Haplotype-Based Analysis of Common Variation in the *Acetyl-CoA Carboxylase* α Gene and Breast Cancer Risk: A Case-Control Study Nested within the European Prospective Investigation into Cancer and Nutrition

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Abstract

A key fatty acid synthesis enzyme, acetyl-CoA carboxylase α (ACC-α), has been shown to be highly expressed in human breast cancer and other tumor types and also to specifically interact with the protein coded by one of two major breast cancer susceptibility genes *BRCA1*. We used a comprehensive haplotype analysis to examine the contribution of the *ACC-α* common genetic variation (allele frequency >5%) to breast cancer in a case-control study (1,588 cases/2,600 controls) nested within the European Prospective Investigation into Cancer and Nutrition. We identified 21 haplotype-tagging polymorphisms efficiently capturing common variation within 325 kb of *ACC-α* and surrounding sequences using genotype data from the HapMap project and our resequencing data. We found an effect on overall risk of breast cancer in homozygous carriers of one common haplotype [odds ratio (OR), 1.74; 95% confidence interval (95% CI), 1.03-2.94]. When the data were subdivided by menopausal status, we found statistical evidence of heterogeneity for two other common haplotypes (*P* value for heterogeneity = 0.016 and 0.045). In premenopausal women, the carriers of these haplotypes, compared with noncarriers, had an altered risk of breast cancer (OR, 0.70; 95% CI, 0.53-0.92 and OR, 1.35; 95% CI, 1.04-1.76). These findings were not significant after adjustment for multiple testing and therefore should be considered as preliminary and evaluated in larger independent studies. However, they suggest a possible role of the *ACC-α* common sequence variants in susceptibility to breast cancer and encourage studies of other genes involved in fatty acid synthesis. (Cancer Epidemiol Biomarkers Prev 2007;16(3):409–15)

Introduction

A Western lifestyle, characterized by low rates of energy expenditure and a high-energy diet rich in saturated fats and refined carbohydrates, is associated with high incidence of breast cancer in women. This type of lifestyle also induces

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Note: O.M. Sinilnikova and J.D. McKay contributed equally to this work.

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storage of excess energy in the form of triglycerides, produced either from the dietary fatty acids or from those synthesized *de novo*. Furthermore, excess energy intake and obesity also cause insulin resistance, which is associated with elevated blood levels of glucose and insulin—factors that induce fatty acid synthesis in different tissues and that have been implicated in the etiology of various cancer types, including that of the breast (1-9).

A number of observations suggest that overexpression of lipogenic enzymes may promote breast tumor development (10). Several studies have shown high levels of key fatty acid synthesis enzymes that catalyze terminal steps in the *de novo* biosynthesis of long-chain fatty acids, fatty acid synthase (FASN) and acetyl-CoA carboxylase α (ACC-α), in human breast cancer (10-12) as well as in other tumor types (13-16). Inhibition of *FASN* or *ACC-α* has been shown to result in growth arrest and tumor cell death (17-24). A highly specific interaction has been shown between ACC-α and the protein coded by the breast cancer susceptibility gene *BRCA1*, which is abolished by disease-causing mutations in the *BRCA1* gene (25). Recent study showed that down-regulation of BRCA1 expression results in increased fatty acid synthesis and suggested that BRCA1 affects lipogenesis through binding to phosphorylated form of ACC-α (26). The above observations led us to hypothesize that genes involved in cellular fatty acid synthesis, particularly *ACC-α*, may be centrally implicated in mammary gland carcinogenesis and that certain alleles may confer breast cancer susceptibility.

The human *ACC-α* gene is located at 17q12, spans ~325 kb, and comprises at least 60 exons. It has a complex organization of regulatory regions, including four tissue-specific promoters (27, 28). In a previous study, we screened for sequence variations in the entire coding sequence, intron-exon junctions, 5′ untranslated region, 3′ untranslated region, and the promoter regions of the *ACC-α* gene in a panel of 49 breast cancer familial cases negative for *BRCA1* and *BRCA2* mutations (29). In this familial study, we identified two potentially disease-associated rare variants, suggesting that there may be mutations in the *ACC-α* gene but they are unlikely to be a major cause of high-risk breast cancer susceptibility. However, a possibility that common *ACC-α* alleles may influence breast cancer risk was suggested in our previous case-control study (453 sporadic breast cancer cases versus 469 controls), assessing the haplotypes formed by the polymorphisms detected in familial cases (29). It is important to note that none of these polymorphisms corresponded to a missense substitution, indicating that *ACC-α* common sequence variation takes place in non-coding regions.

