Genetic Factors Related to Racial Variation in Plasma Levels of Insulin-Like Growth Factor-1: Implications for Premenopausal Breast Cancer Risk

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The oral contraceptive pill is associated with a modest increase in the risk of early-onset breast cancer in the general population, but it is possible that the risk is higher in certain subgroups of women. The relative risk of breast cancer associated with oral contraceptive use has been reported to be higher for African-American women than for white women. African-American women also have a higher incidence of premenopausal breast cancer than white women. Circulating levels of insulin-like growth factor-1 (IGF-I) vary between ethnic groups and are positively associated with the risk of premenopausal breast cancer. In general, the plasma level of IGF-I is lower in women who take oral contraceptives than in women who do not. In an attempt to explain the observed ethnic difference in IGF-I levels with oral contraceptive use, we sought to identify polymorphic variants of genes that are associated with IGF-I levels and estrogen metabolism. We measured IGF-I and IGFBP-3 plasma levels in 503 nulligravid women between the ages of 17 and 35. All women filled out a questionnaire that included information about ethnic background and oral contraceptive use. Samples of DNA were used to genotype the women for known polymorphic variants in the *IGF1, AIB1,* and *CYP3A4* genes. Black women had significantly higher mean IGF-I levels than white women (330 ng/ml versus 284 ng/ml; $P = 0.001$, adjusted for age and oral contraceptive use). IGF-I levels were significantly suppressed by oral contraceptives in white women (301 ng/ml versus 267 ng/ml; $P = 0.0003$), but not in black women. Among oral contraceptive users, the IGF-I level was positively associated with the absence of the *IGF1* 19-repeat allele (338 ng/ml versus 265 ng/ml; $P = 0.00007$), with the presence of the *CYP3A4* variant allele (320 ng/ml versus 269 ng/ml; $P = 0.01$), and with the presence of the *AIB1* 26-repeat allele (291 ng/ml versus 271; $P = 0.08$). After adjusting for genotypes, ethnic group was no longer a significant predictor of the IGF-I level. IGF-I levels are higher among black than white women. Polymorphic variants in the *CYP3A4, IGF1,* and *AIB1* genes are associated with increases in the plasma levels of IGF-I among oral contraceptive users and the variant alleles are much more common in black women than in white women. The high incidence of premenopausal breast cancer among black women may be mediated through genetic modifiers of circulating levels of IGF-I.

Key Words: **insulin-like growth factor-1 (IGF-I); IGF binding protein-3; genotype; oral contraceptives; *IGF1; CYP3A4; AIB1.***

The incidence of early-onset breast cancer is higher for black women than for white women living in the US (1–3) but the reason for this racial difference is unknown. The use of the oral contraceptive pill has been associated with a modest increase in

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the risk of premenopausal breast cancer, particularly among women who begin oral contraceptive use before age 20 or before their first full-term pregnancy (4). Most studies describe the experience of white women (4), but a few studies have reported that African-American women who use oral contraceptives face a higher relative risk of breast cancer than white women (5–7).

Insulin-like growth factor-1 (IGF-I) is a peptide growth factor, which is present in the plasma and the breast tissue, and which promotes the proliferation of breast epithelial cells (8–10). The principal IGF-I binding protein, IGFBP-3, suppresses breast cell proliferation and promotes apoptosis. Plasma levels of IGF-I and of IGFBP-3 vary between ethnic groups (11) and are predictive of the risk of premenopausal breast cancer (12–14). Hankinson *et al.* (12) found a sevenfold increase in the risk of premenopausal breast cancer for women in the highest tertile of serum IGF-I, compared to women in the bottom tertile, after adjustment for age and IGF-BP3 levels.

The plasma level of IGF-I is strongly influenced by exogenous estrogens (15–20). In a recent study of white women, we reported that the mean level of plasma IGF-I was significantly lower in women who took oral contraceptives than in women who did not ($P = 0.001$) (16). However, a minority of women on oral contraceptives demonstrated high levels of IGF-I. These women may constitute a subgroup that is at particularly high risk of premenopausal breast cancer when exposed to the oral contraceptives.

We speculated that differences in plasma IGF-I levels may contribute to the reported difference in the risk of premenopausal breast cancer between white and black women, and that the observed ethnic differences in IGF-I levels may be the consequence of the relative population frequencies of functional polymorphic variants in genes which regulate the metabolism of exogenous estrogens.

