol. We also examined the relationship of *COMT* genotype with sex hormones and other factors known or suspected of influencing mammographic density, including body size, the pituitary hormones prolactin and GH, and with IGF-1 and its main binding protein, IGFBP-3.

Materials and Methods

Methods. The methods used in this study have been published in detail elsewhere (13) and will be only briefly described here.

Source of Subjects. Between 1994 and 1997, potential subjects were identified from the mammographic units of Mount Sinai, Women's College, and St. Michael's Hospital in Toronto. The extent of mammographic density for all of the patients was visually estimated by a radiologist and expressed as a percentage of breast area on a five-point scale. The purpose of our approach to recruitment was to assemble a group of women without breast cancer and with a wide range of density levels by recruiting approximately equal numbers of women in each of five categories of density, with overrepresentation of the extreme categories. The distribution of breast density in this sample of women is, thus, not expected to be the same as in the general population, and expected differences in breast density between pre- and postmenopausal women may be smaller than are usually seen. The number of subjects (n) recruited into each of the five categories were as follows: $<10\%$, $n = 101$; 10 to $<25\%$, $n = 62$; 25 to $<50\%$, $n = 60$; 50 to $<75\%$, $n = 60$; and $\geq 75\%$, $n = 99$.

Recruitment. Potential subjects were sent a letter and subsequently telephoned about the study. Premenopausal subjects were eligible if they were menstruating regularly, not pregnant or breast feeding, and had not had a hysterectomy or oophorectomy. Postmenopausal women were eligible if they had spontaneous amenorrhea for at least 12 months, or had a hysterectomy and were 50 years of age or older, or had a bilateral oophorectomy at any age. A subject was excluded if she was taking any type of exogenous hormone preparation, had breast augmentation or reduction, a personal history of breast cancer, or was being investigated for breast cancer. In total, 382 women agreed to participate in the study, representing 88% of subjects who were contacted and found to be eligible.

Obtaining Consent for Genetic Study. After participating in the above study on determinants of breast density, a letter that described the goals of the genetic component of this study was mailed to subjects, and written consent was obtained to analyze DNA for genetic polymorphisms that might potentially affect breast density levels. Information on ethnicity was also obtained. Of 382 eligible subjects (193 pre- and 189 postmenopausal women), 357 (93%) gave consent for use of their DNA. Eight subjects could not be contacted because they had moved and could not be traced through either telephone directories or their physicians. Sixteen subjects were nonresponders after a minimum of 4 telephone reminders, and 1 did not provide consent.

Ethnicity. By questionnaire (see below), each subject was asked her country of birth, as well as the countries of birth for each of her parents and grandparents. Subjects were also asked the question "What is your ethnic or cultural background?" and given instructions to mark all appropriate categories. Subjects were classified as (*a*) black; (*b*) white (*e.g.,* British, French, European, Latin/South American of European background); (*c*) Native/Aboriginal People of North America (North American Indian, Inuit, Métis); (*d*) East Asian (*e.g.,* Chinese, Japanese, Korean, Vietnamese); (*e*) South Asian (*e.g.,* Indian from India, Pakistani, Punjabi, Tamil); (*f*) Other, with specification; and (*g*) Don't know. Because of low numbers in groups other than Caucasians, the categories were collapsed and described as (*a*) Caucasian (white), (*b*) East Asians, (*c*) Jewish, and (*d*) Other.

Measurements. Data and blood samples were collected after a 12-h overnight fast and, for premenopausal subjects, during the luteal phase of the menstrual cycle (days 20–24). The mammogram closest to the time of the blood draw was used (mean difference was 32 weeks).

Epidemiological and Anthropometric Data. Information about epidemiological risk factors for breast density and breast cancer was collected by questionnaire, and dietary information was obtained using a list-based food frequency questionnaire developed by Block *et al.* (14). Each subject was weighed and measured for height, and waist and hip circumference.

Mammographic Density. Breast density measurements were made using a randomly selected, craniocaudal (viewing from above, down) mammographic view of one breast from each subject. Randomly ordered mammograms were digitized using a Lumisys model 85 and were presented to the observer (N. F. B.) for analysis as an array of 675×925 pixels (0.0676 mm^2/pixel). The observer (N. F. B.) selected a threshold gray value to separate the image of the breast from the background. A second threshold was selected to identify the edges of regions representative of radiographically dense tissue. Summation of pixels within these areas gave a measure of the area of density in the breast. The percentage of radiographic density is the area of dense tissue divided by the entire projected area of the breast multiplied by 100. Additional details of this method are given elsewhere (15).

Measurement of Blood Samples. Measurements were made of serum estradiol, free estradiol, progesterone, FSH, SHBG, prolactin, GH, IGF-1, and IGFBP-3 levels (13).

***COMT* Genotype.** DNA was purified from buffy coats of blood samples using a modified chaotropic method as reported by Wang *et al.* (16) Of 357 participants who consented to participate in the study, DNA samples could not be isolated for 5 subjects, which brought the total number of study subjects down to 352.