Because deep intronic and promoter sequences were not screened in our previous study because of the very large size of the *ACC-α* genomic region, we sought to evaluate in greater depth the linkage disequilibrium (LD) and haplotype structure of *ACC-α*. In the present study, we have systematically estimated a set of single nucleotide polymorphisms (SNP) efficiently capturing common variation in the *ACC-α* gene, using HapMap data and SNP information obtained in our familial cases study (29). With this selected set of haplotype-tagging (ht)SNPs, we have examined the contribution of common genetic variation in the *ACC-α* gene to breast cancer risk, in a large case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC).

Materials and Methods

The EPIC Cohort. The EPIC cohort consists of ~370,000 women and 150,000 men, ages 35 to 69 years, recruited between 1992 and 1998 in 10 Western European countries. The vast majority (>97%) of subjects recruited in the EPIC cohort are of European (Caucasian) origin (30, 31). All EPIC study subjects provided anthropometric measurements, 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. A fully detailed description of the EPIC study has been published elsewhere (30, 31).

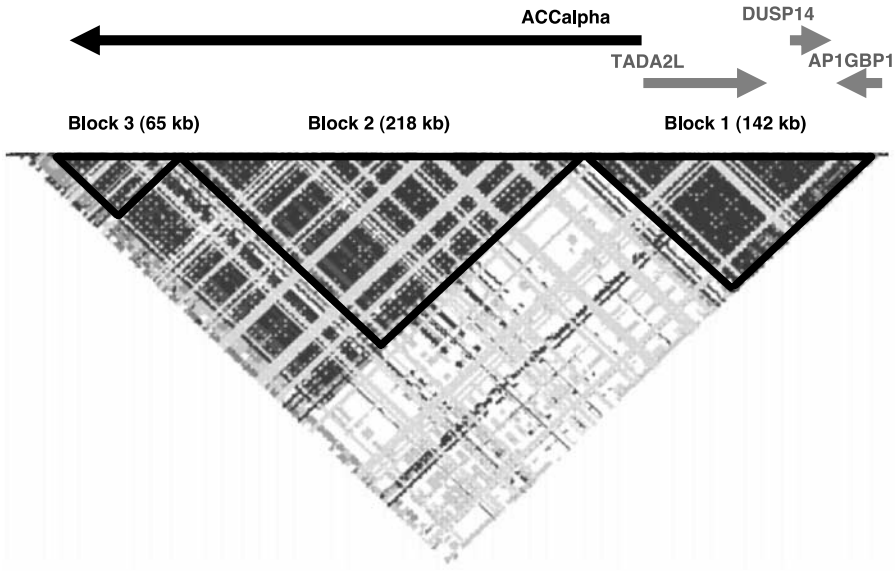

Figure 1. Genomic organization and LD across the *ACC-α* region. Data on 207 SNPs across the extended upstream and downstream sequences in addition to 325 kb of the *ACC-α* gene genotyped in CEPH trios were retrieved from the phase II HapMap database (http://www.hapmap.org, public data release of October 2005). Seven additional informative SNPs identified in our previous study (29) were genotyped in HapMap CEPH trios. Haplotype blocks were defined according to Gabriel et al. (32). Each square in the plot represents the pairwise LD relationships among markers. The intensity of the square color is proportional to the strength of the LD property for the marker pair. *TADA2L,* transcriptional adaptor 2 gene; *DUSP14,* dual specificity phosphatase 14 gene; *AP1GBP1,* AP1γ subunit binding protein 1 gene.

