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IGF1, IGFBP1 and IGFBP3 genes and mammographic density: The Multiethnic Cohort

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Abstract

Insulin-like growth factor-I (IGF-I) has mitogenic properties and stimulates cell growth. In this analysis, we investigated the relation between common genetic variation in *IGF1, IGFBP1*, and *IGFBP3* and mammographic density among 819 women of Hawaiian, European and Japanese ancestry from the Multiethnic Cohort Study. Mammographic density was assessed using a quantitative computer-assisted method. Previously identified tag single nucleotide polymorphisms (SNPs) for *IGF1* (26 tag SNPs) and *IGFBP1/IGFBP3* (22 tag SNPs) were genotyped among the 819 women. Mixed models were conducted to evaluate the associations between genetic variation and mammographic density. Two SNPs were borderline statistical significantly associated with mammographic density; rs35539615 on *IGFBP1* (p=0.05) and rs2453839 on *IGFBP3* (p=0.01). Rs35767on *IGF1* (p=0.03) was also associated with mammographic density, although in opposite direction of what was expected from previous findings with IGF-I levels. The majority of SNPs were, however, not associated with mammographic density. Analyses stratified by ethnicity showed similar results as the overall analyses for *IGF1* and *IGFBP1*. However, for four SNPs in the *IGFBP3* gene, the minor allele was associated with lower mammographic density in Japanese Americans and higher mammographic density in Caucasians. Given the large number of SNPs tested and the few borderline significant results, we only found weak evidence that genetic variations in *IGFBP1* or *IGFBP3* may be related to mammographic density. Ethnicity may modify these relations.

Keywords

Breast cancer; Mammographic density; Multiethnic cohort; SNP; IGF-I

Introduction

Insulin-like growth factor-I (IGF-I) promotes proliferation and inhibits apoptosis in both normal breast cells and breast cancer cell lines. The bioavailability of IGF-I is determined by six binding proteins. IGFBP-3 is the predominant binding protein and has been described to also directly affect breast cancer risk in an IGF-I independent manner (1). In several epidemiologic studies, circulating IGF-I levels were associated with higher breast cancer risk among pre- but not postmenopausal women (2–4). However, more recent results from

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the EPIC study and the Nurses Health Study (NHS)-II did not show an association between IGF-I levels and breast cancer risk among younger women (5;6).

The amount of stromal and glandular tissues of the breast relative to the surrounding fatty tissue can be estimated using mammographic images (7). A high percentage of radiologically dense tissues (percent density) is a strong breast cancer risk factor and is, therefore, often used in etiologic studies as biomarker for breast cancer risk (8). Circulating levels of IGF-I have been found to be related to mammographic density in premenopausal women in past epidemiological studies (9–11), although this was not confirmed in recent reports (12;13). In postmenopausal women, this relation does not seem to exist (9–11;14).

Normal fluctuations of circulating levels over time are a concern in studies of IGF-I and breast cancer risk or mammographic density. Furthermore, IGF-I levels gradually decrease with age (15). Age may, therefore, be an important factor when studying the IGF-I and mammographic density association. In contrast, genetic variation is stable and may reflect lifetime exposure to IGF-I. As polymorphisms of genes in the IGF-I pathway have been associated with circulating levels of IGF-I (16;17) and IGF binding proteins (17), these polymorphisms may also be related to mammographic density. Results of the Nurses' Health Study (NHS) suggested that several SNPs of the IGF1 gene were strongly associated with higher mammographic density (18). However, these SNPs were also found to relate to lower IGF-1 levels in the large Breast and Prostate Cancer Cohort Consortium (BPC3) (17). Two consecutive studies could not replicate the findings from the NHS, but both found a borderline significant association between the minor allele for rs6220 and higher mammographic density (19;20).

So far common genetic variation in the IGF1 gene in relation to mammographic density has only been studied in cohorts with mainly Caucasian women. In women of other ethnicities these relations may be different. In the present study, we investigated the association between common variations in the *IGF-I*, *IGFBP1* and *IGFBP3* genes and mammographic density among Caucasian, Japanese American, and Native Hawaiian women from the Multiethnic Cohort (MEC).

