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Genetic variation in genes of the fatty acid synthesis pathway and breast cancer risk

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Abstract Fatty acid synthase (FAS) is the major enzyme of lipogenesis. It catalyzes the NADPH-dependent condensation of acetyl-CoA and malonyl-CoA to produce palmitic acid. Transcription of the FAS gene is controlled synergistically by the transcription factors ChREBP (carbohydrate response element-binding protein), which is induced by glucose, and SREBP-1 (sterol response element-binding protein-1), which is stimulated by insulin

through the PI3K/Akt signal transduction pathway. We investigated whether the genetic variability of the genes encoding for ChREBP, SREBP and FAS (respectively, *MLXIPL*, *SREBF1* and *FASN*) is related to breast cancer risk and body-mass index (BMI) by studying 1,294 breast cancer cases and 2,452 controls from the European Prospective Investigation on Cancer (EPIC). We resequenced the FAS gene and combined information of SNPs found by

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resequencing and SNPs from public databases. Using a tagging approach and selecting 20 SNPs, we covered all the common genetic variation of these genes. In this study we were not able to find any statistically significant association between the SNPs in the *FAS*, *ChREBP* and *SREBP-1* genes and an increased risk of breast cancer overall and by subgroups of age, menopausal status, hormone replacement therapy (HRT) use or BMI. On the other hand, we found that two SNPs in *FASN* were associated with BMI.

Keywords Fatty acid synthase · Carbohydrate response element-binding protein · Sterol response element-binding protein-1 · Breast cancer · Susceptibility to cancer · Body-mass index

Introduction

International comparison suggests that one of the key factors underlying the high incidence of breast cancer (BC) in industrially developed countries is a Western lifestyle, characterized by low rates of energy expenditure and a high-energy diet, rich in saturated fats, refined carbohydrates and animal protein. However, the physiologic mechanisms of this relationship are poorly understood. Various hormonal and metabolic factors have been postulated to mediate the effects of a Western nutritional

lifestyle on BC risk [1–7]. Breast cancer risk appears to be increased among women who have elevated plasma levels of insulin-like growth factor-I (IGF-I) [1, 2, 4, 5, 7–10]. Excess energy intake and obesity also cause insulin resistance, which is associated with elevated blood levels of glucose and insulin [1, 3–6, 8, 11–13]. In turn, elevated plasma levels of glucose [12] and insulin [3, 14] have been implicated as possible risk factors for both pre- and post-menopausal BC.

Fatty acid synthase (FAS) is the major enzyme of lipogenesis. It catalyzes the NADPH-dependent condensation of acetyl-CoA and malonyl-CoA to produce predominantly the 16-carbon saturated free palmitic acid [15]. Transcription of the *FAS* gene is controlled synergistically by the transcription factors *ChREBP* (carbohydrate response element-binding protein) and *SREBP-1* (sterol response element-binding protein-1). *ChREBP* binds directly to the endogenous promoter of the *FAS* gene in a glucose-dependent but insulin-independent manner and is absolutely required for its induction by glucose [16]. Sterol-responsive element binding protein-1 (*SREBP-1*) is an important transcription factor responsible for lipid accumulation in pancreatic islets in response to high glucose and insulin, through stimulation of acetyl-CoA carboxylase-1 and *FAS* [17, 18]. It is stimulated by insulin through the PI3K/Akt signal transduction pathway [19, 20]. Moreover, *SREBP-1* inhibition also abolishes the induction of *FAS* promoter.

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The FAS gene is highly up-regulated in tumor cells of the breast, as well as colon, prostate, ovary and endometrium [21–25], although this gene is expressed at minimum or undetectable level in most normal tissues. Therefore, FAS overexpression is considered to be one of the most common molecular changes in cancer cells [22, 24, 26–28]. Importantly, treatment of tumor cells with pharmacologic inhibitors of FAS leads to cell cycle arrest, followed by apoptosis of the tumor cells [29]. Moreover, FAS inhibitors have been shown to delay progression of breast and ovarian tumor xenografts, and to induce apoptosis of breast carcinoma cells [15, 23, 30–33]. Thus, the early activation of FAS in malignant and pre-malignant cells could represent a necessary survival strategy, which occurs to compensate for an insufficiency of both oxygen and dietary fatty acids due, for example, to lack of angiogenesis [34]. Thereafter, high levels of FAS are maintained in coordination with increased demand for fatty acid metabolism and/or membrane synthesis in response to cancer-related overexpression of growth factors and/or growth factor receptors [34]. Moreover, FAS hyperactivity and overexpression preferentially occur in the aggressive varieties of cancer and serve as an independent prognostic indicator of adverse clinical outcome [34].