PCR-based RFLP assays were used to determine *COMT*

Table 1 Selected characteristics of study subjects by menopausal status

	Mean (SD)			
	Caucasian[a] Pre $n = 155$ Post $n = 141$	East Asian[b] Pre $n = 5$ Post $n = 9$	Jewish[c] Pre $n = 11$ Post $n = 5$	Other[d] Pre $n = 10$ Post $n = 15$
Premenopausal				
Risk factors				
Age, yr	44.7 (4.7)	47.4 (4.2)	45.6 (3.0)	45.5 (4.6)
Height, cm	163.8 (6.1)	155.2 (6.3)	161.7 (5.1)	162.1 (10.4)
Weight, kg	68.25 (16.2)	57.08 (5.9)	60.32 (10.6)	67.2 (15.3)
BMI, kg/m^2	25.4 (6.0)	23.6 (1.2)	23.0 (3.6)	25.6 (5.5)
Waist:Hip ratio	0.75 (0.06)	0.75 (0.04)	0.73 (0.07)	0.75 (0.06)
Age at menarche, yr	12.7 (1.4)	12.6 (2.7)	12.2 (0.6)	13.8 (1.4)
Age at first birth, yr	28.1 (5.8)	33.3 (6.7)	29.5 (2.7)	24.4 (7.9)
No. of live births	1.4 (1.2)	1.2 (1.1)	1.9 (1.4)	1.8 (1.3)
Mammographic density, %[e]	28.0 (22.7)	49.7 (15.5)	37.0 (24.1)	22.1 (17.5)
Pituitary hormones and growth factors				
Prolactin, μg/liter	16.3 (11.4)	22.0 (15.6)	12.8 (7.5)	25.2 (20.9)
GH, μg/liter	1.7 (2.4)	2.6 (4.5)	2.5 (2.4)	3.6 (6.2)
IGF-1, μg/liter	155.9 (35.8)	149.0 (33.7)	141 (35.0)	143.6 (23.5)
IGFBP-3, mg/liter	2.7 (0.5)	2.6 (0.3)	2.7 (0.6)	2.6 (0.5)
Sex hormones				
Estradiol, pmol/liter	321.2 (219.3)	172.4 (115.8)	239.2 (164.6)	361.5 (165.6)
Free estradiol, %	2.2 (0.7)	1.9 (0.6)	1.9 (0.8)	2.3 (0.6)
SHBG, nmol/liter	55.6 (26.1)	63.6 (21.9)	65.6 (27.8)	49.1 (20.3)
Progesterone, nmol/liter	28.9 (22.7)	30.0 (31.4)	29.3 (21.6)	31.6 (20.0)
FSH, IU/liter	9.3 (15.3)	20.7 (25.9)	10.3 (20.1)	7.2 (8.5)
Postmenopausal				
Risk factors				
Age, yr	56.1 (4.7)	54.3 (3.3)	53.7 (2.3)	56.3 (4.1)
Height, cm	164.9 (6.6)	157.1 (4.7)	164.7 (4.4)	162.2 (6.5)
Weight, kg	71.7 (16.9)	56.4 (8.2)	69.0 (16.6)	65.3 (14.4)
BMI, kg/m^2	26.4 (6.1)	23.0 (3.9)	25.5 (6.2)	24.8 (5.2)
Waist:Hip ratio	0.76 (0.08)	0.77 (0.07)	0.78 (0.10)	0.79 (0.06)
Age at menarche, yr	13.1 (1.6)	12.9 (1.5)	12.7 (2.3)	12.9 (2.2)
Age at first birth, yr	26.0 (5.2)	33.2 (7.1)	23.0 (3.3)	29.7 (8.1)
No. of live births	1.7 (1.5)	1.3 (1.4)	2.2 (1.5)	1.2 (1.2)
Mammographic density,[e] %	21.3 (19.4)	50.7 (11.5)	26.6 (24.3)	30.5 (21.6)
Pituitary hormones and growth factors				
Prolactin, μg/liter	9.7 (5.6)	8.8 (3.7)	7.7 (3.1)	10.4 (3.4)
GH, μg/liter	1.6 (2.6)	1.5 (2.4)	0.5 (0.4)	0.7 (0.8)
IGF-1, μg/liter	128.2 (33.2)	137.2 (38.1)	133.5 (32.2)	142.9 (41.8)
IGFBP-3, mg/liter	2.8 (0.5)	2.8 (0.5)	3.0 (0.5)	2.8 (0.6)
Sex hormones				
Estradiol, pmol/liter	49.7 (98.8)	46.4 (42.4)	26.3 (10.2)	46.9 (32.2)
Free estradiol, %	2.5 (0.6)	2.2 (0.7)	2.3 (0.6)	2.5 (0.5)
SHBG, nmol/liter	42.5 (22.7)	52.1 (26.1)	48.3 (20.1)	41.7 (16.8)
Progesterone, nmol/liter	1.7 (1.1)	1.7 (0.7)	1.6 (1.2)	2.1 (0.9)
FSH, IU/liter	72.1 (30.7)	71.1 (23.2)	72.1 (11.1)	71.7 (31.3)

[a] Premenopausal (Pre): $n = 107$ for age at first birth; $n = 153$ for GH; $n = 154$ for IGF-1, IGFBP-3, and free estradiol. Postmenopausal (Post): $n = 104$ for age at first birth; $n = 127$ for GH; $n = 140$ for IGF-1, IGFBP-3, free estradiol; $n = 119$ for total estradiol.
[b] Pre: $n = 3$ for age at first birth. Post: $n = 6$ for age at first birth; $n = 7$ for GH; $n = 8$ for total estradiol, free estradiol, and SHBG.
[c] Pre: $n = 8$ for age at first birth; $n = 10$ for GH. Post: $n = 5$ for age at first birth.
[d] Pre: $n = 8$ for age at first birth. Post: $n = 10$ for age at first birth.
[e] Proportion of breast area occupied by dense tissue.

genotype. PCR primers 5′-TCG TGG ACG CCG TGA TTC AGG-3′ and 5′-AGG TCT GAC AAC GGG TCA GGC-3′ were used to amplify a 217-bp fragment of *COMT* that contains the polymorphic *Nla*III site as well as one other constant *Nla*III site. Each 25-μl PCR reaction mixture contained 60 ng of DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 μM deoxynucleoside triphosphate, 0.25 μM of each primer, and 0.5 units Amplitaq DNA polymerase. The reaction mixture was first denatured at 94°C for 5 min and then amplified by PCR for 32 cycles at 94°C for 30 s, at 55°C for 30 s, and at 72°C for 1 min, followed by a 10-min extension at 72°C. Twelve μl of PCR product was incubated with 10 units of *Nla*III in a volume of 20 μl at 37°C overnight. The same amount of PCR product was incubated without *Nla*III in the same buffer side by side. Two μg of ethidium bromide and 5 μl of 6× Ficoll Loading Buffer were added to each tube and loaded on a 12% PAGE gel. The electrophoresis was carried out in 1× TBE (Tris-borate EDTA) buffer at 180 V for 2.5 h. The gel was then photographed under a UV transluminator. *Nla*III cuts only the low-activity variant (*COMT**2) in addition to a second constant cleavage site in the PCR product. Thus, homozygotes for *COMT**1 generated fragments of 136 and 81 bp, heterozygotes

gave 136, 96, 81, and 40-bp fragments, and homozygotes for *COMT**2 generated 96, 81, and 40-bp fragments. (The 40-bp fragment ran off the gel during electrophoresis.)

