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Association of lifestyle and demographic factors with estrogenic and glucocorticogenic activity in Mexican American women

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Abstract

Breast cancer risk is higher in US-born than in foreign-born Hispanics/Latinas and also increases with greater length of US residency. It is only partially known what factors contribute to these patterns of risk. To gain new insights, we tested the association between lifestyle and demographic variables and breast cancer status, with measures of estrogenic (E) and glucocorticogenic (G) activity in Mexican American women. We used Chemical-Activated LUciferase gene eXpression assays to measure E and G activity in total plasma from 90 Mexican American women, without a history of breast cancer at the time of recruitment, from the San Francisco Bay Area Breast Cancer Study. We tested associations of nativity, lifestyle and sociodemographic factors with E and G activity using linear regression models. We did not find a statistically significant difference in E or G activity by nativity. However, in multivariable models, E activity was associated with Indigenous American ancestry (19% decrease in E activity per 10% increase in ancestry, $P = 0.014$) and with length of US residency (28% increase in E activity for every 10 years, $P = 0.035$). G activity was associated with breast cancer status (women who have developed breast cancer since recruitment into the study had 21% lower G activity than those who have not, $P = 0.054$) and alcohol intake (drinkers had 25% higher G activity than non-drinkers, $P = 0.015$). These associations suggest that previously reported breast cancer risk factors such as genetic ancestry and alcohol intake might in part be associated with breast cancer risk through mechanisms linked to the endocrine system.

Introduction

Breast cancer risk in US Latina women, although lower than that of non-Latina Whites (1), is higher in those born in the USA, and risk increases with younger age at migration (2). Age-adjusted incidence rates for the period 1988–2004 showed 38% higher rates for US-born than for foreign-born Latinas (3). It is only partially known what factors contribute to these patterns of increasing risk (2,3).

Previous studies have attempted to explain the changes in breast cancer incidence among Latina immigrants using measures of exposure obtained through questionnaires or through record linkage to census data to evaluate the effect of socioeconomic status (SES) or neighborhood effects (2,3). However, by themselves, these results are limited because they are bound to provide information about exposures that have already

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Abbreviations

BMI	body mass index
E	estrogenic
ER	estrogen receptor
FBS	fetal bovine serum
G	glucocorticogenic
GR	glucocorticoid receptor
HT	hormone therapy
IQR	interquartile range
OC	oral contraceptive
RLU	relative light unit
SES	socioeconomic status

been associated with breast cancer risk, or because they do not tell us much about the possible precursors and the biological effects underlying the associations. Furthermore, it is known that individuals with similar reported exposures are not equally susceptible to disease, due to inter-individual variation in the metabolism of endogenous and exogenous compounds (4,5).

Conducting analysis of endocrine disruptors by measuring elevated or reduced hormone activity in plasma is a novel way to understand the differences in breast cancer risk between Latina women born in the USA compared with foreign-born Latinas. Enzyme activation by exposure to hormone receptor binding compounds can lead to increased hormone catabolism and compromise hormone signaling (6). Breast cancer risk has been directly linked to hormone receptor disruptors in animal models (7,8) and in occupational exposure studies (9,10). There is also evidence linking endocrine disruptors to breast cancer risk through regulation of microRNAs' expression (11), as well as through their involvement in the formation of reactive electrophiles such as reactive oxygen species and subsequent DNA adduct formation (12).

Cell-based reporter bioassays have been commonly used to identify estrogenic (E) compounds present in the environment (13–16), but few studies have used them to test the association between overall E activity in human blood and breast cancer risk (17–19), as was originally proposed by Brouwers *et al.* (20). An analysis conducted in samples collected prospectively from the Singapore Chinese Health Study tested the associations between levels of estrogens and estrogen receptor (ER)-mediated bioactivity and breast cancer risk among postmenopausal women and found results suggesting that factors other than estrone and estradiol may activate ER-mediated signaling pathways to increase breast cancer risk (19). There is extensive evidence for the role of estrogens in breast cancer risk and prognosis. Selective estrogen receptor modulators, such as tamoxifen and raloxifene, as well as aromatase inhibitors, are cornerstones of breast cancer treatment and have been shown in randomized trials to prevent breast cancer, particularly ER-positive disease (21–24). Epidemiologic studies have documented about a 2-fold higher risk of breast cancer in postmenopausal women in the top versus bottom 20–25% of plasma estradiol, estrone or estrone sulfate levels (25,26). Estrogens affect breast tissue largely through binding ER, which in turn leads to expression of ER target genes (27). However, multiple other compounds can also bind ER and either activate or suppress downstream signaling, including metals (e.g. cadmium), chemicals for industrial or household use (e.g. bisphenol A, parabens and phthalates), natural food components (e.g. isoflavones) and endogenous compounds (e.g. 27-hydroxycholesterol and estrogen metabolites) (27–32).

Exposure to endogenous and exogenous glucocorticoid receptor (GR) modulators is also likely to contribute to breast cancer development. Glucocorticoids are adrenocortical steroid hormones involved in several physiological and cellular processes, including cell differentiation, metabolism and programmed cell death by interacting with the GR (33). Reduced expression of the GR gene was observed in a panel of human liver, lung, prostate, colon and breast cancers and found to play an important role in promoting accurate chromosome segregation during mitosis, which highlights its role as a tumor suppressor (34). In addition, GR expression in breast cancer tissue has been associated with smaller tumor size and lower grade (35). In addition, glucocorticogenic (G) activity might reflect cortisol levels, which, given their link to stress (36), could be particularly relevant to this population of immigrant women.