The above observations led us to hypothesize that ChREBP, SREBP, and FAS, the key molecules involved in cellular fatty acid synthesis, may be centrally implicated in mammary gland carcinogenesis, and that polymorphic alleles of their encoding genes that modify their expression or activity confer altered BC susceptibility. In this report we investigated the genetic variability of the genes encoding for ChREBP, SREBP and FAS. We tested association of tagging SNPs in the three candidate genes with BC risk in a study of 1,294 invasive BC cases and 2,452 controls nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). Since weight gain and obesity are well-known risk factors for postmenopausal BC [35], we have tested association of tagging SNPs in our three candidate genes with body-mass index (BMI) as well. To our knowledge this is the first report on polymorphisms of these genes and BC risk.

Materials and methods

The EPIC cohort

A fully detailed description of the EPIC cohort has been published elsewhere [36]. Briefly, EPIC consists of about 370,000 women and 150,000 men, aged 35–69, recruited between 1992 and 2005 in 10 Western European countries.

The vast majority (>97%) of subjects recruited in the EPIC cohort are of European ('Caucasian') origin. All

EPIC study subjects provided anthropometric measurements (height, weight, and waist and hip circumferences) and extensive, standardized questionnaire information about medical history, diet, physical activity, smoking, and other lifestyle factors. Women also answered questions about menstrual and reproductive history, hysterectomy, ovariectomy, and use of exogenous hormones for contraception or treatment of menopausal symptoms. About 260,000 women and 140,000 men provided a blood sample.

Cases of cancer occurring after recruitment into the cohort and blood donation are identified through local and national cancer registries in 7 of the 10 countries, and in France, Germany, and Greece by a combination of contacts with national health insurances and/or active follow-up through the study subjects or their next of kin. Follow-up on vital status is achieved through record linkage with mortality registries.

Selection of case and control subjects

Case subjects were selected among women who developed BC after blood collection. Control subjects (1–2 controls per case) were selected randomly by incidence density sampling, matching the cases for centre of recruitment, age at blood donation, duration of follow-up, menopausal status at the time of blood donation and use of exogenous hormones. This study did not include women who were using hormone replacement therapy (HRT) at the time of blood donation. A total of 1,294 invasive BC cases and 2,452 controls were included in the present study. Each control should have been free of cancer up to the duration of follow-up of the index case. The study was approved by the ethical review boards of the International Agency for Research on Cancer, and of the collaborating institutions responsible for subject recruitment in each of the EPIC recruitment centres.

Resequencing analysis

The coding exons flanked by approximately 30 bp of proximal intronic sequence, 5' UTR, and proximal promoters of *FASN* were resequenced on 46 samples of BC cases of Caucasian origin not carriers of germline BRCA1/BRCA2 mutations, using previously established procedures [37]. The genomic sequences of each locus of interest were downloaded from the UC Santa Cruz genome browser (<http://genome.ucsc.edu/>) (Sequences of the mutation screening primers and experimental conditions are available upon request). Sequencing reaction products were electrophoresed on a Spectrumedix capillary sequencer.

The resulting chromatograms were analyzed using the program Java SnpScreen (S. V. Tavtigian, unpublished).

The program aligns all of the chromatograms to the canonical text sequence of each amplicon, normalizes the signal strength from all of the chromatograms, and then displays them as aligned forward–reverse chromatogram pairs. Potentially heterozygous positions are spotted, based on the joint data from the target sequence and each forward and reverse chromatogram pair.

Selection of tagging SNPs

We aimed at surveying the entire set of common genetic variants in the *FASN*, *MLXIPL* and *SREBF1* genes. We followed a tagging approach [38]. Gene regions were defined as the sequence between the beginning of the first known exon and the end of the last known exon. When an LD block covering a part of the gene extended outside the gene we also considered any additional sequence to include the whole block. See Figs. 1, 2 and 3 for the exact regions that were considered. We included all polymorphisms in each gene region with minor allele frequency (MAF) $\geq 5\%$ in Caucasians from the International HapMap Project (version 22; <http://www.hapmap.org>) and, for the *FASN*

gene, from our resequencing work. The SNPs detected by resequencing were genotyped in the HapMap Caucasian samples. Tagging SNPs were selected with the use of the Tagger program within Haploview (<http://www.broad.mit.edu/mpg/haploview/>; <http://www.broad.mit.edu/mpg/tagger/>; [39]), using pairwise tagging with a minimum r^2 of 0.8. We used, as input for Tagger, genotypes downloaded from the HapMap database and genotypes of our resequencing SNPs typed on HapMap samples.