METHODS

Study Subjects

Healthy volunteer women, between the ages of 17 and 35 years, were recruited from the University of Toronto, from the Bay Centre for Birth Control, and from the Toronto community by flyers and e-mail. The protocol was approved by the University of Toronto ethics committee. We have previously described 311 white women in this sample who reported never to have been pregnant (16). The present study deals with the results on 503 women of the four most common ethnic groups, white, black, Asian, and Indian-Pakistani. Asian women included those of Japanese, Chinese, Korean, Vietnamese, or Thai decent. Forty-one women of other, or mixed, ethnicity were excluded. Only women who reported never to have been pregnant, to have no past history of cancer, and who did not have diabetes were included. The study protocol included a morning visit to the study center on a random day of the menstrual cycle. All women completed a self-administered questionnaire, detailing information on age, ethnic group, menstrual history, current diet (vegetarian or not), exercise, alcohol consumption, and smoking. A brief medical history was taken and included the current use of medications, including oral contraceptives. Samples of various packages of oral contraceptive brands were displayed at the center and the women were asked to identify their current brand. All contraceptive formulations contained 17-α ethinyl estradiol as estrogen, but the type of progestin varied. Each type of contraceptive was coded as to the amount of estrogen and type of progesterone it contained. The average daily dose of ethinyl estradiol was calculated for women who used triphasic pills. Seven different types of progestins were recorded.

Blood samples were taken for analysis of plasma levels of IGF-1 and IGFBP-3, and for DNA extraction. Levels of IGF-I and IGFBP-3 in plasma were assayed using ELISA methodology as previously described (21). The level of free IGF-I is believed to be a better predictor of breast cancer risk than the total IGF-I (12). In the present study the ratio of IGF-I to IGFBP-3 was taken as a surrogate for free IGF-I.

Genotyping

Genomic DNA was extracted from 20 ml of peripheral blood using Puregene DNA extraction kits.

IGF1 polymorphism. The polymorphism in the *IGF1* gene is a variable number tandem repeat (VNTR) type polymorphism (22), consisting of eight alleles, ranging in size from 16 to 23 repeat units. The polymorphism was evaluated by a PCR-based assay using the following primers: sense 5′-GCT AGC CAG CTG GTG TTA TT-3′ and antisense 5′-ACC ACT CTG GGA GAA GGG TA-3′. The forward primer was end-labeled with Cy5 (Amersham Life Sciences, Oakville, Ontario). PCR were performed using 25 ng of template DNA for each reaction, 0.4

mM of each primer, 200 mM of each deoxynucleotide triphosphate, 1.0 mM $MgCl_2$, 2% dimethyl sulfoxide, 1.25U *Taq* polymerase (Gibco, Fredrick, MD), and the manufacturer's standard buffers in a final reaction volume of 25 μl. To evaluate the genotype the amplified PCR products were subjected to acrylamide gel electrophoresis. The gels were dried and scanned on a Storm 860 Phosphorimaging System (Molecular Dynamics, Sunnyvale, CA) to visualize and score the repeat alleles. The number of repeat elements was determined by measuring the size of the PCR products in comparison to known standards.

AIB1 polymorphism. The variable number tandem repeat polymorphism in the *AIB1* gene contains seven alleles and was evaluated using the following PCR primers: sense 5′-TCA TCA CTT CCG ACA ACA GAG G and antisense 5′-TGG GGG AAG CAG TCA CAT TAG GA. PCR were performed using 25 ng of template DNA for each reaction, 0.4 mM of each primer, 200 mM of each deoxynucleotide triphosphate, 1.0 mM $MgCl_2$, 2% dimethyl sulfoxide, 1.25 U *Taq* polymerase (GIBCO), and the manufacturer's standard buffers in a final reaction volume of 25 μl. To evaluate the VNTR in the *AIB1* gene the amplified PCR products were subjected to electrophoresis on Spreadex 500 gels (Helixx, Toronto, Ontario) and visualized with ethidium bromide staining. The number of repeat elements was determined by measuring the size of the PCR products in comparison to known standards.

CYP3A4. The *CYP3A4* polymorphism is a biallelic single nucleotide polymorphism in the nifedipine responsive element of the gene (23). PCR was performed to amplify a 592-bp fragment in the upstream coding region of the CYP3A4 gene. The primers are 5′-AAC AGG GGT GGA AAC ACA-3′ and 5′-CTT TCC TGC CCT GCA CAG-3′. The forward primer was end-labeled with Cy5 (Amersham Life Sciences, Oakville, ON). The PCR amplification was performed in 25-μl reaction volumes and consisted of 35 cycles (94°C/min, 64°C/30 s, and 72°C/30 s). To identify homozygous wild-type (W/W), heterozygous variant (W/V), and homozygous variant (V/V) genotypes, two samples for each subject were separated by conformation-sensitive gel electrophoresis (CSGE) (24). In one well, 8 μl of PCR product was loaded in the absence of PCR product generated from a known homozygous variant subject (−V/V DNA). In a second well 4 μl of PCR product was loaded with 4 μl of V/V DNA (+V/V DNA). As described by Rebbeck *et al.*, the absence and presence of V/V DNA do not affect the separation pattern for W/V and V/V subjects (23). Homoduplexes and heteroduplexes were always observed for W/V subjects, while only a single homoduplex was observed for V/V subjects. For W/W subjects, the absence of V/V DNA resulted in homoduplex formation, while the addition of V/V DNA produced both homoduplex and heteroduplex bands. Following electrophoresis, gels were dried and scanned on a Storm 860 Phosphorimaging System (Molecular Dynamics) to visualize the bands and genotype of the subjects.