Statistical Methods. Data were analyzed using the SAS statistical software package (version 6.12; 17). Data were inspected for normality and, when necessary, were transformed to approximate a normal distribution. This included square-root transformations of percentage breast density, area of dense breast tissue, total estradiol levels in premenopausal women, SHBG, progesterone in premenopausal women, IGF-1, and IGFBP-3. The following variables were natural-log transformed before analyses: area of nondense breast tissue; progesterone in postmenopausal women; FSH; prolactin; GH; BMI; subscapular skinfold; waist circumference; and hip circumference. For postmenopausal women, a power transformation was used for total estradiol levels, where $y = [1 - (1/\sqrt{estradiol + 0.5})]*2$. Free estradiol values for pre- and postmenopausal women were squared-transformed before analyses. All of the transformed variables were back-transformed for presentation in Tables and Figures, and 95% CIs were calculated.

The allelic distribution in each ethnic group was determined and tested for Hardy-Weinberg equilibrium. Relationships between *COMT* genotype, breast density, hormone levels, and anthropometric measurements were explored using analysis of covariance. Age and ethnicity were included in all of the models. Additional adjustments were made to control for potential confounding variables; details are given in the Tables and Figures.

Differences in results were found for pre- and postmenopausal subjects; thus, all of the data are presented stratified by menopausal status. Bonferroni *t* tests were used when a significant main effect of genotype was evident in the analysis and the data were being analyzed to determine differences among the three genotypes (*i.e., *1/*1, *1/*2,* and *2/*2). Differences between genotype pairs were considered significant if they were equal to or below $P = 0.017$ (0.05/3).

Values for GH were missing in 19 (premenopausal, $n = 3$; postmenopausal, $n = 16$) and undetectable in 120 (premenopausal, $n = 53$; postmenopausal, $n = 67$) of 357 (34%) subjects in the study. A missing value occurred when the volume of serum available for a subject was insufficient for both a GH and IGF-1 analysis. In such instances, IGF-1 values were determined and GH assays were not performed. Nondeterminate values were assumed to be caused by the episodic and pulsatile nature of GH release, which renders basal hormone levels with considerable variability. Consequently, for all statistical analyses that involved GH, the analyses were first performed on the subset of 218 women with GH values and then were repeated in the entire set of subjects by assigning a value of 0.2 ng/liter to the 120 undetectable measurements. The assigned value of 0.2 ng/liter represents the lower limit of sensitivity for the assay. In all instances, the results were not different and are, therefore, presented here with all of the study subjects included. To further ensure that we were not introducing a bias into the results, we examined for a relationship between GH detectability and *COMT* genotype and found that none existed.

Results

Characteristics of Subjects by Ethnicity. There were 181 pre- and 171 postmenopausal subjects who participated in the study. Selected characteristics of these subjects according to ethnicity are shown in Table 1. Eighty-four % of the subjects were Caucasian, 3.9% were East Asian, 4.8% were Jewish, and 7.1% were from other ethnic groups. The mean age was 45 years in pre- and 56 years in postmenopausal subjects. The two groups were similar in height, total number of live births, and age at menarche. Postmenopausal subjects had a higher BMI, were on average younger at their first birth, had a lower percentage mammographic density, and had lower blood levels of prolactin and IGF-1. Blood levels of GH and IGFBP-3 were similar in pre- and postmenopausal subjects.

Compared with Caucasians, pre- and postmenopausal East Asian women were shorter, lighter, older at first birth, and had greater levels of percentage breast density. Premenopausal Jewish subjects had greater levels of percentage breast density than Caucasians. Compared with Caucasians, premenopausal East Asians and Jewish subjects had lower levels of total and free estradiol and higher levels of SHBG, and East Asians also had higher levels of circulating FSH. In postmenopausal women, compared with Caucasians, East Asians had higher IGF-1 levels, Jewish subjects had lower estradiol levels, and both groups had higher SHBG levels.

Table 2 *COMT* genotype and allele frequency distribution

	n	*COMT* genotype distribution[a]			Allele frequency	
		**1/*1*	**1/*2*	**2/*2*	*COMT*1*	*COMT*2*
Premenopausal						
All women	181	45	98	38	0.52	0.48
Caucasian	155	38	86	31	0.52	0.48
East Asian	5	2	2	1	0.60	0.40
Jewish	11	2	5	4	0.41	0.59
Other	10	3	5	2	0.55	0.45
Postmenopausal						
All women	171	46	79	46	0.50	0.50
Caucasian	141	28	71	42	0.45	0.55
East Asian	9	7	2	0	0.89	0.11
Jewish	6	2	2	2	0.50	0.50
Other	15	9	4	2	0.73	0.27

[a] *COMT*1/*1* is associated with the high-activity phenotype, **1/*2* with the intermediate-activity phenotype, and **2/*2* with the low-activity phenotype.

Ethnicity and Allele Frequencies. Table 2 shows the observed allelic frequencies for each ethnic group. None differed significantly from Hardy-Weinberg expected values. The frequency of the *COMT*2* allele was 0.48 among premenopausal subjects and 0.50 among postmenopausal subjects, similar to published values for Caucasian populations of mixed European ancestry (18, 28). East Asians had the lowest frequency of *COMT*2*, 0.21 overall, which is similar to previously published values (28), although the frequency differed between menopausal groups (40% in pre, 11% in post). A low frequency of *COMT*2* alleles was also observed for the 15 postmenopausal subjects from "other" ethnicities.