DNA extraction and genotyping

DNA was extracted from blood samples on an Autopure instrument (Qiagen, Hilden, Germany) with Puregene chemistry (Qiagen, Hilden, Germany). The order of DNAs from cases and controls was randomized on PCR plates in order to ensure that an equal number of cases and controls could be analyzed simultaneously. All the genotyping was carried out using the Taqman assay. The MGB Taqman probes and primers were synthesized by Applied Biosystems (Foster City, CA). The reaction mix included 5 ng genomic DNA, 10 pmol each primer, 2 pmol each probe

Fig. 1 *FASN* gene and its SNPs. From top to bottom, schematic representation of the gene (boxes represent exons, lines represent introns), position of the SNPs in the region, LD structure of the gene region (color intensity is proportional to r^2 , while numbers represent

D' ; squares where numbers are not reported have $D' = 100\%$). LD blocks covering regions larger than 10 kb outside genes were kept intact when they overlapped parts of the gene or the promoter region

Fig. 2 *MLXIPL* gene and its SNPs. For details, see Fig. 1

and 2.5 μ l of 2 $\times$ master mix (Applied Biosystems) in a final volume of 5 μ l. The thermocycling included 40 cycles with 30 s at 95°C followed by 60 s at 60°C. PCR plates were read on an ABI PRISM 7900HT instrument (Applied Biosystems).

All samples that did not give a reliable result in the first round of genotyping were resubmitted to up to three additional rounds of genotyping. Data points that were still not filled after this procedure were left blank. Repeated quality control genotypes (8% of the total) showed an average concordance of 99.7%.

Statistical analysis

The frequency distribution of genotypes was examined for cases and controls. For each SNP, deviation of genotype frequencies from the Hardy–Weinberg equilibrium was assessed in the controls by χ^2 test (threshold of $P < 0.01$).

We used conditional logistic regression for multivariate analyses to assess the main effects of the genetic polymorphism on BC risk assuming dominant, recessive and codominant models of inheritance. The most common allele in the controls was held as the reference category in calculating the odds ratio (OR). All relative risk analysis were performed with the exclusion of cases with carcinomas in situ ($n = 93$ cases and 100 controls). For this analysis incomplete case–control sets could not be used and therefore were excluded. Subgroup analyses were performed based on menopausal status and age (with cut-point at 50 or 55 years of age at diagnosis). Additional analyses were performed by including cases of carcinoma in situ, by excluding cases diagnosed shortly (6 months, 1 or 2 years) after blood drawing, or subjects who reported intake of hexogenous hormones.

Relationships of polymorphic gene variants with BMI were estimated by standard regression models and percentage of difference in geometric means in each genotype

Fig. 3 *SREBF* gene and its SNPs. For details, see Fig. 1

category compared to the major homozygote category was calculated. An additional analysis was performed by categorizing subjects with $\text{BMI} \geq 30$ as obese $25 \leq \text{BMI} < 30$ as overweight and <25 as normal weight, and calculating odds ratios for obese or overweight versus normal weight with unconditional logistic regression. All BMI analyses were adjusted for age at blood donation, centre and BC case-control status. For this analysis we could use the genotypes of all the subjects, regardless of case-control and matching status.

Polymorphisms selected as tagging SNPs had a low level of residual linkage disequilibrium (on average $r^2 = 0.15$ for the *FASN* gene, $r^2 = 0.14$ for *MLXIPL* and $r^2 = 0.60$ for *SREBF1*), therefore we assumed that haplotypes were adequately captured by our tagging SNPs, and we did not attempt a haplotype analysis.

Results

The study explored the association between 20 DNA polymorphisms in three key genes involved in the fatty acid synthesis, namely *FASN*, *MLXIPL* and *SREBF1* and BC risk, as well as with BMI. We included 1,294 incident BC cases from the EPIC cohort and 2,452 matched controls in the study. Table 1 summarizes the baseline characteristics of cases and controls.