Data Analysis

The correlations between age and plasma levels of IGF-I were measured using the Pearson correlation coefficient. The effects of ethnic group and oral contraceptive use on these hormone levels were measured using multivariate linear regression models, adjusting for age.

All comparisons between ethnic groups and between women with different genotypes of the three candidate genes were adjusted for age (continuous), for current oral contraceptive use (yes/no), and for estrogen dose (continuous) among oral contraceptive users, except where otherwise indicated.

The 19-repeat allele of the *IGF1* polymorphism has been previously associated with the serum IGF-I level (22). The number of tandem repeats of each of the two alleles of the *IGF1* polymorphism was therefore determined for each woman. The women were categorized by the presence of zero, one, or two copies of the 19-repeat allele. Similarly, for the *AIB1* polymorphism, the women were categorized according to the presence of zero, one, or two copies of the 26-repeat allele. The number of variant alleles of the bi-allelic *CYP3A4* polymorphism was determined for each woman.

RESULTS

The total sample of 503 women included 329 white women (65%), 78 African-American women (15%), 71 Asians (14%), and 25 women of Indian or Pakistani origin (5%). All women were between the ages of 17 and 35 years. The characteristics of the women in the four ethnic groups are described in Table 1.

Among the entire study group, plasma IGF-I levels declined continuously with age ($r = -0.41$; $P = 10^{-21}$). Black women had significantly higher mean IGF-I levels than white women ($P = 0.001$, adjusted for age and oral contraceptive use) (Fig. 1).

TABLE 1
Characteristics of Women by Ethnic Background

	White (n = 329)		Black (n = 78)		Asian (n = 71)		Indian-Pakistani (n = 25)	
	Mean	(Range)	Mean	(Range)	Mean	(Range)	Mean	(Range)
Age (years)	25.4	(17–35)	24.8	(17–35)	24.3	(19–31)	22.9	(19–30)
Height (cm)	166	(147–188)	165	(150–182)	160	(150–173)	159	(154–166)
Weight (kg)	63.3	(41.3–114.9)	63.5	(44.5–95.3)	55.7	(39.5–78.5)	54.2	(40.4–79.4)
BMI (kg/m^2)	23.1	(16.93–46.32)	23.3	(15.83–32.37)	21.6	(16.45–27.38)	21.6	(16.03–32.56)
Age at menarche (years)	12.8	(9–18)	12.2	(8–17)	12.1	(8–16)	12.4	(10–15)
Current OC user	50%		21%		25%		36%	
Current smoker	15%		10%		14%		16%	
Current vegetarian diet	15%		3%		4%		20%	
Highest level of education								
Attended high school	1.5%		2.6%		—		—	
Graduated high school	3.6%		5.1%		2.8%		8%	
Attended college/university	23.7%		43.6%		39.7%		36%	
Graduated degree/diploma	71.1%		47.4%		57.7%		56%	

Note. Missing values: Height was missing for 2 white women, weight for 15 white women, BMI for 17 white women, age at menarche for 2 white women, and smoking status was missing for 4 white women. Weight and BMI were missing for 1 black woman. Height and BMI were missing for 4 Asian women. Height and BMI were missing for 1 Indian-Pakistani woman.

The age-adjusted mean IGF-I levels were lower in white women who used oral contraceptives than in white women who were not on oral contraceptives (266 ng/ml versus 301 ng/ml; P = 0.0003). We found that the mean IGF-I levels were inversely related to the average daily estrogen dose (P = 0.01), but were not related to the progesterone content of the oral contraceptives. In contrast, oral contraceptive use was not associated with lower IGF-I levels in black women (P = 0.80), in Asian women (P = 0.22), or in Indian-Pakistani women (P = 0.71). Among users of oral contraceptives, black women had significantly higher levels of plasma IGF-I than white women (341 ng/ml versus 267 ng/ml; P = 0.001) (Fig. 1).