Material and Methods

Study participants

Female MEC participants who were included in both a nested case-control study on mammographic density (21) and in a nested case-control study on genetic variation (22;23) were considered for the present analysis. The MEC is a prospective investigation that was established to study diet and cancer. Rationale and design are described in detail elsewhere (24). In brief, over 215,000 participants were recruited between 1993 and 1996 in Hawaii and Los Angeles. All participants completed a baseline questionnaire on dietary habits, demographic background, anthropometric measures and lifestyle factors. The study was designed to include African Americans, Latinos, Caucasians, Japanese Americans, and Native Hawaiians.

Mammographic density was measured in a breast cancer case-control study of Caucasian, Japanese American, and Native Hawaiian women nested within the Multiethnic Cohort. Incident cases diagnosed with invasive breast cancer by the end of December 2000, having one or more prediagnostic mammograms available were randomly selected (n=607) and frequency matched to controls (n=667) on ethnicity and by 5-year age groups. Additional information on breast surgery, mammography history, menopausal status and hormone replacement therapy use was collected for these subjects. Of the 1274 women in this study, 821 had been genotyped as part of a previous genetic association study of *IGF1*, *IGFBP1*,

and *IGFBP3* and breast cancer risk (22;23). Two women of other ethnicity were excluded, resulting in a total study population of 819 Caucasian, Japanese American, and Native Hawaiian women

Informed consent form was obtained from all participants. This study was approved by the Institutional Review Boards of the University of Hawaii and the University of Southern California (as applicable).

Tag SNP selection

Selection criteria for single nucleotide polymorphisms (SNP) in the *IGF-I*, *IGFBP-I and IGFBP3* genes used in the present study, are described in detail in two previous reports (22;25). Spanning 156 kb at a density of one SNP every 2.4 kb, 64 SNPs of the *IGF-I* gene (minor allele frequency ≥5%) were genotyped in a multiethnic panel of 349 controls (25). A subset of 29 SNPs was then selected to capture the common haplotypes in LD blocks defined by these 64 SNPs among all five major ethnicitic/racial groups in the original study population, i.e., African Americans, Latinos, Caucasians, Japanese Americans and Native Hawaiians. Using this panel of tagging SNPs, the proportion of the genetic variation that was captured at a pairwise r^2 >0.8 was 90% for Caucasians, 96% for Japanese Americans and 98% for Hawaiians (26). In the same multiethnic population used to select *IGF-I* haplotype-tagging SNPs, 36 SNPs with a minor allele frequency ≥5% spanning the 71-kb *IGFBP-I/IGFBP3* locus were genotyped. 89% of the genetic variation was captured by a subset of 23 tagging SNPs at a pair wise r^2>0.8 among Caucasians. For Japanese Americans and Hawaiians this proportion was 91% and 84% respectively (26). For the present study, three *IGF1* SNPs (rs5742634; rs1520219; rs4764882) and one *IGFBP1* SNP (rs1065781) specific for racial/ethnic groups that were not included in this study were excluded.

Genotyping—The Taqman allelic discrimination assay (Applied Biosystem, Foster City, CA) was used by the Multiethnic Cohort laboratory at the University of Southern California to genotype all tagging SNPs. When tested among control subjects of the previously mentioned breast cancer case-control study, all SNPs were in Hardy-Weinberg equilibrium (HWE) in at least four ethnic groups (at p>0.01) (22;25). Genotype concordance across replicate samples was 99.7% for *IGF1* and 99.8% for *IGFBBP1/IGFBP3*. On average, *IGF1* SNPs were successfully genotyped in 97.9% of the samples. For the *IGFBP1/IGFBP3* SNPs, this figure was 97.4% (22;25).

Mammographic density analysis—Mammographic assessment within the nested case-control study has been described previously (21). Prediagnostic mammograms were used for cases; for controls, images during a similar time period were selected. Cranial caudal views were scanned with a Kodak LS85 Film Digitizer (absorbance range, 0.001–4.1; Eastman Kodak Company, Rochester, New York) at a resolution of 98 pixels per inch (pixel size equal to 260μm). Using a computer-assisted method based on grey levels of pixels in the digitized mammogram, one of the authors (GM) quantified the total breast area on the mammogram as well as the area of dense tissue within the breast. The ratio between these two breast measures, i.e., percent breast density was calculated. To assess reader reliability, approximately 10% of the films were read in duplicate. Intraclass correlation coefficients (ICC) were 0.96 (95% confidence interval (CI): 0.95, 0.97) and 0.996 (95% CI: 0.995, 0.997) for the size of the dense and the total breast area respectively. This resulted in an ICC of 0.974 for percent density (95% CI: 0.968, 0.978).