Table 1 Baseline characteristics of BC cases and control subjects

Variable	Cases	Controls
Number	1521 ^a	2555 ^a
Women with carcinoma in situ	93 ^a	100 ^a
Pre-menopausal women	395 ^a	755 ^a
Peri-menopausal women	139 ^a	228 ^a
Post-menopausal women	987 ^a	1572 ^a
Mean age at blood donation	55.2(40.7–67.5) ^b	54.9(40.2–68.1) ^b
Mean age at diagnosis	57.5(43.0–70.0) ^b	–
Height	161.2(151.0–172.0) ^b	160.6(150.0–171.8) ^b
Weight	67.6(51.7–89.0) ^b	67.2(51.0–89.5) ^b
Body mass index	26.0(20.3–34.4) ^b	26.1(20.1–35.0) ^b

^a Number of subjects

^b Mean (5th–95th percentiles)

We covered all the common genetic variants in the region of our candidate genes with a tagging approach. For the *FASN* gene, which is considerably larger than the two others, and located in a region of higher LD complexity, we integrated HapMap data with our own resequencing data. We found that all common ($\text{MAF} \geq 0.05$) SNPs identified through resequencing were either already present in HapMap or tagged by HapMap SNPs, with the exception of

Table 2 Associations between BC risk and SNPs in *FASN*, *MLXIPL* and *SREBF1*

Gene name	rs#	Homozygotes common allele		Heterozygotes				Homozygotes rare allele				P_{trend}^c
		Ca ^a	Co ^a	Ca	Co	OR ^a	95% CI ^a	Ca	Co	OR	95% CI	
<i>FASN</i>	rs2228309	404	676	633	1087	1.00	(0.85–1.17)	238	439	0.92	(0.75–1.12)	0.445
	rs4969465	730	1262	449	772	0.99	(0.86–1.15)	83	131	1.10	(0.82–1.48)	0.701
	rs2229425	962	1606	281	519	0.89	(0.75–1.05)	23	46	0.83	(0.50–1.37)	0.108
	rs1140616	349	573	637	1077	0.98	(0.83–1.16)	299	567	0.87	(0.72–1.06)	0.230
	rs2229422	575	1014	559	930	1.05	(0.90–1.22)	136	238	1.01	(0.80–1.27)	0.751
	rs4969486	1187	2048	85	144	1.00	(0.76–1.32)	1	2	0.99	(0.09–10.9)	0.978
	FASN_A0866 ^b	781	1353	408	674	1.04	(0.90–1.21)	42	76	0.96	(0.65–1.42)	0.766
	rs4246445	669	1164	496	822	1.04	(0.90–1.20)	98	173	0.95	(0.73–1.24)	0.985
	rs4485435	877	1441	357	652	0.92	(0.78–1.07)	34	74	0.79	(0.52–1.19)	0.141
	rs11867806	607	1055	537	898	1.03	(0.89–1.20)	117	204	1.00	(0.79–1.28)	0.813
<i>MLXIPL</i>	rs12949488	814	1323	393	739	0.88	(0.76–1.03)	57	97	0.99	(0.70–1.39)	0.235
	rs799157	1145	1939	91	148	1.05	(0.80–1.38)	2	1	3.95	(0.36–43.5)	0.552
	rs1051921	847	1419	336	611	0.91	(0.78–1.07)	33	56	0.96	(0.61–1.50)	0.317
	rs6967028	1027	1742	219	392	0.93	(0.77–1.12)	12	29	1.05	(0.51–2.18)	0.582
	rs7808877	659	1074	452	831	0.87	(0.75–1.02)	99	147	1.07	(0.81–1.42)	0.539
	rs11772762	775	1326	401	656	1.05	(0.89–1.23)	46	78	1.00	(0.68–1.47)	0.666
<i>SREBF1</i>	rs17145750	926	1556	293	515	0.95	(0.80–1.12)	19	26	1.26	(0.69–2.29)	0.836
	rs9899634	475	809	598	1011	1.02	(0.87–1.19)	181	338	0.93	(0.72–1.20)	0.644
	rs11868035	598	1011	545	937	1.01	(0.87–1.17)	116	214	0.93	(0.72–1.20)	0.744
	rs12951376	545	956	560	903	1.10	(0.95–1.28)	133	244	0.98	(0.77–1.23)	0.694