***COMT* Genotype and Mammographic Measures.** Mean values for the mammographic measures of percentage density, dense area, and nondense area according to *COMT* genotype, and adjusted for age and ethnicity, are shown in Fig. 1. In premenopausal women, each additional *COMT*2* allele was associated with lower levels of percentage breast density after adjustment for age and ethnicity ($F = 3.19$, $P = 0.04$). Mean percentage breast density was 35.5% (95% CI, 25.0–47.9) in the homozygous *COMT*1* group, 30.4% (95% CI, 21.7–40.2) in heterozygotes, and 21.3% (95% CI, 13.1–31.6) in homozygous *COMT*2* subjects. These associations with percentage density were attributable to associations of the low-activity *COMT*2* allele with a smaller area of dense breast tissue ($P =$

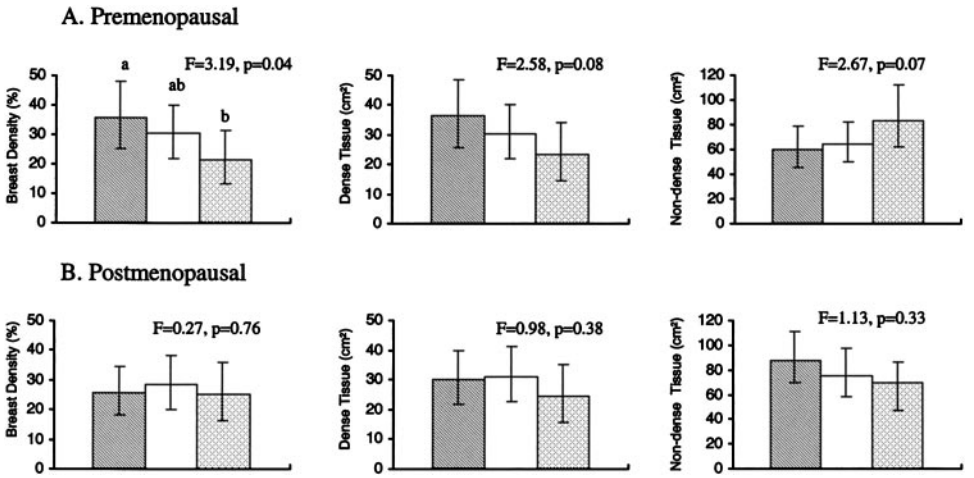

Fig. 1. *COMT* genotype and mammographic features. Values are least square means (±95% CI). All of the analyses are adjusted for age and ethnicity. In each genotype group, the number of subjects (*n*) are as follows: *A,* premenopausal group **1/*1*, $n = 45$; group **1/*2*, $n = 98$; group **2/*2*, $n = 38$; *B,* postmenopausal group **1/*1*, $n = 46$; group **1/*2*, $n = 79$; group **2/*2*, $n = 46$. *a, b, ab,* genotypes not sharing the same superscripts are significantly different, *i.e., a* and *b* are significantly different, but *a* and *ab* (or *b* and *ab*) are not significantly different (Bonferroni *t* test, $P < 0.016$). ▨, group **1/*1*; □, group **1/*2*; ▩, group **2/*2*.

0.08) and with a larger area of nondense tissue ($P = 0.07$). In postmenopausal subjects, the *COMT*2* allele was not associated with any mammographic measure ($P > 0.33$).

***COMT* Genotype, Sex Hormones, and Growth Factors.** Serum levels of sex hormones and growth factors according to *COMT* genotype are shown in Table 3. All of the results were adjusted for age and ethnicity, and those further adjusted for BMI and WHR are shown in italics.

Premenopausal subjects hetero- and homozygous for the *COMT*2* allele had mean progesterone levels that were, respectively, 32.1 and 43.4% lower than homozygotes for the *COMT*1* allele. These differences were statistically significant both before ($F = 9.17$, $P = 0.0002$) and after ($F = 6.81$, $P = 0.001$) adjustment for BMI and WHR. The low-activity *COMT*2* allele was also associated with lower FSH levels ($F = 2.98$, $P = 0.05$), although this relationship was no longer significant after adjustment for BMI and WHR ($P = 0.20$). None of the other measured sex hormones varied according to *COMT* genotype. In postmenopausal subjects, no associations were observed between *COMT* genotype and measured sex hormones.

Prolactin and GH levels did not vary with *COMT* genotype in pre- or postmenopausal subjects. The *COMT*2* allele was, however, associated with IGF-1 and IGFBP-3 levels in premenopausal subjects. Mean IGF-1 levels were 5.5% lower in heterozygotes and 13.9% lower in *COMT*2* homozygotes, compared with *COMT*1* homozygotes. In contrast, IGFBP-3 levels were 5.1% higher in heterozygotes and 10.3% higher in *COMT*2* homozygotes compared with *COMT*1* homozygotes. Additional adjustments for BMI and WHR attenuated these relationships slightly, and the association between *COMT* genotype and IGFBP-3 was no longer statistically significant ($P = 0.19$). In postmenopausal subjects, *COMT* genotype was not associated with levels of IGF-1 or IGFBP-3.

***COMT* Genotype and Anthropometric Characteristics.** *COMT* genotype was not related to height in pre- or postmenopausal subjects (data not shown; $P \geq 0.11$). Selected results showing the relationship of *COMT* genotype and other measurements of body size are shown in Fig. 2. In premenopausal subjects, the low activity *COMT*2* allele was associated with greater BMI ($F = 3.31$, $P = 0.04$), WHR ($F = 8.33$, $P = 0.0003$), and subscapular skinfold thickness ($F = 4.77$, $P = 0.01$) after adjustment for age and ethnicity. The *COMT*2* allele was not associated with BMI in postmenopausal subjects but was associated with lower mean WHR ($F = 3.42$, $P = 0.04$) and subscapular skinfold thickness ($F = 5.04$, $P = 0.008$). The menopausal differences observed for WHR and subscapular skinfold were both statistically significant (WHR: $F = 9.37$, $P = 0.0001$ for interaction; subscapular skinfold: $F = 8.31$, $P = 0.0003$ for interaction).

Because the GH-IGF axis is known to be associated with body fat, we reanalyzed the relationships between *COMT* and measurements of body size, adjusting for GH, IGF-I, and IGFBP-3, to determine whether the associations of genotype and body size were independent of these factors. Results are shown in Fig. 2 (*viewer's right hand side of each panel*).