Statistical analyses—To account for subjects with multiple mammographic readings over time, mixed models were used to estimate mean values of percent density, dense area and non-dense area by genotype. Differences between mean values were tested for three

modes of inheritance, i.e., co-dominance (trend over the three genotypes), dominant (homozygous for the major allele versus other genotypes), and recessive (homozygous for the minor allele versus other genotypes). As the IGF-1 and mammographic density relationship is probably stronger among premenopausal women, a model including only mammograms taken while the women were premenopausal was analyzed. Additional analyses were conducted stratified by case status and by ethnicity. The analyses were adjusted for ethnicity, age, the square of age, and body mass index (BMI) at the time of each mammogram. These variables were selected based on their strong relationship with mammographic density. The square of age was added as the decrease of mammographic density with increasing age is not constant over time (27). Additional adjustment for parity, age at first child birth, menopausal status and HRT use did not attenuate the results and were excluded from the models. Parity and age at first child birth were added to the premenopausal model as this changed the associations.

To maintain statistical power, co-dominant and recessive effects were only tested when at least 20 women had two minor alleles for a specific SNP. Otherwise women being homozygous for the minor allele and heterozygous women were grouped together and only the dominant mode of inheritance was tested. Power to detect a difference of 5 percent mammographic density via the dominant mode of inheritance for SNPs with a minor allele frequency of 10%, 20% and 30% was 0.72, 0.87 and 0.90 respectively. All statistical tests and corresponding p-values were two-sided, and p-values < 0.05 were considered statistically significant. All statistical analyses were done using the SAS software package, version 9.1 (SAS Institute, Cary, NC, USA).

Results

Mean breast area and dense area were 140.3 cm^2 (SD=63.6) and 32.2 cm^2 (25.2) for Hawaiian women, 138.0 cm^2 (69.8) and 35.6 cm^2 (31.5) for Caucasian women and 89.1 cm^2 (32.4), and 29.7 cm^2 (18.7) for Japanese American women (Table 1). As a result, percent density was 27.3 (20.8) for Hawaiian women, 31.0 (23.5) for Caucasian women, and 36.6 (21.3) for Japanese American women.

Only one of the *IGF1* SNPs (rs35767) was significantly related to percent density (Table 2). Women with one or two copies of the minor allele had 3.2% lower densities (p=0.03). Results for the dense area were very similar (34.2 cm^2 (95% CI: 31.8 – 36.5), 30.7 cm^2 (27.7 – 33.8) and 30.8 cm^2 (24.0 – 37.6) for 0,1 or 2 copies of the minor allele respectively). Rs35767 was not clearly related to the non-dense area. Hawaiian and Caucasian women with at least one copy of the minor allele for rs35767 had lower percent densities with p-values of 0.04 and 0.05, respectively (Supplemental Table 1). This effect was not observed in Japanese American women. Although not statistically significant, women homozygous for the minor allele of rs2139572 or rs1996656 had 7.3% and 5.0% lower mammographic density, respectively, as compared to the rest of the women. There were not enough women with two copies of the minor alleles for SNPs rs2139572 and rs1996656 to allow for stratified analyses by ethnic group. Hawaiian women homozygous for the minor allele of rs2946834 had 5.8% lower mammographicdensity (p-recessive=0.08), which was not, however, seen in the other ethnic groups. In Caucasian women, the minor allele of rs35765 was associated with a 6.6% lower density via the dominant mode of inheritance (p-value=0.05), but again there was no indication for a relation in the other ethnic groups.