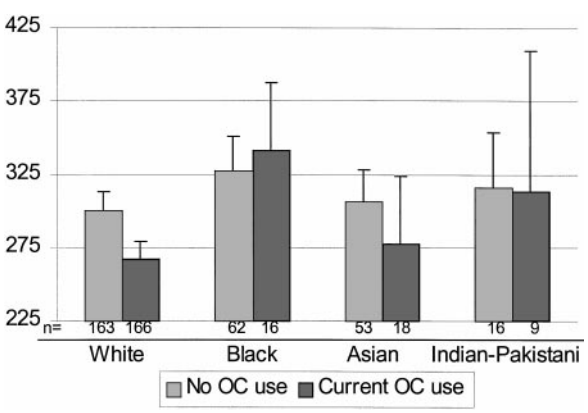

FIG. 1. Mean IGF-I levels among White, Black, Asian, and Indian-Pakistani women classified by current oral contraceptive use. Black women had significantly higher mean IGF-I levels than white women (P = 0.001, adjusted for age and oral contraceptive use). The IGF-I levels were significantly suppressed by oral contraceptives in white women (P = 0.0003, adjusted for age). Among oral contraceptive users, black women had higher levels of plasma IGF-I than white women (P = 0.001, adjusted for age). Error bars represent 95% CI.

IGF-1 Levels and the IGF1 Gene

Among users of oral contraceptives, the mean IGF-I level was significantly higher in women who lacked a copy of the 19-repeat allele of the *IGF1* gene, compared to women with one or two copies of the allele (338 ng/ml versus 266 ng/ml; P = 0.00007) (Fig. 2). No association between the *IGF1* polymorphism and the plasma IGF-I levels was present in women who did not use oral contraceptives. The interaction between the use of oral contraceptives and the presence of the *IGF1* 19-repeat allele on the plasma IGF-I level was highly significant (P = 0.002) and was present in all four ethnic groups (data not shown).

Polymorphic Variants of the CYP3A4 Gene

Among women who took oral contraceptives, the mean IGF-I levels were higher for women who carried one or two variant CYP3A4 alleles than among

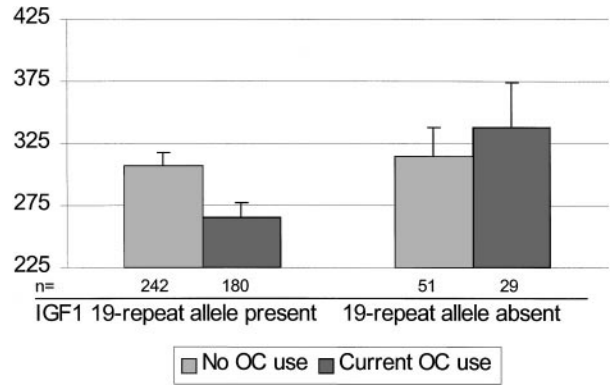

FIG. 2. Mean IGF-I levels in relation to *IGF1* genotype and oral contraceptive use. Among users of oral contraceptives, the mean IGF-I level was significantly higher in women who lacked a copy of the 19-repeat allele of the *IGF1* gene, compared to women with one or two copies ($P = 0.00007$, adjusted for age and estrogen dose). Error bars represent 95% CI.

women who did not (320 ng/ml versus 269 ng/ml; $P = 0.01$). There was a significant trend in the mean IGF-I level by the number of CYP3A4 alleles ($P = 0.005$) (Fig. 3). In nonusers, there was no significant trend in the mean IGF-I level by number of CYP3A4 variant alleles ($P = 0.20$).

Polymorphic Variants of the AIB1 Gene

Among users of oral contraceptives, women who carried one or more copies of the 26-repeat allele of the *AIB1* gene had modestly higher mean IGF-I levels than women who lacked the 26-repeat allele (291 ng/ml versus 271 ng/ml). Although the difference did not achieve statistical significance ($P = 0.08$) the effect was present at all exposure levels of exogenous estradiol (Fig. 4). Among nonusers, no association was seen (306 ng/ml versus 309 ng/ml; $P = 0.76$).