The association of *COMT* genotype with BMI (further adjusted for WHR) and subscapular skinfold (further adjusted for BMI and WHR) in premenopausal women were no longer significant, but WHR (further adjusted for BMI) remained significantly associated with *COMT* genotype ($F = 3.91$, $P = 0.02$). In postmenopausal subjects, the *COMT*2* allele continued to be associated with lower mean subscapular skinfold thickness ($F = 5.06$, $P = 0.008$).

***COMT* Genotype, Body Size, Hormones, Growth Factors, and Mammographic Density.** We examined the possibility that the association of *COMT* with body size, sex hormones, and growth factors, as described above, were responsible for mediating the relationship between *COMT* genotype and breast density levels. This was done by introducing these factors, singly and in combination, into a regression model that contained *COMT* as an independent variable and mammographic density as the dependent variable. If these factors mediate the relationship between genotype and mammographic density, we expect the strength of the relationship between *COMT* and breast density to be reduced as these factors are added to the model and displace *COMT* in explaining variations in density. Mutually adjusted values for the GH-IGF-I axis, body size, and sex hormones were determined because we sought to obtain independent measures of the effects of these variables. For example, adjusted values for body size were obtained by regressing measures of body size (BMI or WHR) with sex hormones and growth factors to obtain predicted values. We then subtracted predicted values from observed values to obtain adjusted values (residuals), which were then used in regression analyses.

The influence of adjustment for body size, the GH-IGF-I axis, and sex hormones on the association of *COMT* genotype

Table 3 *COMT* genotype, sex hormones, and growth factors

Values are least square means (95% CI), and all of the models are adjusted for age and ethnicity. Results provided in italics have been further adjusted for BMI and WHR.[a,b,ab]

	Premenopausal					Postmenopausal				
	Genotype			*F*	*P*	Genotype			*F*	*P*
	*1/*1 $n = 45$[c]	*1/*2 $n = 98$[d]	*2/*2 $n = 38$[e]			*1/*1 $n = 46$[f]	*1/*2 $n = 79$[g]	*2/*2 $n = 46$[h]		
Sex hormones										
Total estradiol	282.2 (209.9–365.3)	237.8 (180.1–303.4)	257.6 (185.5–341.5)	0.88	0.42	30.4 (24.6–38.4)	32.2 (25.9–41.4)	30.4 (24.6–38.4)	0.38	0.68
(pmol/liter)	*291.0 (217.5–375.3)*	*241.5 (183.4–307.6)*	*242.7 (171.9–325.8)*	*1.11*	*0.33*	*30.7 (26.2–35.7)*	*33.9 (28.4–41.2)*	*32.9 (27.3–40.4)*	*0.58*	*0.56*
Free estradiol (%)	2.02 (1.73–2.27)	2.19 (197–2.40)	2.21 (1.94–2.45)	1.28	0.28	2.58 (2.38–2.76)	2.37 (2.15–2.57)	2.40 (2.16–2.62)	1.90	0.15
	2.14 (1.89–2.36)	*2.23 (2.03–2.41)*	*2.13 (1.57–2.36)*	*0.62*	*0.54*	*2.57 (2.41–2.72)*	*2.40 (2.22–2.57)*	*2.53 (2.34–2.70)*	*2.16*	*0.12*
SHBG (nmol/liter)[j]	62.3 (52.2–73.2)	55.5 (47.1–64.6)	54.5 (44.6–65.4)	1.28	0.28	39.6 (32.7–47.0)	46.0 (37.9–54.9)	46.8 (37.6–56.9)	1.39	0.25
	57.9 (48.8–67.8)	*54.0 (46.3–62.4)*	*58.2 (48.5–68.8)*	*0.67*	*0.51*	*39.9 (34.0–46.4)*	*45.3 (38.2–53.0)*	*42.1 (34.6–50.4)*	*1.16*	*0.32*
Progesterone (nmol/liter)[k]	36.8 (29.2–45.4)[a]	25.0 (19.5–31.2)[b]	20.8 (14.9–27.7)[b]	9.17	0.0002	1.5 (1.2–1.8)	1.6 (1.3–2.0)	1.6 (1.3–2.0)	0.44	0.65
	34.5 (27.3–42.5)[a]	*24.3 (19.1–30.2)[b]*	*21.4 (15.6–28.1)[b]*	*6.81*	*0.001*	*1.5 (1.2–1.8)*	*1.6 (1.3–2.0)*	*1.6 (1.3–2.0)*	*0.32*	*0.73*
FSH (IU/liter)[l]	6.8 (5.2–9.0)[a]	6.2 (4.9–7.9)[ab]	4.8 (3.6–6.4)[b]	2.98	0.05	64.1 (55.9–73.5)	61.0 (53.1–69.9)	73.0 (61.2–87.0)	2.69	0.07
	6.4 (4.9–8.5)	*6.0 (4.8–7.6)*	*4.9 (3.7–6.5)*	*1.61*	*0.20*	*63.8 (55.9–72.9)*	*61.2 (53.0–70.6)*	*69.5 (59.4–81.3)*	*1.56*	*0.21*
Growth factors										
Prolactin (μg/liter)	16.1 (13.0–20.0)	14.3 (11.8–17.4)	16.0 (12.6–20.2)	0.97	0.38	8.0 (6.9–9.3)	8.7 (7.4–10.1)	8.3 (6.9–9.9)	0.49	0.61
	16.0 (12.9–19.8)	*14.3 (11.8–17.3)*	*15.7 (12.5–19.7)*	*0.87*	*0.42*	*8.0 (6.9–9.2)*	*8.5 (7.3–9.9)*	*7.9 (6.7–9.4)*	*0.52*	*0.60*
GH (μg/liter)[m]	1.41 (0.85–2.34)	0.86 (0.56–1.33)	0.96 (0.56–1.64)	2.24	0.11	0.46 (0.30–0.72)	0.46 (0.29–0.72)	0.53 (0.32–0.88)	0.22	0.80
	1.29 (0.78–2.11)	*0.82 (0.53–1.25)*	*1.06 (0.62–1.81)*	*2.18*	*0.12*	*0.45 (0.29–0.69)*	*0.44 (0.28–0.69)*	*0.47 (0.29–0.78)*	*0.06*	*0.94*
IGF-1 (μg/liter)[n]	159.0 (147.5–170.9)[a]	150.2 (140.5–160.1)[a]	136.9 (125.8–148.4)[b]	6.25	0.002	129.0 (119.2–139.2)	132.9 (122.5–143.7)	134.5 (122.9–146.6)	0.40	0.67
	156.2 (145.4–167.3)[a]	*148.6 (139.6–157.8)[a]*	*139.9 (129.2–151.1)[b]*	*3.51*	*0.03*	*129.0 (119.4–139.0)*	*134.3 (123.7–145.3)*	*132.2 (120.9–144.1)*	*0.44*	*0.65*
IGFBP-3 mg/liter[o]	2.53 (2.37–2.70)[a]	2.66 (2.52–2.80)[ab]	2.79 (2.61–2.97)[b]	3.83	0.02	2.77 (2.62–2.92)	2.85 (2.70–3.01)	2.73 (2.56–2.91)	1.27	0.28
	2.58 (2.42–2.73)	*2.68 (2.55–2.82)*	*2.73 (2.56–2.90)*	*1.70*	*0.19*	*2.76 (2.61–2.91)*	*2.82 (2.63–3.02)*	*2.72 (2.55–2.90)*	*0.80*	*0.45*