Rs35539615 for *IGFBP1* was associated with higher percent density via the recessive mode of inheritance (p=0.05) (Table 3) resulting in 5.0% higher mammographic density. A similar association was seen with the dense area (33.3 cm^2 (95%CI: 30.9 – 35.7), 31.1 cm^2 (28.2 – 33.9) and 37.7 cm^2 (31.5 – 44.0) for 0,1 or 2 copies of the minor allele respectively), The

non-dense area was unrelated. We found the same result for Japanese American women (7.3% difference, p=0.04) (Supplemental Table 2). Small numbers in the other ethnic groups did not allow testing for the recessive mode of inheritance for this SNP. An increase in copy number of the minor allele for rs2453839 (*IGFBP3*) was related to lower percent density (p=0.01) (Table 2). The association with percent density was mainly driven by the dense area (33.9 cm^2 (95%CI: 31.8 – 36.1), 30.8 cm^2 (27.6 – 34.1) and 27.2 cm^2 (17.0 – 37.4) for 0,1 or 2 copies of the minor allele respectively), but to a lesser extent also by the non-dense area (84.6.2 cm^2 (80.9 – 88.4), 88.7 cm^2 (83.1 – 94.3) and 94.2 cm^2 (76.5 – 111.8)). Similar results were seen in Caucasians and Japanese Americans (p-values; 0.02 and 0.29 respectively); in Hawaiian women, however, percent density was not different between genotypes (Supplemental Table 2). The analyses of *IGFBP1/3* within ethnic group showed some associations that were not seen in the overall analyses. In Hawaiians only, women with at least one copy of the minor allele for rs1874479, rs1496495, RS10228265, rs1496497 or rs2270628 had higher percent density (differences; 5.7%–7.6%, p-values; 0.02, 0.01, 0.03, 0.01 and 0.02 respectively). Opposite effects were seen for Caucasian and Japanese American women for four SNPs for the *IGFBP1* gene. In Japanese American women, an increase in copy number of these SNPs, rs3110697, rs2854747, rs2854746 and rs2854744, was related to lower percent density (differences between homozygotes of major and minor alleles; 6.7%–8.2%, p=values, 0.04, 0.02, 0.01 and 0.02, respectively). In Caucasian women, however, an increase in number of these SNPs was related to higher percent density (differences; 4.8%–7.8%, p-values: 0.03, 0.06, 0.18 and 0.06 respectively) (Supplemental Table 2). Tests for heterogeneity showed borderline statistically significant difference across ethnic subgroups for SNPs rs3110697 (p-value=0.07) and rs2854747 (p-value=0.07). Heterogeneity for rs2854746 and rs2854744 was statistically not significant (p-values: 0.27 and 0.15 respectively).

Analyses with the dense area as measure of mammographic density showed very similar results compared to results with percent density (data not shown). Premenopausal mammograms were available for only 189 women. As a consequence, confidence intervals were wide and none of the SNPs was statistically significantly associated with either percent density or the dense area in premenopausal women. The strongest and statistically most significant association was found for rs12821878. Women carrying one or two copies of the minor allele had on average 6.3 % lower density (p-value=0.10). Case status stratified analyses showed similar results for cases and controls (data not shown). Results of haplotype analyses were mostly in the same direction as results for analyses with single SNPs and did not provide important additional information (data not shown).

Discussion

The minor allele of rs35767 (*IGF1*) was significantly associated with lower percent density and its association with dense area was of borderline statistical significance. Two polymorphisms at the *IGFBP1/3* locus were also significantly related to mammographic density. Rs35539615 (*IGFBP1*) was associated with higher percent density via the recessive mode of inheritance. An increase in the number of the minor allele for rs2453839 (*IGFBP3*) was related to lower percent density. Four SNPs (*IGFBP3*) were related to mammographic density in opposite directions among Caucasians as compared to Japanese Americans.

We had hypothesized that genetic variants in *IGF1*, *IGFBP1* or *IGFBP3* are related to mammographic density. Only one of the SNPs in *IGF1* (rs35767) was significantly associated with lower percent density via the dominant mode of inheritance. However, in the large Breast and Prostate Cancer Cohort Consortium (BPC3) study, carriers of at least one copy of the minor allele of rs35767 had modestly, but statistically significantly higher IGF-I levels (17). As IGF-I induces mitogenesis and inhibits apoptosis, we expected genetic

variants that are positively related to IGF-I levels to be also associated with increased mammographic density. The only other study of this SNP found no relation with mammographic density (18). These observations and the fact that we tested a large number of genetic variants make it likely that the association with rs35767 in our study was a chance finding. Although statistically non-significant, women homozygous for the minor allele of rs2139572 or rs1996656 had 7.3% and 5.0% lower mammographic density, respectively. To the best of our knowledge, rs2139572 has never been described in relation to circulating levels of IGF-I or mammographic density, but rs1996656, which is in strong linkage disequilibrium with rs2139572, was found not to be related to circulating levels (17;26) or mammographic density (18).