Selection of Case and Control Subjects. Case subjects were selected among women who developed breast cancer after blood collection. Cases with carinomas *in situ* were excluded from the analyses. Control subjects were selected randomly by incidence density sampling, matching the cases for center of recruitment, age at blood donation, duration of follow-up, menopausal status, and exogenous hormones use. As the controls had been matched to the cases in two study phases—a first (earlier) study phase with two controls per case and a later phase with only one control per case—the overall control-to-case ratio was ~1.7. A total of 1,610 invasive breast cancer cases and 2,628 controls were included in the present study. The study was approved by the ethical review boards of the IARC and of the collaborating institutions responsible for subject recruitment in each of the EPIC recruitment centers.

Determination of Haplotypes Blocks and Selection of htSNPs. Data on SNPs within the *ACC-α* locus genotyped in Centre d'Etude du Polymorphisme Humain (CEPH) trios (Utah residents with ancestry from Northern and Western Europe) were retrieved from the phase II HapMap database

Table 1. Association between *ACC-α* haplotypes and breast cancer risk by menopausal status

LD block	htSNP haplotype no	htSNP haplotype	Allelic frequency in controls*	Copies of haplotype	OR (95% CI)			*P* het†
					Entire sample (1,588 cases/2,600 controls)	Premenopausal women (373 cases/700 controls)	Postmenopausal women (1,072 cases/1664 controls)	
1	h1-1	12112	0.323	None	1	1	1	
				One	0.97 (0.84-1.11)	1.08 (0.82-1.41)	0.94 (0.79-1.11)	
				Two	1.11 (0.90-1.38)	1.34 (0.86-2.10)	1.04 (0.80-1.35)	0.321
1	h1-2	11111	0.197	None	1	1	1	
				One	1.02 (0.89-1.18)	1.00 (0.74-1.34)	1.08 (0.90-1.29)	
				Two	1.11 (0.79-1.55)	0.98 (0.49-1.95)	1.06 (0.70-1.61)	0.635
1	h1-3	21121	0.164	None	1	1	1	
				One	0.96 (0.83-1.11)	0.65 (0.48-0.87)	1.08 (0.90-1.29)	
				Two	0.84 (0.57-1.26)	1.36 (0.62-2.99)	0.73 (0.45-1.21)	0.016
1	h1-4	11211	0.121	None	1	1	1	
				One	0.99 (0.85-1.17)	1.01 (0.74-1.39)	0.94 (0.77-1.15)	
				Two	1.74 (1.03-2.94)	1.58 (0.65-3.83)	1.85 (0.85-4.00)	0.674
1	h1-5	11121	0.084	None	1	1	1	