[a,b,ab] Genotypes not sharing the same superscripts are significantly different, *i.e.*, *a* and *b* are significantly different, but *a* and *ab* (or *b* and *ab*) are not significantly different (Bonferroni *t* test, $P < 0.016$).
[c] $n = 44$ for free estradiol, GH, IGF-1, and IGFBP-3.
[d] $n = 97$ for total estradiol, SHBG, and GH.
[e] $n = 37$ for total estradiol, SHBG, GH, IGF-1, and IGFBP-3.
[f] $n = 43$ for total estradiol and SHBG; $n = 44$ for free estradiol and FSH; $n = 42$ for GH, IGF-1, and IGFBP-3.
[g] $n = 62$ for total estradiol and SHBG; $n = 69$ for GH, IGF-1, and IGFBP-3.
[h] $n = 40$ for total estradiol and SHBG; $n = 43$ for GH, IGF-1, and IGFBP-3.
[i] Adjusted for SHBG.
[j] Adjusted for total estradiol levels.
[k] Models adjusted for FSH.
[l] Adjusted for progesterone levels in premenopausal women and for free estradiol values in postmenopausal women.
[m] Adjusted for IGF-1 and IGFBP-3.
[n] Adjusted for GH and IGFBP-3.
[o] Adjusted for GH and IGF-1.

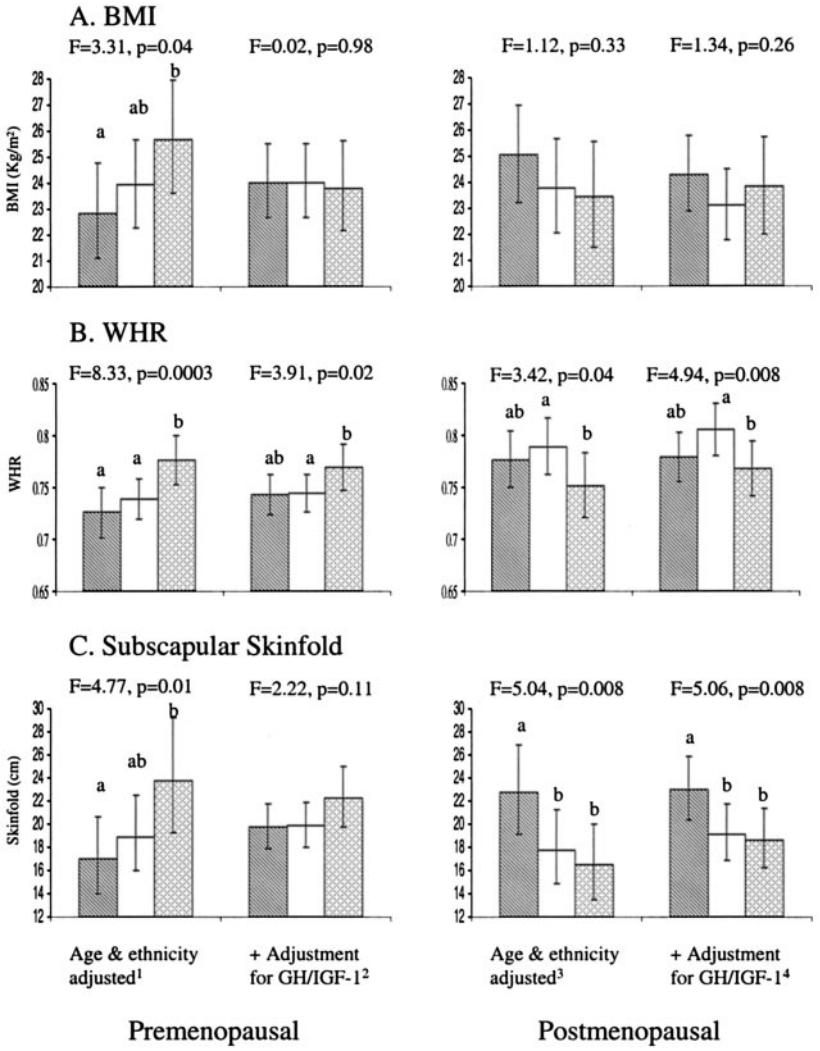

Fig. 2. COMT genotype and anthropometric variables. Values are least square means (95% CI), and all of the analyses are adjusted for age and ethnicity. [1] Premenopausal subjects (*n*) of group **1/*1, n* = 45; group **1/*2, n* = 98; group **2/*2, n* = 38 [*n* = 37 for subscapular skinfold (*C*)]. [2] Results (premenopausal) are further adjusted for GH, IGF-1, and IGFBP-3. *A,* BMI analyses are also adjusted for WHR; *B,* WHR analyses are also adjusted for BMI; *C,* subscapular skinfold analyses are also adjusted for BMI and WHR. Premenopausal group **1/*1, n* = 44; group **1/*2, n* = 96; group **2/*2, n* = 37, except for subscapular skinfold (*C*), *n* = 36. [3] Postmenopausal group **1/*1, n* = 46 except for subscapular skinfold (*C*), *n* = 45; group **1/*2, n* = 79; group **2/*2, n* = 46. [4] Postmenopausal group **1/*1, n* = 42 except for subscapular skinfold (*C*), *n* = 41; group **1/*2, n* = 69; group **2/*2, n* = 43. *a, b, ab,* genotypes not sharing the same superscripts are significantly different, i.e. *a* and *b* are significantly different, but *a* and *ab* (or *b* and *ab*) are not significantly different (Bonferroni *t* test, $P < 0.016$). ▧, group **1/*1*; □, group **1/*2*; ▨, group **2/*2*.