In addition to rs35767, four other SNPs examined in the present study were previously found to be associated with IGF-I levels in the BPC3 study. None of these four SNPs was associated with percent density or the dense area in the present study. Three previous studies on *IGF-I* polymorphisms and mammographic density found inconclusive results (18–20). None of the SNPs that were found to be significantly associated to mammographic density in one of these studies (including the present) were replicated in another, although not all studies used the same set of SNPs. Moreover, the two SNPs that were most strongly related to lower mammographic density (rs1520220, rs2946834)(18), were related to higher circulating levels in the BPC3 (17), which seems biologically implausible. Given these findings and the observation that the greatest difference in IGF-I levels between SNP genotypes was only 4.8% in the BPC3 study (17), it appears unlikely that common variation in the *IGF-I* gene would be substantially related to mammographic density. An exception to this may be the borderline significant association between the minor allele of rs6220 and higher mammographic density that was found in a Dutch and in a Canadian study (19;20). This SNP was also found to be significantly related to higher IGF-I circulating levels (16;20). Unfortunately, this SNP was not genotyped in the present study.

Only the NHS has published data on common variation in *IGFBP1/3* and mammographic density. The association between higher percent density and the minor allele of rs2453839 (*IGFBP3*) was also observed in the NHS, although it did not reach statistical significance (18). In both studies, there was a trend of increased density with number of minor alleles, but the effect was strongest via the recessive mode of inheritance. Although there were insufficient numbers of subjects to analyze a recessive effect in each ethnic group, lower percent density was seen in both Caucasians and Japanese American women further supporting a possible true relation. However, unpublished results of a large, prospective Dutch study with over 1900 participants did not show this SNP to be associated with premenopausal mammographic density or with postmenopausal density in a subgroup of approximately one third these women which had become postmenopausal during follow-up (Taverne, personal communication). Furthermore, rs2453839 was not associated with IGF-I or IGFBP-3 levels in BPC3 (17). RS10228265 was not associated with mammographic density in the NHS (18), but it was not analysed in the Dutch study. The only significant association in the NHS between the genetic variation in the IGFBP1/3 locus (rs1065780) and mammographic density was not confirmed in the present study.

Most results from the ethnic-specific analyses were in the same direction as for the total population. However, some statistically significant associations were observed in specific groups only. BMI, which strongly influences percent density, differed by ethnicity and was included in the models. Although numbers in the stratified analyses were small, allelic distributions did not materially differ between ethnic groups. True effect modification by ethnicity is thus possible. Given the multiple comparisons in this study, these findings may also have been due to chance.

A strength of our study was the relatively large sample size. Furthermore, using a set of SNPs to tag underlying haplotypes ensures capturing common variation of the genes under study. Case-control status may influence the *IGF1*- mammographic density relation. Mammograms of patients were, however, collected before the date of diagnosis (on average 3.8 years before diagnosis) and separate analyses did not attenuate the results materially.

Conclusions

In our study we found no indication that common variation in the IGF1 gene is substantially related to mammographic density. Rs2453839 (*IGFBP3*) may be related to lower mammographic density, but this requires confirmation in future studies. We found some SNPs in *IGFBP3* to be possibly differentially related to mammographic density among Caucasians and Japanese Americans. This may indicate that ethnicity modifies the *IGF1* and mammographic density relations to some extent, but the smaller sample size in the ethnic/racial groups in this study limit our ability to draw strong conclusions.

Statements on novelty and impact

The present research for the first time describes the association between common genetic variation in the *IGF1*, *IGFBP1* and *IGFBP3* genes and mammographic density in a multiethnic population.