				One	0.85 (0.71-1.03)	0.88 (0.62-1.25)	0.88 (0.70-1.11)	
				Two	1.21 (0.57-2.59)	0.23 (0.03-1.90)	2.12 (0.84-5.31)	0.642
2	h2-1	1111111111	0.329	None	1	1	1	
				One	1.08 (0.94-1.24)	1.39 (1.05-1.83)	0.98 (0.83-1.16)	
				Two	1.00 (0.80-1.24)	1.21 (0.77-1.90)	1.02 (0.78-1.33)	0.045
2	h2-2	1211111111	0.189	None	1	1	1	
				One	1.04 (0.90-1.20)	0.93 (0.70-1.23)	1.10 (0.92-1.31)	
				Two	0.95 (0.66-1.37)	0.66 (0.29-1.48)	1.00 (0.64-1.56)	0.247
2	h2-3	2112211122	0.115	None	1	1	1	
				One	1.06 (0.91-1.23)	1.01 (0.74-1.39)	1.07 (0.89-1.30)	
				Two	0.75 (0.40-1.41)	0.67 (0.20-2.19)	0.57 (0.25-1.31)	0.776
2	h2-4	2111211111	0.085	None	1	1	1	
				One	0.94 (0.79-1.13)	0.75 (0.52-1.08)	0.98 (0.79-1.22)	
				Two	0.67 (0.29-1.56)	0.62 (0.12-3.18)	0.80 (0.29-2.22)	0.204
2	h2-5	1121211221	0.066	None	1	1	1	
				One	1.02 (0.84-1.24)	1.00 (0.69-1.46)	0.94 (0.74-1.19)	
				Two	1.46 (0.54-3.95)	2.27 (0.37-13.9)	1.25 (0.33-4.72)	0.685
2	h2-6	1121211121	0.061	None	1	1	1	
				One	0.83 (0.67-1.03)	0.96 (0.64-1.45)	0.88 (0.68-1.15)	
				Two	1.68 (0.65-4.36)	8.52 (0.89-81.3)	0.56 (0.11-2.72)	0.511
2	h2-7	2112221122	0.052	None	1	1	1	
				One	0.86 (0.68-1.07)	0.73 (0.45-1.19)	0.85 (0.66-1.11)	
				Two	0.60 (0.16-2.30)	3.62 (0.33-40.4)	0.21 (0.03-1.76)	0.776
2	h2-8	1111212111	0.050	None	1	1	1	
				One	1.10 (0.89-1.36)	1.07 (0.70-1.64)	1.17 (0.90-1.52)	
				Two	1.72 (0.69-4.29)	2.12 (0.29-15.2)	1.58 (0.52-4.77)	0.771
3	h3-1	111111	0.439	None	1	1	1.00	
				One	1.02 (0.88-1.18)	1.07 (0.80-1.43)	0.99 (0.83-1.19)	
				Two	0.97 (0.80-1.17)	0.84 (0.57-1.23)	1.09 (0.87-1.37)	0.924
3	h3-2	212111	0.163	None	1	1	1.00	
				One	0.97 (0.85-1.12)	0.97 (0.73-1.29)	0.96 (0.81-1.15)	
				Two	0.70 (0.44-1.10)	0.50 (0.18-1.40)	0.73 (0.42-1.27)	0.946
3	h3-3	211121	0.155	None	1	1	1.00	
				One	1.03 (0.89-1.20)	1.02 (0.76-1.38)	1.01 (0.84-1.22)	
				Two	0.83 (0.54-1.30)	1.09 (0.51-2.33)	0.59 (0.32-1.08)	0.758
3	h3-4	111211	0.119	None	1	1	1.00	
				One	1.07 (0.91-1.25)	1.21 (0.88-1.66)	1.04 (0.85-1.26)	
				Two	1.16 (0.65-2.09)	2.15 (0.75-6.17)	1.06 (0.51-2.20)	0.321
3	h3-5	121111	0.051	None	1	1	1.00	
				One	1.12 (0.91-1.39)	1.02 (0.66-1.57)	1.22 (0.94-1.59)	
				Two	1.71 (0.68-4.26)	2.01 (0.28-14.3)	1.60 (0.53-4.84)	0.516