with percentage mammographic density is shown in Fig. 3. In premenopausal women, adjustment for measures of body size (BMI and WHR), for the GH-IGF-1 axis, or for hormones (FSH and progesterone), had little influence on the association when these groups of variables were included in the model, one or two (data not shown) at a time. However, when all of these variables were included together, the association of mammographic density with *COMT* genotype was removed completely ($F = 0.01$, $P = 0.99$). These findings, thus, suggest that body size, the GH-IGF-1 axis, and sex hormones mediate the relationship between *COMT* and breast density. In postmenopausal women, no statistically significant association between genotype and percentage density was seen before or after adjustment for these variables.

Discussion

Results from this study show that the low-activity *COMT*2* allele is associated with lower levels of mammographic density in pre- but not in postmenopausal women. In premenopausal women, the *COMT*2* allele, after adjustment for age and ethnicity, was also associated with lower levels of IGF-1, progesterone, and FSH, and with higher levels of IGFBP-3, WHR, BMI, and subscapular skinfold. All of these associations showed evidence of increasing effect with each additional low-activity allele. The relationship of *COMT* with mammographic density was entirely explained by the association of the genotype with body size, the GH-IGF-1 axis, and with the sex hormones progesterone and FSH. These observations suggest that, with the exception of the relationship of *COMT* with WHR, the low-activity *COMT*2* allele is associated with a reduced risk of breast cancer in premenopausal women. This was in direct contrast to our hypothesis that the *COMT*2* allele would be associated with increased breast density levels and, therefore, with increased risk of breast cancer.

Variations in breast density, sex hormones, the GH-IGF-1 axis, and body size are all known to influence the risk of breast cancer. Extensive mammographic density has been found to be associated with an increased risk of breast cancer in both pre- and postmenopausal women (3); and increased levels of mammographic density and/or breast cancer risk have been associated with higher blood levels of IGF-1 (in premenopausal women; Refs. 13, 19, 20) and progesterone (21), as well as with the use of hormone replacement therapies that contain both progestins and estrogen, rather than estrogen alone (6, 22). The

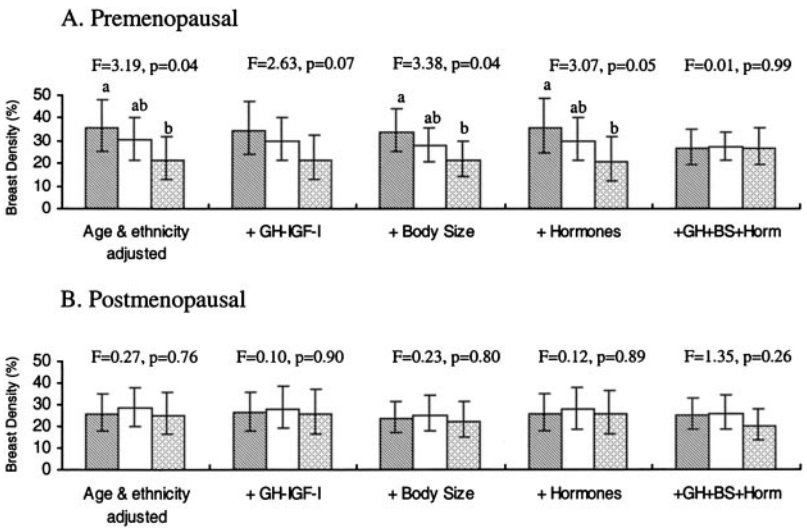

Fig. 3. *COMT* genotype and percentage breast density adjusted individually and in combination for the GH-IGF-I axis, body size, and hormones. Values are least square means (± 95% CIs). Number of subjects in each genotype group: premenopausal group **1/*1*, $n = 44$ for all analyses except age and ethnicity adjusted, for which $n = 45$; group **1/*2*, $n = 96$ for all analyses except age and ethnicity adjusted, in which $n = 98$; group **2/*2*, $n = 37$ in all analyses except age and ethnicity adjusted, in which $n = 38$. Postmenopausal group **1/*1*, $n = 42$ in all analyses except age and ethnicity adjusted, in which $n = 46$; group **1/*2*, $n = 69$ in all analyses except age and ethnicity adjusted, in which $n = 79$; group **2/*2*, $n = 43$ in all analyses except age and ethnicity adjusted, in which $n = 46$. The three categories of variables are mutually adjusted (see results section, *COMT* Genotype, Body Size, Hormones, Growth Factors, and Mammographic Density for explanation). GH analyses (*GH*) adjusted for age, GH, IGF-1, and IGFBP-3. Body size analyses (*BS*) adjusted for age, BMI, and WHR. Hormones analyses (*Horm*) adjusted for age, FSH, and progesterone. *a, b, ab,* genotypes not sharing the same superscripts are significantly different, i.e. *a* and *b* are significantly different, but *a* and *ab* (or *b* and *ab*) are not significantly different (Bonferroni *t* test, $P < 0.016$). ▧, group *1/*1; □, group *1/*2; ▨, group *2/*2.

association of FSH with *COMT* genotype was no longer significant after adjustment for body size, but the association of progesterone with *COMT* genotype remained significant after adjustment. All blood measurements were made between days 20 and 24 of the menstrual cycle and progesterone levels of 5 nmol/liter or greater were detected in 82% of the subjects, which confirmed that ovulation had occurred, and, therefore, the association observed is not likely to be the result of sampling error, but could still be so because of chance.