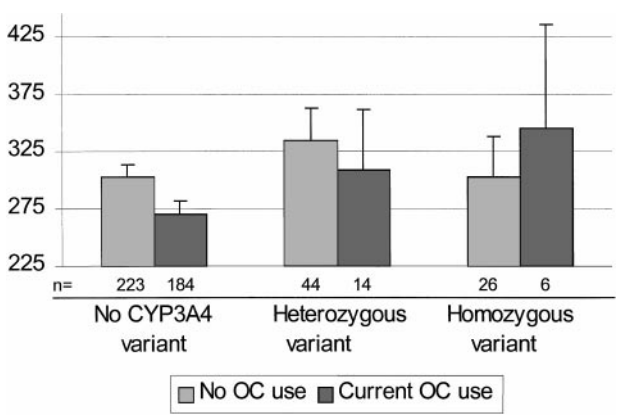

FIG. 3. Mean IGF-I levels in relation to *CYP3A4* genotype and oral contraceptive use. Among oral contraceptive users, there was a trend in the mean IGF-I level by the number of CYP3A4 alleles ($P = 0.005$, adjusted for age and estrogen dose). Error bars represent 95% CI.

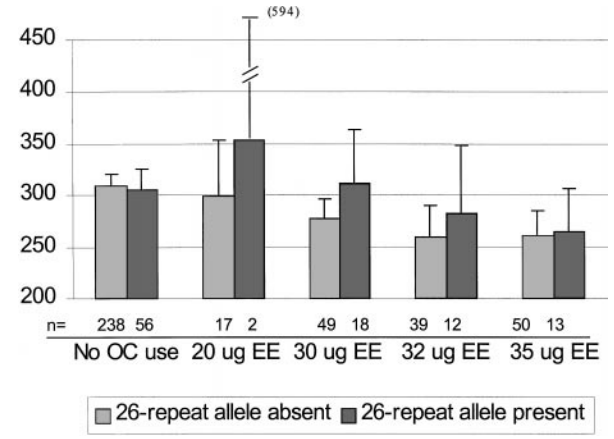

FIG. 4. Mean IGF-I levels in relation to *AIB1* genotype and oral contraceptive status by dose of 17-α-ethinyl estradiol. Among oral contraceptive users, women who carried one or more copies of the 26-repeat allele of the *AIB1* gene had higher mean IGF-I levels than women who lacked the 26-repeat allele ($P = 0.08$, adjusted for age and estrogen dose). Error bars represent 95% CI.

Cumulative Number of Variant Alleles and IGF-I Levels

The frequencies of the specific alleles of the *IGF1* gene, the *CYP3A4* gene, and the *AIB1* gene varied by ethnic group (Table 2). We assigned a score from zero to four to each woman, depending on the total number of variants present (i.e., the absence of the IGF-1 19-repeat allele, the presence of at least one copy of the AIB1 26-repeat allele, and the presence of one or two copies of the CYP3A4 variant (counted as one for heterozygotes and as two for homozygotes). Black women had, on average, 1.7 variants, compared to 0.4 for the white women, 0.5 for the Asian women, and 0.4 for the Indian-Pakistani women ($P < 0.001$ for the difference between black versus white women) (Table 3). Among oral contraceptive users, a strong correlation was present between the total variant score and the mean IGF-I level ($r = 0.31$; $P = 4 \times 10^{-6}$) (Figs. 5 and 6). Among women who did not use oral contraceptives, no significant association was seen ($r = 0.06$; $P = 0.16$) (Fig. 5).

To evaluate concurrently the effect of ethnic background and the genotypes of the three candidate genes on the mean plasma IGF-I level, a series of

TABLE 2
Allele Frequency Distribution of Polymorphic Genetic Variants by Ethnic Background

	White (n = 329)		Black (n = 78)		Asian (n = 71)		Indian-Pakistani (n = 25)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
IGF1 genotype (VNTR)								
16	1	(0.2%)	7	(4.5%)	1	(0.7%)	—	
17	4	(0.6%)	6	(3.8%)	7	(4.9%)	—	
18	38	(5.8%)	25	(16.0%)	27	(19.0%)	5	(10%)
19	460	(70.2%)	59	(37.8%)	58	(40.8%)	28	(56%)
20	110	(16.8%)	30	(19.2%)	13	(9.1%)	9	(18%)
21	29	(4.4%)	22	(14.1%)	32	(22.5%)	8	(16%)
22	13	(2.0%)	5	(3.2%)	4	(2.8%)	—	
23	—		2	(1.3%)	—		—	
AIB1 genotype (VNTR)								
22	—		—		1	(0.7%)	—	
26	84	(12.8%)	10	(6.4%)	6	(4.2%)	5	(10%)
27	1	(0.2%)	—		—		—	
28	254	(38.8%)	55	(35.3%)	29	(20.4%)	12	(24%)
29	311	(47.6%)	88	(56.4%)	101	(71.1%)	31	(62%)
30	4	(0.6%)	3	(1.9%)	3	(2.1%)	2	(4%)
31	—		—		2	(1.4%)	—	
CYP3A4								
No variant	305	(94.1%)	9	(11.5%)	69	(97.1%)	24	(100%)
Heterozygous variant	18	(5.6%)	38	(48.7%)	2	(2.8%)	—	
Homozygous variant	1	(0.3%)	31	(39.7%)	—		—	