The results of this large study did not confirm previously published relationships between genetic variants in the *IGF1* gene and mammographic density. These results show that the influence of genetic variation in the *IGF1* gene on mammographic density is probably very small.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BMI	body mass index
BPC3	Breast and Prostate Cancer Cohort Consortium
CI	confidence interval
IGF-I	insulin-like growth factor-I
IGFBP1/3	insulin-like growth factor binding protein I/3
MEC	Multiethnic cohort
NHS	Nurses' Health Study
SD	standard deviation
SNP	single nucleotide polymorphisms

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Table 1

Baseline characteristics of the study population, N=819

	Native Hawaiian (N=202)		Caucasian (N=259)		Japanese American (N=358)	
Characteristic	**N (%)**	**Mean (SD)**	**N (%)**	**Mean (SD)**	**N (%)**	**Mean (SD)**
Age (y)		54.5 (9.0)		56.9 (9.4)		58.7 (9.2)
BMI (kg/m^2)		28.1 (6.5)		25.1 (5.6)		23.6 (4.1)
Menopausal status						
Premenopausal	66 (33)		62 (24)		81 (23)	
Postmenopausal	136 (67)		197 (76)		277 (77)	
Case status						
Case	54 (27)		122 (47)		180 (50)	
Control	148 (73)		137 (53)		178 (50)	
Percent density		29.1 (22.2)		33.6 (25.2)		37.0 (21.7)
Dense area (cm^2)		33.5 (27.1)		36.3 (32.2)		29.0 (19.0)
Total breast area (cm^2)		136.8 (64.9)		131.8 (71.0)		85.1 (32.6)

SD=standard deviation; BMI=body mass index

Table 2

Common genetic variation of *IGF1* and percent breast density in the Multiethnic Cohort, N=819

Haplotype block	SNP	N[1]	Mean (95% CI)	p-value trend[2]	p-value dominant[3]	p-value recessive[4]
1	rs7965399	546	33.0 (31.4 ; 34.6)	0,47	0,41	0,92
		210	31.7 (29.0 ; 34.4)			
		32	32.1 (25.5 ; 38.8)			
	rs35765	691	32.8 (31.4 ; 34.3)		0,60	
		105	31.8 (28.1 ; 35.4)			
	rs35767	435	34.0 (32.2 ; 35.8)	0,06	0,03	0,82
		267	30.6 (28.3 ; 33.0)			
		52	31.9 (26.7 ; 37.1)			
	rs2288377	553	33.2 (31.6 ; 34.8)	0,22	0,18	0,73
		201	31.0 (28.2 ; 33.8)			
		35	31.1 (24.7 ; 37.6)			
2	rs12821878	611	32.9 (31.3 ; 34.5)		0,64	
		169	32.1 (29.1 ; 35.1)			
	rs1019731	703	32.8 (31.3 ; 34.3)		0,95	
		75	32.9 (28.4 ; 37.5)			
	rs12423791	555	33.1 (31.5 ; 34.7)		0,51	
		181	32.0 (28.9 ; 35.0)			
	rs2195239	338	33.1 (31.1 ; 35.2)	0,26	0,48	0,22
		348	32.7 (30.6 ; 34.7)			
		96	30.3 (26.5 ; 34.1)			
	rs2195240	335	33.2 (31.2 ; 35.3)	0,46	0,57	0,51
		351	32.7 (30.6 ; 34.7)			
		93	31.5 (27.6 ; 35.4)			
3	rs10735380	458	32.9 (31.2 ; 34.7)	0,27	0,47	0,20