NOTE: Block 1 htSNPs: rs1714987, 32840588T>C, rs7211875, rs712039, rs2158235 (for the second SNP: position on chromosome 17, human genome assembly March 2006). Block 2 htSNPs: rs725038, rs11655013, rs2305098, rs2305097, rs11659129, rs3848462, rs9330251, rs2302803, 32731494G>T, 32770917T>C (for the last two SNPs: position on chromosome 17, human genome assembly March 2006). Block 3 htSNPs: rs1266180, rs2285655, rs3815059, rs17573357, rs2017571, rs2411161.

*Haplotypes observed with frequency >5% among controls.

†*P* value for heterogeneity of effect between subgroups of premenopausal and postmenopausal women under codominant model.

Table 2. Interaction between the effect of *ACC-α* haplotypes and BMI on breast cancer risk

LD block	htSNP haplotype no.	htSNP haplotype	Allelic frequency in controls*	Entire sample (1,564 cases/2,580 controls)		
				OR (95% CI) BMI <25 kg/m^2	OR (95% CI) BMI >25 kg/m^2	*P* inter †
1	h1-1	12112	0.323	1.05 (0.91-1.21)	1.00 (0.88-1.14)	0.61
1	h1-2	11111	0.197	0.98 (0.83-1.15)	1.11 (0.94-1.30)	0.302
1	h1-3	21121	0.164	1.06 (0.89-1.26)	0.85 (0.72-1.01)	0.083
1	h1-4	11211	0.121	1.08 (0.88-1.32)	1.07 (0.88-1.30)	0.962
1	h1-5	11121	0.084	0.80 (0.62-1.03)	0.97 (0.77-1.22)	0.269
2	h2-1	1111111111	0.329	0.92 (0.80-1.06)	1.16 (1.01-1.32)	0.022
2	h2-2	1211111111	0.189	1.15 (0.97-1.37)	0.90 (0.77-1.07)	0.05
2	h2-3	2112211122	0.115	1.08 (0.89-1.32)	0.96 (0.78-1.18)	0.406
2	h2-4	2111211111	0.085	0.91 (0.71-1.16)	0.93 (0.74-1.16)	0.914
2	h2-5	1121211221	0.066	1.10 (0.85-1.44)	0.99 (0.77-1.27)	0.552
2	h2-6	1121211121	0.061	0.86 (0.64-1.17)	0.89 (0.69-1.15)	0.879
2	h2-7	2112221122	0.052	0.85 (0.63-1.15)	0.84 (0.62-1.13)	0.95
2	h2-8	1111212111	0.050	1.13 (0.85-1.51)	1.14 (0.87-1.49)	0.97
3	h3-1	111111	0.439	0.98 (0.86-1.13)	1.00 (0.88-1.13)	0.888
3	h3-2	212111	0.163	0.99 (0.83-1.18)	0.89 (0.75-1.06)	0.418
3	h3-3	211121	0.155	1.08 (0.90-1.30)	0.91 (0.77-1.09)	0.187
3	h3-4	111211	0.119	0.96 (0.78-1.17)	1.20 (0.98-1.46)	0.115
3	h3-5	121111	0.051	1.11 (0.83-1.48)	1.19 (0.91-1.55)	0.725

NOTE: Block 1 htSNPs: rs1714987, 32840588T>C, rs7211875, rs712039, rs2158235 (for the second SNP: position on chromosome 17, human genome assembly March 2006). Block 2 htSNPs: rs725038, rs11655013, rs2305098, rs2305097, rs11659129, rs3848462, rs9330251, rs2302803, 32731494G>T, 32770917T>C (for the last two SNPs: position on chromosome 17, human genome assembly March 2006). Block 3 htSNPs: rs1266180, rs2285655, rs3815059, rs17573357, rs2017571, rs2411161.
*Haplotypes observed with frequency >5% among controls.
† *P* value for multiplicative interaction between the effects of *ACC-α* genetic variation and BMI on breast cancer risk under codominant model.

(public data release of October 2005).[32] Seven additional informative SNPs identified in our original study (29) were genotyped in HapMap CEPH trios. Haplotype blocks, defined according to Gabriel et al. (32), were determined using the program ''Haploview'' (33). HtSNPs, defining haplotypes with allele frequencies >5%, were selected using the ''tagSNPs'' program described by Stram et al. (34).

Genotyping. DNA extraction and SNPs genotyping were done as described by Canzian et al. (35). Briefly, genotyping of the selected htSNPs was done using the TaqMan technique on the ABI PRISM 7900 sequence detection system (Applied Biosystems, France). PCR was carried out in 5 μL using 10 ng of DNA template, TaqMan universal PCR master mix (Applied Biosystems or Eurogentech, France), SNP-specific primers, and probes designed by Applied Biosystems or Proligo Assay-by-Design services. Sequences of PCR primers/probes and PCR conditions are available on request.

Statistical Analysis. For each SNP, deviation of genotype frequencies from the Hardy-Weinberg equilibrium was assessed in the controls by χ^2 test.

Individuals' expected numbers of copies (''dosages'') of each haplotype, given genotypes of each of the htSNPs, were estimated using the tagSNPs program by Stram et al. (34). Individuals' haplotype dosage values were related to breast cancer risk through conditional logistic regression models, assuming dominant, recessive, and codominant models of inheritance. In these analyses, each haplotype was compared with all other haplotypes as the reference in calculating the odds ratios (OR). A global test of association for each of the haplotype blocks was conducted using the likelihood-ratio test based on conditional logistic regression.

Possible heterogeneity of effect between premenopausal and postmenopausal women was tested using a χ^2 test. To assess whether body mass index (BMI) modifies the association of breast cancer risk with the *ACC-α* haplotypes, we calculated risk stratum-specific ORs (BMI <25 and >25 kg/m^2) and tested for departures from a multiplicative interaction model.