Increased BMI and WHR are strongly associated with less extensive mammographic density in both pre- and postmenopausal subjects (3, 23, 24), but the relationship of body size to the risk of breast cancer is complex. Obesity is associated with an increased risk of breast cancer after the menopause (25) but with a lower risk of the disease before the menopause (26). Increased WHR has also been found to be associated with increased postmenopausal breast cancer (26) and might also be associated with increased risk of premenopausal breast cancer (26, 27).

Our results are not likely to be influenced by bias in the selection of subjects or by population stratification. We recruited only 382 (20%) of the 1874 women to whom letters were sent, but most who did not participate could either not be contacted (40%), or were evaluated as ineligible (28%). Of the 434 subjects who were contacted and who were eligible, 382 (88%) agreed to participate. Among the Caucasian subjects who did participate, the overall frequency of the *COMT*2* allele was 0.51, compared with the expected frequency of 0.5 for a codominant allele in a Caucasian population of mixed European ancestry (18, 28). Ethnicity was controlled for in all of our statistical analyses, and results were also similar when all of the analyses were repeated on the 296 subjects identified as Caucasians (155 premenopausal, 141 postmenopausal; data not shown). A potential limitation of this study, however, is the relatively small sample size for each menopausal group, which increases the possibility of false positive and negative findings. Although this study was cross-sectional in design, because genotype is fixed, the causal direction of the relationship with phenotype is not in doubt.

The mechanisms by which *COMT* genotype influences the variables described here are unknown, although they are presumably related to the metabolic functions controlled by this gene, and the alterations in exposure to hormones or their metabolites that result from differences in activity. The enzyme encoded by the *COMT* gene inactivates by methylation the 2- and 4-hydroxyestrogens, as well as the catecholamines dopamine, norepinephrine, and epinephrine. In addition, the influence of these effects on the variables identified in this study are likely to be influenced by ovarian hormones, because the associations of *COMT* genotype with risk factors differed according to menopausal status.

The biological properties of 2-hydroxyestrogens, which are nonestrogenic (9) and antioxidant (29–31), and of 4-hydroxyestrogens, which are estrogenic and prooxidant (8, 9, 32), might account for an association with breast density because malondialdehyde, an end product of lipid peroxidation, is positively associated with density (33). The role of COMT in the methylation and inactivation of dopamine, norepinephrine, and epinephrine may, in part, explain the genotype's association with both body size and the GH–IGF-I axis because these neurotransmitters are important in regulating a number of physiological systems, including GH and body size (34–38). Secretion of GH, which stimulates production of IGF-I, is also influenced by both estrogen and catecholestrogens (39, 40); and, GH and IGF-1, in turn, stimulate progesterone production (41, 42). In our study, GH ($P = 0.02$) and IGF-1 ($P = 0.0001$) were strongly related to progesterone levels in pre- but not in postmenopausal subjects ($P > 0.18$).

The relationship of *COMT* genotype with anthropometric variables may also be mediated, at least in part, by an underlying relationship with the GH-IGF-I axis, which is known to participate in the regulation of body fat stores (43). In premenopausal women, the association of BMI with *COMT* genotype was no longer significant after adjustment for variables in the GH-IGF-I axis, although WHR remained significantly associated with genotype, even after adjustment for overall adiposity. The continued relationship of *COMT* with WHR suggests that *COMT* may play an independent role in determining aspects of adiposity, such as body fat distribution. Independent associations between *COMT* genotype and indicators of body fat distribution (WHR and subscapular skinfold), and not overall body fat, were also observed in postmenopausal women.

Several authors have hypothesized that the low-activity *COMT*2* allele would increase breast cancer risk (9), but re-

sults have been conflicting. To date, two studies have examined the relationship of *COMT* genotype to breast density, and neither found a statistically significant association in pre- or postmenopausal subjects. However, in one of these studies, all of the subjects had breast cancer (44), and because mammographic density is a risk factor for breast cancer, its relationship with genotype may not be the same in those with the disease as in the general population. The other study was in healthy subjects but contained only 94 premenopausal women, in whom there was a (nonsignificantly) higher level of density in those homozygous for the low-activity allele than in those with the high-activity allele (45).

Nine case-control studies have been reported that examined the relationship of *COMT* genotype with breast cancer risk (18, 46–53). In premenopausal women, five studies had odds ratios less than unity (none significant; Refs. 18, 47, 49–51), and four had odds ratios greater than unity [Refs. 46, 48, 52, 53; significantly so in one (46)] when low-activity *COMT*2* homozygotes were compared with high activity *COMT*1* homozygotes. In the studies with more than 200 cases, odds ratios comparing homozygotes for the low- and high-activity alleles of *COMT*, were less than unity for two of the three studies in premenopausal women (Refs. 47, 50; none statistically significant), and in all three of the studies in postmenopausal women [Refs. 46, 47, 50; significantly so in one (46)]. These findings, together with our own, do not support the hypothesis that the low-activity variant of COMT leads to increased breast cancer risk. Moreover, if, as our present results suggest, *COMT* genotype influences breast cancer risk, at least in part, by affecting levels of exposure to other risk factors that themselves have only modest effects on risk, then weak associations between the genotype and the risk of breast cancer are expected, and large sample sizes will be required to detect them.

One factor that might complicate the understanding of the relationship between *COMT* and breast cancer risk is body size. Our results suggest that in premenopausal women *COMT i*s associated with body size, and that this association in part mediates the relationship between *COMT* genotype and breast density. If this is so, then adjusting for BMI may be overadjustment, and lead, incorrectly, to the conclusion that *COMT* genotype is unrelated to breast cancer risk.

The present results add to the evidence that mammographic density has genetic determinants (7), and that, these genes may exert their effects by influencing other phenotypic features that are related to the risk of breast cancer and other diseases. Blood levels of IGF-I, as well as body weight and the WHR, are quantitative traits that are known to be, in part, heritable (54, 55). The present findings suggest that estrogen metabolism genes are among the determinants of these factors, and may, therefore, be involved in the several diseases that have been associated with them. In addition to breast cancer, these include cancers of the prostate (56) and colon (57), coronary heart disease (58), and diabetes (59).

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