Note. Missing values: Three *IGF1* alleles, four *AIB1* alleles, and five *CYP3A4* genotypes were missing among white women. *CYP3A4* genotype was missing for one Indian-Pakistani woman.

multiple linear regressions was performed. In the absence of genotype, ethnic background was a significant predictor of the IGF-I levels among users of oral contraceptives. Similarly, in the absence of ethnic group, the number of genetic variants was a significant predictor of the IGF-I level. However, when both the ethnic group and the total number of variants were introduced into the model, the effect of ethnic group was no longer significant (P_{trend} = 0.0002 for number of variants, adjusted for age, estrogen dose, and ethnic group). This was also true if the genetic variants were entered into the model individually, rather than as a score. Among nonusers, the total number of variants did not predict the mean IGF-I levels ($P_{\text{trend}} = 0.74$, adjusted for age and ethnic group).

These data are based on the total plasma IGF-I levels. We repeated all analyses using the IGF-I to IGFBP-3 ratio as the outcome, in place of the total IGF-I. The results remained essentially the same.

TABLE 3
Cumulative Number of Variant Alleles by Ethnic Background

Cumulative number of variants	White (n = 329)		Black (n = 78)		Asian (n = 71)		Indian-Pakistani (n = 25)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
0	209	(64.9%)	6	(7.7%)	43	(60.6%)	16	(66.7%)
1	104	(32.2%)	21	(26.9%)	24	(33.8%)	7	(29.2%)
2	8	(2.5%)	40	(51.3%)	4	(5.6%)	1	(4.2%)
3 or more	1	(0.3%)	11	(14.1%)	—		—	

Note. Missing values: Cumulative numbers of variants were missing for five white women and one Indian-Pakistani woman.

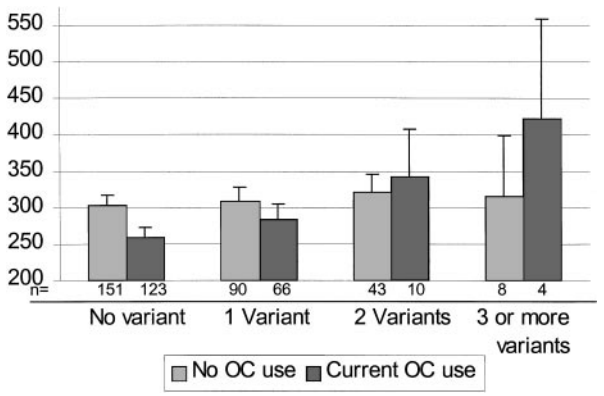

FIG. 5. Mean IGF-I levels in relation to cumulative number of variant alleles and oral contraceptive use. Among oral contraceptive users, a strong correlation was present between the total variant score and the mean IGF-I level ($r = 0.31$; $P = 4 \times 10^{-6}$, adjusted for age and estrogen dose). No significant effect was seen among nonusers. Error bars represent 95% CI.

DISCUSSION

Epidemiologic investigations of circulating IGF-I levels in the healthy female population to date have been based largely on white women. These studies may not be generalizable to other ethnic groups. We found that on average, black women had significantly higher IGF-I levels than white women, and that they did not show the characteristic suppression of IGF-I associated with oral contraceptive use. Black women who used oral contraceptives actually had higher mean IGF-I levels than black women who were not on oral contraceptives. In white women, IGF-I levels were inversely correlated with the average estrogen dose contained in oral contraceptives, but were not related to progesterone content. All oral contraceptives used by the women in the present study contained 17-α ethinyl estradiol.

In girls, the highest levels of circulating IGF-1 are observed between the ages of 11 and 13 (25). A study of 136 girls between the ages of 9 to 17 reported that African-American girls had higher free IGF-I concentrations and higher ratios of free to total IGF-I in serum than white girls. The African-American girls were older and more sexually mature. After controlling for sexual maturation and fat mass, the adjusted levels of total IGF-I, bound IGF-I, and IGF-BP3 in the white girls were significantly higher than in the black girls (26). After puberty, IGF-I levels decline continually with age, and the decrease is pronounced after menopause in white women (27). It appears that the effects of exogenous estrogen on serum IGF-I depend on the route of administration. In general, endogenous and transdermal estrogens increase IGF-I levels, whereas oral estrogens decrease the levels. For example, hormone replacement therapy in the form of transdermal estradiol resulted in a slight increase in serum IGF-I; but the administration of oral estradiol to postmenopausal women resulted in a significant fall in the mean IGF-I level in an (ethnically undefined) Australian population (17). The finding was confirmed in an Italian study (28), but to our knowledge no studies have investigated the effect of transdermal estrogen in postmenopausal black women. We observed a similar effect on IGF-I levels from oral estrogen when oral contraceptives were taken by premenopausal white women (15,16). Estrogen causes distinct, route-dependent effects on the somatotrophic axis. The dissociation of the GH/IGF-I axis by the oral route is likely to arise from impaired hepatic IGF-I production which causes increased GH secretion through reduced feedback inhibition (29).