[32] http://www.hapmap.org

Significant *P* values were adjusted for multiple hypotheses testing by permutation testing (36). A method of false-positive response rate probabilities has also been used to account for the multiple comparisons (37). For the false-positive response rate probabilities calculation, a prior probability of true association of <0.01 and a threshold for noteworthiness of <0.2 were used.

Results

The HapMap project has genotyped 207 informative SNPs across the extended upstream and downstream sequences in addition to 325 kb of the *ACC-α* gene, and we augmented this genotyping with seven informative SNPs from our original study (29). Using the criteria of Gabriel et al. (32), we defined three haplotype blocks in this region. There was significant correlation between blocks 2 and 3, which under a more relaxed haplotype block definition are likely to be a single block. Blocks 2 (218 kb) and 3 (65 kb) were found to span almost the entire *ACC-α* gene from intron 1 through 3.8 kb downstream of the last exon (Fig. 1). Block 1 (142 kb) spanned the *ACC-α* 5′ extremity (5′ half of the intron 1, exon 1, and promoter PI) and three neighbor genes, *TADA2L* (transcriptional adaptor 2), *DUSP14* (dual specificity phosphatase 14), and 3′ portion of the *AP1GBP1* gene (AP1γ subunit binding protein 1; Fig. 1). Blocks 1, 2, and 3 contained 67, 81, and 35 HapMap SNPs (average spacing ~ 2.4 kb), respectively, with minor allele frequency >5% estimated in CEPH trios. Estimation of the smallest set of htSNPs predicting common haplotypes (frequency >5%) using the tagSNPs program (34) resulted in selection of 21 SNPs (Table 1). The squared correlation R^2_h, reflecting the reliability of haplotype assignment (34), was above 0.80 for each of the three haplotype blocks. We used the htSNPs to construct the haplotypes across the three blocks and then analyzed the effects of common haplotypes (frequency >5%) within each block on breast cancer risk. Haplotype dosages were determined for 1,588 cases and 2,600 matched controls. Table 1 presents the haplotype associations for *ACC-α* and breast cancer risk in the entire study sample and by menopausal status. There were no significant global haplotype effects in any of the three blocks:

Table 2. Interaction between the effect of *ACC-α* haplotypes and BMI on breast cancer risk (Cont'd)

Premenopausal women (372 cases/700 controls)			Postmenopausal women (1,049 cases/1,644 controls)		
OR (95% CI) BMI <25 kg/m^2	OR (95% CI) BMI >25 kg/m^2	*P* inter[§]	OR (95% CI) BMI <25 kg/m^2	OR (95% CI) BMI >25 kg/m^2	*P* inter[§]
1.13 (0.87-1.46)	1.12 (0.82-1.53)	0.962	1.04 (0.86-1.25)	0.96 (0.83-1.12)	0.538
0.91 (0.68-1.22)	1.17 (0.79-1.73)	0.299	1.08 (0.87-1.35)	1.06 (0.87-1.28)	0.868
0.91 (0.67-1.24)	0.57 (0.37-0.89)	0.088	1.04 (0.83-1.31)	0.96 (0.79-1.17)	0.616
1.11 (0.78-1.59)	1.09 (0.73-1.63)	0.944	0.99 (0.75-1.31)	1.05 (0.83-1.33)	0.749
0.79 (0.52-1.19)	0.86 (0.50-1.50)	0.792	0.83 (0.59-1.16)	1.05 (0.80-1.36)	0.277
0.99 (0.77-1.28)	1.53 (1.13-2.07)	0.031	0.92 (0.76-1.10)	1.09 (0.93-1.28)	0.160
1.05 (0.77-1.43)	0.69 (0.47-1.02)	0.100	1.23 (0.98-1.53)	0.95 (0.78-1.15)	0.09
0.94 (0.65-1.37)	0.99 (0.63-1.53)	0.880	1.10 (0.85-1.43)	0.94 (0.74-1.20)	0.393
0.79 (0.50-1.23)	0.75 (0.45-1.23)	0.872	0.88 (0.64-1.21)	1.01 (0.77-1.31)	0.536
1.07 (0.69-1.66)	1.04 (0.57-1.91)	0.948	1.06 (0.73-1.53)	0.88 (0.66-1.18)	0.457
1.11 (0.67-1.83)	1.09 (0.64-1.87)	0.967	0.82 (0.54-1.25)	0.88 (0.65-1.20)	0.798
1.03 (0.56-1.90)	0.66 (0.32-1.32)	0.332	0.77 (0.53-1.12)	0.84 (0.60-1.19)	0.719
1.13 (0.68-1.89)	1.10 (0.59-2.06)	0.951	1.15 (0.79-1.68)	1.21 (0.88-1.67)	0.847
0.89 (0.69-1.14)	1.01 (0.76-1.33)	0.519	1.07 (0.90-1.28)	1.02 (0.88-1.19)	0.689
0.94 (0.67-1.31)	0.85 (0.58-1.26)	0.727	0.96 (0.76-1.22)	0.91 (0.75-1.12)	0.745
1.08 (0.78-1.48)	0.94 (0.64-1.40)	0.600	1.01 (0.78-1.30)	0.88 (0.71-1.08)	0.401
1.21 (0.85-1.72)	1.33 (0.84-2.11)	0.743	0.89 (0.68-1.17)	1.16 (0.92-1.45)	0.150
1.01 (0.60-1.71)	1.17 (0.63-2.16)	0.738	1.17 (0.81-1.70)	1.27 (0.92-1.74)	0.757