Haplotype block	SNP	N[1]	Mean (95% CI)	p-value trend[2]	p-value dominant[3]	p-value recessive[4]
		280	32.5 (30.2 ; 34.7)			
		55	29.4 (24.4 ; 34.3)			
	rs2373722	749	32.8 (31.4 ; 34.2)		0,61	
		42	31.3 (25.4 ; 37.1)			
	rs5742657	577	32.9 (31.3 ; 34.4)	0,59	0,63	0,72
		185	32.2 (29.2 ; 35.1)			
		22	31.1 (23.1 ; 39.1)			
	rs5742665	710	32.7 (31.3 ; 34.2)		0,86	
		83	32.3 (28.2 ; 36.5)			
	rs9308315	296	33.2 (31.0 ; 35.4)	0,30	0,52	0,27
		337	32.7 (30.7 ; 34.8)			
		112	30.8 (27.3 ; 34.3)			
	rs1549593	705	32.5 (31.1 ; 34.0)		1,00	
		85	32.5 (28.3 ; 36.8)			
	rs1520220	327	33.4 (31.3 ; 35.5)	0,17	0,30	0,21
		349	32.4 (30.4 ; 34.4)			
		108	30.3 (26.7 ; 34.0)			
	rs6218	598	32.7 (31.1 ; 34.2)	0,55	0,55	0,80
		167	31.7 (28.5 ; 34.8)			
		21	31.2 (23.0 ; 39.5)			
	rs5742723	569	32.7 (31.1 ; 34.3)	0,86	0,64	0,56
		177	31.6 (28.5 ; 34.6)			
		25	34.5 (27.0 ; 42.0)			
4	rs2946834	270	33.1 (30.8 ; 35.4)	0,44	0,60	0,44
		367	32.7 (30.7 ; 34.6)			
		149	31.5 (28.4 ; 34.6)			

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Haplotype block	SNP	N[1]	Mean (95% CI)	p-value trend[2]	p-value dominant[3]	p-value recessive[4]
	rs2139573	352	32.5 (30.4 ; 34.6)	0,81	0,83	0,43
		323	33.3 (31.2 ; 35.4)			
		104	31.3 (27.6 ; 35.0)			
	rs4764880	604	33.2 (31.6 ; 34.7)	0,86	0,55	0,40
		146	31.5 (28.1 ; 34.8)			
		23	36.0 (28.2 ; 43.8)			
	rs2139572	590	33.0 (31.4 ; 34.6)	0,22	0,50	0,06
		173	32.8 (29.8 ; 35.8)			
		25	25.7 (18.2 ; 33.2)			
	rs2139570	304	32.2 (30.0 ; 34.4)	1,00	0,52	0,36
		351	33.7 (31.6 ; 35.7)			
		104	31.2 (27.5 ; 34.8)			
	rs4764876	291	33.0 (30.8 ; 35.2)	0,65	0,89	0,50
		351	33.2 (31.2 ; 35.2)			
		141	31.9 (28.7 ; 35.1)			
	rs4764695	304	31.7 (29.5 ; 33.9)	0,56	0,18	0,49
		354	34.2 (32.1 ; 36.2)			
		117	31.7 (28.3 ; 35.2)			
	rs1996656	466	32.6 (30.9 ; 34.4)	0,62	0,98	0,14
		230	33.3 (30.9 ; 35.8)			
		31	27.8 (21.1 ; 34.4)			

Mean levels and CIs were adjusted for ethnicity, age, age^2 and BMI at the time of each mammogram

BMI=body mass index; CI=confidence interval; SNP=single nucleotide polymorphism

[1] number of women for each genotype

[2] p-value for additive mode of inheritance; percent breast density was linearly related to the number of minor alleles (0, 1, or 2)

[3] p-value for dominant mode of inheritance; percent breast density was compared between women who were heterozygous or homozygous for the minor allele and all others

[4] p-value for recessive mode of inheritance; percent breast density was compared between women who were homozygous for the minor allele and all others

Table 3

Common genetic variation of *IGFBP1* and *IGFBP3* and percent breast density in the Multiethnic Cohort, N=819