Both oral contraceptive use and high levels of plasma IGF-I are believed to increase the risk for breast cancer in premenopausal women, and it is therefore important to elucidate the relationships between the two. Because oral contraceptives decrease IGF-I levels in most young women, and because IGF-I levels appear to be positively correlated with breast cancer risk, we might expect oral contraceptive use to be associated with a reduced risk of premenopausal breast cancer. However, it is possible that the risk is not equal in all women, and the hazard may be restricted to women who are genetically predisposed. In our study, plasma levels of IGF-I were associated with genotype for three candidate genes (*IGF1*, *CYP3A4*, and *AIB1*). Among carriers of two or more risk alleles, the average level of IGF-I was actually higher if they took oral contraceptives than if they did not (Fig. 5). The ethnic differences in the IGF-I levels among users of oral contraceptives could be explained to a large degree by population genetic differences in the three candidate genes.

The three genes were chosen on the basis of previous epidemiologic studies and on their known biologic activities. We studied the *IGF1* polymorphism because of a prior report that it was related to circulating IGF-I level. Rosen and colleagues reported lower mean IGF-I levels in men and women who were homozygous for the 19-repeat allele (22) compared to heterozygous carriers and noncarriers of the 19-repeat allele. However, a subsequent report

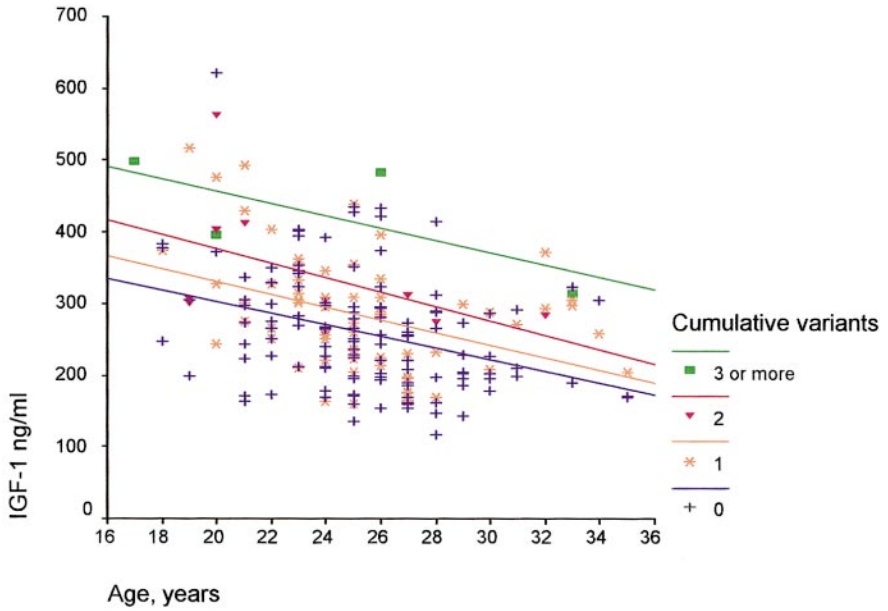

FIG. 6. Mean IGF-I levels in relation to age and cumulative number of variant alleles among oral contraceptive users.

failed to find a relationship between this polymorphism and bone mineral density (30), but no IGF-I levels were measured in that study (31). In the present study, the effect of the *IGF1* genotype on IGF-I levels was restricted to oral contraceptive users. Oral contraceptive users who lacked a copy of the 19-repeat allele had significantly higher mean levels of IGF-I than women with at least one copy of the allele. This interaction between oral contraceptives and the *IGF1* polymorphism was seen in women of all ethnic backgrounds. The *IGF1* polymorphism is located in the promoter region of the *IGF1* gene (22). This microsatellite polymorphism is located 1 kb upstream of the *IGF1* transcription start site (32,33) and contains specific regulatory elements (34). *In vitro* studies of rat osteoblasts have shown a 100- to 300-fold difference in suppressing the stimulatory effect of prostaglandin E_2 in response to 17-α and 17-β estradiol when they bind to the estrogen response elements within the *IGF1* promoter region (34). The difference in IGF-I levels among oral contraceptive users with and without the 19-repeat allele suggests that this allele may be associated with a conformational change in the region of the *IGF1* promoter, possibly involving the estrogen response element.