block 1 (P = 0.62), block 2 (P = 0.36), and block 3 (P = 0.69). One of the haplotypes in block 1 showed significant association with breast cancer, fitting a recessive genetic model. Homozygous carriers of haplotype h1-4 were at increased risk of breast cancer [OR, 1.74; 95% confidence interval (95% CI), 1.03-2.94; P = 0.038].

When the data were stratified based on menopausal status, we found statistical evidence of heterogeneity for one haplotype in block 1 (h1-3, P value for heterogeneity between premenopausal and postmenopausal women = 0.016) and one haplotype in block 2 (h2-1, P value for heterogeneity = 0.045; Table 1). Compared with noncarriers, the premenopausal heterozygotes of the haplotype h1-3 had an OR for reduction of breast cancer risk of 0.65 (95% CI, 0.48-0.87), whereas the h1-3 homozygotes had an OR of 1.36 (95% CI, 0.62-2.99) although the number of homozygotes was small (Table 1). The protective effect of the haplotype h1-3 in premenopausal women is compatible with dominant model, with an OR of 0.70 (95% CI, 0.53-0.92) for h1-3 heterozygous and homozygous carriers compared with noncarriers. An elevated risk of breast cancer was observed in heterozygous (OR, 1.39; 95% CI, 1.05-1.83) and homozygous carriers (OR, 1.21; 95% CI, 0.77-1.90) of the most prevalent (32.9%) haplotype h2-1 in block 2. This association seems to fit a dominant model (h2-1 heterozygous and homozygous carriers versus noncarriers: OR, 1.35; 95% CI, 1.04-1.76); however, a codominant model cannot be rejected.

We also tested for statistical interaction between *ACC-α* genotypes and BMI. The subjects were dichotomized into two groups with BMI less and more than 25 kg/m^2. The only *ACC-α* haplotype showing a significant interaction with BMI of its effect on breast cancer risk was the haplotype h2-1 (P = 0.02; Table 2). An increased breast cancer risk associated with this haplotype was observed in the higher BMI group (OR, 1.16; 95% CI, 1.01-1.32). A test for interaction with BMI was also significant in premenopausal women (P = 0.031), the haplotype h2-1 being associated with breast cancer in overweight and obese (OR, 1.53; 95% CI, 1.13-2.07) but not in lean individuals (OR, 0.99; 95% CI, 0.77-1.28).

No association was observed between haplotypes in block 3 and breast cancer risk.We attempted to replicate breast cancer risk associations of common *ACC-α* alleles suggested in our previous study that was carried out on breast cancer cases and control subjects from France (29). The frequencies of the *ACC-α* haplotypes formed by the four SNPs used in this study (5′ untranslated region−86T>C; PIII−724G>T; rs2302803; rs2252757) were estimated in the EPIC sample. There was no evidence for association with breast cancer.