^a Ca, Cases; Co, Controls; OR, Odds ratios estimated by conditional logistic regression with matching of cases and controls for age at blood donation centre of recruitment, menopausal status at blood donation and past use of HRT; 95%CI, 95% confidence intervals

^b Polymorphism FASN_A0866 is not represented in NCBI dbSNP. It is located in intron 20 of *FASN* and its flanking sequence is GCCCCGCCCC AGCCCCAGCC CAGTGCCCCC ACCCAAGTAT GCCGCACTCA [C/T] CCAGCCTGTC CCCACAGTGG CTGACGTGGT GGTGAGCAGG TGGCTGAGGG

^c P_{trend} for codominant model

one (FASN_A0866), which was genotyped on the complete series of cases and controls.

The genotype distributions at all SNPs typed in the EPIC samples were in Hardy–Weinberg equilibrium in controls, with non-significant χ^2 values (data not shown).

The frequencies and distribution of the genotypes and the odds ratios for the association of each polymorphism with BC risk and BMI level are described in Tables 2 and 3 respectively. We observed no statistically significant association in the codominant, dominant or recessive models between the SNPs in the *FASN*, *MLXIPL* and *SREBF1* genes and BC risk overall and stratified by age or menopausal status.

Additional analyses performed by including cases of carcinoma in situ, or excluding cases diagnosed shortly after blood drawing, or subjects who reported intake of exogenous hormones, showed essentially the same results as using all cases and controls (data not shown).

We performed also an analysis of the geometric means of BMI by SNP genotypes. We found that two SNPs in the *FASN* gene were associated with the level of BMI

(Table 3). SNP rs1140616 was associated with a higher mean of BMI (P_{trend} 0.0061), while SNP FASN_A0866 was associated with a lower mean of BMI (P_{trend} 0.0270) (Table 3).

There was no significant heterogeneity in association of any of the SNP variants with breast cancer with any category of BMI (<25 , $25 \leq \text{BMI} < 30$ or ≥ 30 kg/m²). For *SREBF1* and *MLXIPL* no associations with BMI were observed.

Discussion

We postulated that genetic polymorphisms in the *FASN*, *MLXIPL* and *SREBF1* genes might play an important role in development of BC given their key function in lipogenesis. Up-regulation of genes involved in lipogenesis is a common feature of many cancers, as documented by the fact that many human tumor cells synthesize fatty acids instead of using fatty acids from the diet [40].

We tested in a large-scale association study, nested within the EPIC cohort, the involvement in BC risk of

Table 3 Associations between BMI and SNPs in *FASN*, *MLXIPL* and *SREBF1*

Gene name	rs#	Homozygotes common allele		Heterozygotes		Homozygotes rare allele		P_{trend}^c
		<i>N</i>	BMI (95% CI) ^a	<i>N</i>	BMI (95% CI)	<i>N</i>	BMI (95% CI)	
<i>FASN</i>	rs2228309	1236	25.96(25.71–26.22)	1959	26.04(25.84–26.24)	785	26.15(25.83–26.47)	0.366
	rs4969465	2289	26.03(25.84–26.22)	1415	26.04(25.80–26.27)	240	26.11(25.54–26.69)	0.863
	rs2229425	2935	26.02(25.85–26.19)	940	26.06(25.77–26.35)	81	26.12(25.13–27.10)	0.770
	rs1140616	1031	25.73(25.45–26.00)	1971	26.05(25.85–26.26)	986	26.27(25.98–26.55)	0.0071
	rs2229422	1812	26.06(25.84–26.27)	1713	25.99(25.78–26.21)	433	26.15(25.72–26.58)	0.922
	rs4969486	3702	26.02(25.87–26.17)	268	26.18(25.64–26.72)	4	26.13(21.70–30.56)	0.586
	FASN_A0866 ^b	2504	26.15(25.97–26.33)	1236	25.89(25.64–26.15)	139	25.49(24.74–26.24)	0.0275
	rs4246445	2107	25.93(25.74–26.13)	1527	26.16(25.93–26.39)	311	26.01(25.51–26.52)	0.278
	rs4485435	2658	25.98(25.80–26.15)	1164	26.15(25.88–26.41)	127	26.32(25.53–27.11)	0.200
	rs11867806	1915	26.01(25.81–26.22)	1646	26.01(25.78–26.23)	377	26.25(25.79–26.71)	0.519
<i>MLXIPL</i>	rs12949488	2450	25.96(25.78–26.14)	1311	26.13(25.88–26.38)	179	26.24(25.57–26.90)	0.214
	rs799157	3613	26.06(25.91–26.21)	272	25.89(25.35–26.43)	5	25.40(21.42–29.38)	0.506
	rs1051921	2674	26.01(25.84–26.19)	1121	26.19(25.93–26.46)	108	25.54(24.68–26.39)	0.7440
	rs6967028	3210	26.06(25.89–26.22)	694	25.94(25.60–26.28)	36	27.04(25.56–28.52)	0.990
	rs7808877	2063	25.95(25.75–26.15)	1520	26.15(25.92–26.38)	292	25.92(25.40–26.45)	0.468
	rs11772762	2475	26.13(25.95–26.32)	1243	26.02(25.76–26.27)	146	25.30(24.56–26.04)	0.0732
<i>SREBF1</i>	rs17145750	2901	25.95(25.78–26.12)	937	26.28(25.98–26.57)	54	26.04(24.83–27.25)	0.0877
	rs9899634	1487	26.02(25.79–26.25)	1855	26.02(25.81–26.23)	610	26.21(25.85–26.57)	0.475
	rs11868035	1872	26.14(25.93–26.34)	1706	26.00(25.78–26.22)	382	25.80(25.34–26.25)	0.152
	rs12951376	1745	25.95(25.73–26.17)	1710	26.10(25.88–26.32)	444	26.07(25.65–26.50)	0.403