We chose to study *CYP3A4* because it codes for one of the key enzymes involved in the metabolism of the 17-α ethinyl estradiol, which is the estrogen contained in oral contraceptives (35,36). *CYP3A4* is the member of the cytochrome P450 family which is most abundantly expressed in the liver (37). An *in vivo* study showed that oral contraceptive steroids inhibited CYP3A4 activity (38). We found that IGF-I levels were higher among oral contraceptive users with the *CYP3A4* variant allele and a dose-response relationship was present. To our knowledge the effect of different *CYP3A4* variant alleles on ethinyl estradiol metabolism has not been studied. Based on an *in vitro* promotor assay, the *CYP3A4* variant allele was found to increase transcriptional activity of the gene (39). The variant allele of *CYP3A4* is much more common among black women than among women of other ethnic backgrounds. It is not clear how the association between the CYP3A4 variant allele and increased IGF-I level is mediated, but our observations suggest that this may be mediated by the metabolism of the synthetic ethinyl estradiol.

The third gene that was associated with circulating IGF-I levels among oral contraceptive users was *AIB1*. We chose to study *AIB1* because it is a coactivator of estrogen signaling, because of its reported involvement in breast cancer, and because of its relationship with bone mineral density. *AIB1* codes for a nuclear receptor protein which interacts with the estrogen receptor in a ligand-dependent fashion (40). Transfection of *AIB1* in a model system resulted in enhancement of estrogen-dependent tran-

scription (40). The *AIB1* gene is amplified in approximately 10% of breast cancers (40). The CA repeat polymorphism of the *AIB1* gene codes for an expressed polyglutamine tract. Rebbeck *et al.* reported a higher frequency of the 26-repeat allele of this polymorphism among BRCA1 carriers with breast cancer compared with healthy BRCA1 carriers (41). No association was seen between the 26-repeat allele and breast cancer risk in a group of unselected (nonhereditary) patients and controls (42).

Serum IGF-I levels have been reported to be positively correlated with bone mineral density (43,44), and high bone density is also a risk factor for breast cancer (45–47). The *AIB1* polymorphism has been reported to influence bone density in young white women (22,48). Bone density was higher in women with at least one copy of the 26-repeat *AIB1* allele than in women with no 26-repeat allele (48).

In the present study, we found that contraceptive users who carried the 26-repeat allele of the *AIB1* gene had higher IGF-I levels than women who lacked this allele. However, the association failed to reach statistical significance and requires confirmation. A possible explanation for this finding is that the 26-repeat is a more potent coactivator of the estrogen signal than are alleles of other lengths. Furthermore, the degree of coactivation by *AIB1* may vary with the estrogen type. Ligand specificity may potentially explain the differences we saw between women exposed primarily to endogenous 17-β estradiol and to 17-α ethinyl estradiol in the form of oral contraceptives.

In women on oral contraceptives, breast tissue levels of IGF-I mRNA were found to be strongly correlated with breast cell proliferation rates (49) and proliferation rates have been directly linked to cancer risk (50). Women who demonstrate an idiosynchratic increase in circulating IGF-I when exposed to oral contraceptive may therefore face an increased risk of breast cancer during periods of oral contraceptive use, assuming that tissue gene expression varies with circulating IGF-I levels. This increase appears to correlate with the cumulative number of genetic variants of the three candidate genes. We found large differences in the observed frequencies of the individual genotypes of each the three genes between the four ethnic groups. Over 65% of black women had two or more variant alleles, compared with only 3% of white women, 5% of Asian women, and 4% of Indian-Pakistani women. When the cumulative number of variant genes was taken into consideration, the effect of ethnic background was not a significant determinant of IGF-I levels among oral contraceptive users. It is therefore likely that much of the variation in IGF-I levels in users of oral contraceptives in different ethnic groups is attributable to the genotypes at these three loci.

Our finding of higher overall IGF-I levels among black women than among women of other ethnic backgrounds may explain to some degree why premenopausal breast cancer is more common among black than white women (1–3). IGF-I levels were particularly high among black women who used oral contraceptives, and the role of oral contraceptives in contributing to breast cancer rates among black women deserves further study. Our data are cross-sectional and repeated measures were not taken on the same individuals. It will be important in future studies to measure IGF-I levels before and after starting oral contraceptives in order to confirm the findings reported here.

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