Discussion

We postulated that the *ACC-α* gene might play an important role in development of breast cancer given its key function in lipogenesis whose up-regulation is a common feature of many cancers and also in view of specific interaction between ACC-α and BRCA1 (25). We tested in a large-scale association study, nested within the EPIC cohort, the involvement in breast cancer risk of common alleles of *ACC-α*, which might be related to variations in its activity. The strengths of this study are a large size of the sample drawn from the prospective cohort and application of a comprehensive approach for examining *ACC-α* common variation. It is likely that common variation in the *ACC-α* region could be well captured given the strong LD within each of the three blocks and across blocks 2 and 3, as well as due to a dense SNP coverage of the *ACC-α* locus.

Our previous study in a sample of modest size (453 breast cancer cases and 469 controls) observed significant associations of breast cancer risk with several common *ACC-α* haplotypes defined by four SNPs (29). Although the present study was much larger, with a statistical power >0.90, at a significance level of 0.05, to detect a relative risk of 1.5 for a dominant allele with a frequency of ~5%, or for a recessive allele with a frequency of ~20%, we were unable to replicate the initial findings. It seems unlikely that our previous study result was caused by hidden population stratification, as simulation studies have shown that in ethnically highly homogeneous populations, such as Western Europeans, biases due to population stratification are very unlikely to occur (38, 39). A further difference is that the previous study was strongly enriched for the early-onset breast cancer cases diagnosed before the age 45 years (245 of 453 cases), whereas in the EPIC sample there were only 123 of such cases.

Three positive although not highly statistically significant associations were observed in the present study. The haplotype h1-4 in the LD block 1 showed overall recessive association with breast cancer (OR, 1.74; 95% CI, 1.03-2.94). This LD block encompasses 5′ extremity of the *ACC-α* gene, *TADA2L* gene, and two other genes. The haplotype h1-4 holds

almost exclusively the rare allele of the tagging SNP rs7211875 corresponding to a Pro6Ser missense substitution in the *TADA2L* gene. Seven close proxies of this SNP ($r^2 > 0.8$) are available in the HapMap data. Because the intergenic region between *ACC-α* and *TADA2L* has been shown to function as a shared bidirectional promoter that operates to coregulate expression of both genes (40), it is possible that certain haplotype h1-4 polymorphisms, even located outside of *ACC-α*, may influence its expression.

The other breast cancer associations were detected among the subset of premenopausal women. A dominant protection effect was detected for the haplotype h1-3 in the LD block 1 (OR, 0.70; 95% CI, 0.53-0.92). The SNP rs1714987 residing uniquely on this haplotype tags 18 other SNPs ($r^2 > 0.8$). One of these proxies, rs829163, located in intron 1 of *ACC-α*, 326 nucleotides downstream of exon 1, might create an acceptor splice site according to estimation of the SpliceSiteFinder program[33] and result in activation of a pseudoexon splicing.

We also found evidence of a dominant risk effect in premenopausal women of the haplotype h2-1 in the LD block 2 spanning major part of the *ACC-α* genomic region (218 kb). No specific SNP residing uniquely on this haplotype could be identified. The effect of this haplotype was found to differ by BMI in the whole study sample and particularly in premenopausal patients. Premenopausal carriers of the haplotype h2-1 with BMI >25 kg/m^2 were at increased risk of breast cancer (OR, 1.53; 95% CI, 1.13-2.07), whereas carriers with BMI <25 kg/m^2 were not. Given a role of *ACC-α* in fatty acid synthesis, this finding does not seem unexpected, although data on association between breast cancer and BMI in premenopausal women are controversial (41-43). Although the *ACC-α* haplotype h2-1 is likely to be associated with modest increase of breast cancer risk, its overall health effect could be substantial due to the high frequency of this allele in general population.

One problem with studies of large genes like *ACC-α* is that the many haplotypes contained in the genome region mean that multiple testing is inherent in the analysis. As a result, we expect a significant number of false-positive associations. Indeed, using permutation test (36) or correction by false-positive response rate probabilities (37), none of the tests here remained significant, indicating that our results may be the result of statistical noise.

The findings of our survey should be considered as preliminary and evaluated in larger independent studies. Further investigation would be needed to identify the causal variant(s) carried by risk haplotypes and to understand their functional relevance.

[33] http://www.genet.sickkids.on.ca/~ali/splicesitefinder.html

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