Statistically significant results ($P < 0.05$) are reported in bold

^a 95%CI, 95% confidence intervals

^b Polymorphism FASN_A0866 is not represented in NCBI dbSNP. It is located in intron 20 of *FASN* and its flanking sequence is GCCCGCCCC AGCCCCAGCC CAGTGCCCC ACCCAAGTAT GCCGCACTCA [C/T] CCAGCCTGTC CCCACAGTGG CTGACGTGGT GGTGAGCAGG TGGCTGAGGG

^c P_{trend} for codominant model

common polymorphisms of the three genes. We did not observe any significant association between polymorphisms of *FASN*, *MLXIPL* and *SREBF1* and an increased risk of cancer.

However, we did observe a statistically significant association between BMI and SNPs rs1140616 and FAS_A0866 for the *FASN* gene. When we stratified subjects according to age or menopausal status, the two *FASN* SNPs also showed differential association with BMI, i.e., rs1140616 resulted associated only in younger/pre-menopausal women, while FAS_A0866 was associated with BMI only in older/post-menopausal subjects. This finding is relevant because, although we did not find an association with the final end point, BC, we found an association with an intermediate end point, since BMI is considered to be a risk factor for BC [35]. It is possible that the relatively modest change of BMI associated to *FASN* SNPs is not sufficient to have an impact on BC risk. In this view, it would be interesting to include these SNPs together with those of other genes in multifactorial models to predict BMI and BC risk.

In order to take into account the large number of tests performed in this project, we calculated for each gene the number of effective independent variables, M_{eff} , by use of the SNP Spectral Decomposition approach [41, 42] (<http://gump.qimr.edu.au/general/daleN/SNPSPdsuperlite/>). The M_{eff} values for *FASN*, *MLXIPL* and *SREBF1* are 8, 5, and 2, respectively. The experiment-wide significance threshold is therefore $0.05/(8 + 5 + 2) = 0.0034$. If we use this threshold no association between SNPs and BMI remains significant.

The strengths of this study are a large sample size and use of a comprehensive tagging approach for examining *FASN*, *MLXIPL* and *SREBF1* common variation.

Although over 97% of the EPIC subjects are estimated to be of Caucasian origin, differences in allelic frequencies across Europe could in theory cause confounding by population stratification. However, we did not observe major variations in allele frequencies across countries for the SNP studied here. Moreover, cases and controls were systematically matched for EPIC recruitment center.

In conclusion, we have studied association of polymorphisms of three key genes in the lipogenesis pathway with BC risk and BMI within the EPIC study. We can confidently exclude a major role of these polymorphisms in BC risk, although two SNPs in *FASN* show an association with BMI that should be further evaluated in more comprehensive studies of polymorphisms and obesity.

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