Haplotype block	SNP	N[1]	Mean (95% CI)	p-value trend[2]	p-value dominant[3]	p-value recessive[4]
1	rs10228265	378	32.4 (30.5 ; 34.4)	0,64	0,35	0,64
		315	34.1 (32.0 ; 36.2)			
		86	32.2 (28.2 ; 36.2)			
	rs1553009	452	33.1 (31.3 ; 34.8)	0,45	0,55	0,50
		281	32.5 (30.2 ; 34.7)			
		55	31.0 (26.1 ; 36.0)			
	rs35539615	415	32.9 (31.1 ; 34.8)	0,48	0,87	0,05
		295	31.8 (29.6 ; 34.0)			
		61	37.4 (32.6 ; 42.2)			
	rs2201638	703	32.9 (31.4 ; 34.4)		0,50	
		97	31.5 (27.7 ; 35.3)			
	rs1065780	262	34.0 (31.7 ; 36.3)	0,53	0,09	0,37
		365	30.8 (28.8 ; 32.7)			
		143	33.7 (30.6 ; 36.8)			
2	rs3793344	255	33.6 (31.2 ; 35.9)	0,98	0,29	0,22
		375	31.2 (29.2 ; 33.1)			
		156	34.2 (31.2 ; 37.1)			
	rs1874479	587	32.3 (30.7 ; 33.9)		0,55	
		197	33.3 (30.6 ; 35.9)			
	rs4988515	778	32.7 (31.3 ; 34.1)		0,70	
		33	34.0 (27.5 ; 40.6)			
	rs4619	276	33.9 (31.6 ; 36.2)	0,79	0,18	0,25
		350	31.1 (29.0 ; 33.1)			
		143	34.3 (31.2 ; 37.4)			

Haplotype block	SNP	N[1]	Mean (95% CI)	p-value trend[2]	p-value dominant[3]	p-value recessive[4]
	rs1908751	332	33.3 (31.2 ; 35.3)	0,97	0,61	0,40
		347	32.1 (30.0 ; 34.1)			
		89	34.4 (30.5 ; 38.4)			
	rs1496495	564	32.4 (30.7 ; 34.0)	0,55	0,39	0,60
		209	33.9 (31.4 ; 36.5)			
		21	30.6 (22.5 ; 38.7)			
	rs1496497	563	32.3 (30.7 ; 33.9)	0,62	0,44	0,59
		209	33.8 (31.2 ; 36.4)			
		22	30.5 (22.6 ; 38.4)			
	rs2270628	584	32.2 (30.7 ; 33.8)		0,49	
		214	33.3 (30.7 ; 35.8)			
3	rs3110697	333	32.8 (30.7 ; 34.9)	0,97	0,63	0,54
		353	31.8 (29.8 ; 33.7)			
		100	33.5 (29.8 ; 37.1)			
	rs6953668	682	32.3 (30.8 ; 33.7)		0,20	
		124	34.7 (31.3 ; 38.2)			
	rs2854747	349	33.0 (31.0 ; 35.1)	0,73	0,47	0,69
		349	31.8 (29.8 ; 33.8)			
		92	33.2 (29.3 ; 37.0)			
	rs2854746	297	33.7 (31.3 ; 36.1)	0,43	0,16	0,80
		333	31.3 (29.2 ; 33.3)			
		159	32.6 (29.6 ; 35.6)			
	rs2854744	320	33.9 (31.6 ; 36.1)	0,68	0,16	0,32
		333	31.1 (29.1 ; 33.1)			
		142	34.0 (30.8 ; 37.2)			
	rs2132570	477	33.2 (31.4 ; 34.9)	0,17	0,39	0,09
		276	32.6 (30.3 ; 34.8)			

Haplotype block	SNP	N[1]	Mean (95% CI)	p-value trend[2]	p-value dominant[3]	p-value recessive[4]
		41	27.8 (22.0 ; 33.5)			
	rs2471554	653	32.0 (30.5 ; 33.6)		0,15	
		158	34.7 (31.6 ; 37.8)			
Outside blocks	rs6670	684	32.6 (31.1 ; 34.1)		0,75	
		121	33.2 (29.7 ; 36.8)			
	rs2453839	548	33.7 (32.0 ; 35.3)	0,01	0,04	0,02
		225	31.4 (28.9 ; 33.8)			
		23	23.2 (15.4 ; 31.0)			

Mean levels and CIs were adjusted for ethnicity, age, age^2 and BMI at the time of each mammogram

BMI=body mass index; CI=confidence interval; SNP=single nucleotide polymorphism

[1] number of women for each genotype

[2] p-value for additive mode of inheritance; percent breast density was linearly related to the number of minor alleles (0, 1, or 2)

[3] p-value for dominant mode of inheritance; percent breast density was compared between women who were heterozygous or homozygous for the minor allele and all others

[4] p-value for recessive mode of inheritance; percent breast density was compared between women who were homozygous for the minor allele and all others