In the present study, we used cell-based assays (37,38) to measure overall E and G activity in plasma of 90 Mexican American women who participated as controls in the San Francisco Bay Area Breast Cancer Study (SFBCS), a population-based case–control study of women aged 35–79 years. The specific goal of our study was to test if nativity (ref. US-born) and other breast cancer risk factors were associated with E and G activity in total plasma.

Materials and methods

Study samples

The San Francisco Bay Area Breast Cancer Study (SFBCS), described elsewhere (2,39), is a multiethnic population-based case–control study of breast cancer initiated in 1995, and with biospecimen collection added for cases diagnosed between 1 April 1997 and 30 April 2002 and matching controls. Briefly, participating women aged 35–79 years resided in the San Francisco Bay Area when diagnosed with a first primary histologically confirmed invasive breast cancer between April 1995 and April 2002. Controls identified by random-digit dialing were frequency matched to cases based on race/ethnicity and the expected 5 year age distribution of cases. Trained interviewers administered a structured questionnaire in English or Spanish at a home visit and took anthropometric measurements. Trained phlebotomists collected a fasting blood sample. Since for some women, the blood was collected a few years after recruitment into the study, a phlebotomy questionnaire was administered at the time of blood draw to update some key variables.

For the present study, 90 women were selected from the set of 603 Latina controls with stored plasma if they had developed breast cancer since the time of blood collection or if they remained free of breast cancer and were of Mexican origin. Through linkage with the California Cancer Registry in 2013, 15 Latina women were identified who developed breast cancer after blood collection. The remaining 75 women were randomly selected within subsets according to age at migration to the USA if foreign-born (balancing the number of younger and older age at migration) and menopausal status (balancing the number of premenopausal and postmenopausal women within each demographic category). The final sample included 60 foreign-born women (8 cases and 52 controls) and 30 US-born women (7 cases and 23 controls) (Supplementary Figure S1). Overall, 33 women were premenopausal and 57 were postmenopausal. Since we included all Latina women who developed breast cancer after blood collection, some of them were not of Mexican origin. Of the 15 cases, one was from Colombia, one from Puerto Rico and two from Nicaragua. Given that 97% of the women included in the present analysis were of Mexican origin, we refer to them generally as Mexican American throughout the manuscript, despite the fact that three women had different national origins.

Measures

The questionnaire for the main study obtained data on demographic background (education in years, country of birth, and age at migration if not US born) and known or suspected breast cancer risk factors. For the present analysis, we selected specific risk factors that we hypothesized could be associated with E or G activity at the time of blood draw, such as use of menopausal hormone therapy (HT), alcohol intake, body mass index (BMI)

or socioeconomic and sociocultural background. The phlebotomy questionnaire collected information on use of oral contraceptives (OCs), menopausal HT and alcohol (beer, wine, hard liquor) during the 6 months prior to the blood draw. OC and HT use at the time of blood draw was categorized as current, former, and never. For alcohol intake, grams per day were calculated. BMI was obtained by dividing measured weight (kg) by measured height (m) squared. Neighborhood level SES was estimated using a composite index including income, education, poverty, unemployment, occupation and housing and rental values, based on 2000 Census block-group data (40,41). Individual proportion of Indigenous American genetic ancestry was available for 86 of the 90 samples and was included in the analyses as a proxy for unmeasured sociocultural and/or biological differences. Details about the procedure for ancestry estimation have been previously reported (42). Briefly, we estimated global individual ancestry as the average locus-specific ancestry across 59 211 loci for each individual. Locus-specific ancestry estimates obtained with the HAPMIX software (43) were available from a previous genome-wide genotyping effort described elsewhere (42) and were estimated based on a three-way admixture model (African, European and Indigenous American components).

Luciferase assay overview

Chemically Activated LUciferase gene eXpression, or CALUX, bioassays are highly sensitive and reliable high throughput screenings used to measure biologically relevant exposures in various media including sediment (13), house dust (14), drinking water (15,16) and human blood (19,20). CALUX assays are used to identify receptor-mediated signaling pathways of gene expression by specific compounds such as estrogens and androgens (44,45). We relied on these bioassays to agnostically measure ER and GR agonist and antagonist activity profiles. Two breast cancer cell lines, T47D-Kbluc and MDA-Kb2, were stably transfected with a luciferase promoter gene construct to detect total E and G activity, respectively, for endogenous and exogenous compounds in human plasma. The process is initiated when compounds found in the plasma enter the cell and bind to the hormone receptor in the cytoplasm. If the compound that is bound to the receptor is an agonist, the agonist will cause the ligand bound receptor to translocate to the nucleus. The DNA binding domain of the receptor will then bind to its respective responsive element and transcription of the luciferase gene will take place. Upon cell lysis and substrate addition, promoter activity is measured by the amount of emitted light, referred to as relative light units (RLUs). Higher RLUs usually indicate agonists are binding the receptor and producing more luciferase protein. A decrease in RLUs can result if agonists are scarce or when an antagonist binds to the receptor and blocks nuclear translocation or inhibits transactivation, which leads to less production of the luciferase protein.

Cell culture and treatments for the ER bioassay

The methods used were similar to those previously described by Wilson *et al.* (46). The transfected breast cancer cell line T47-Kbluc was used to measure total endogenous and exogenous estrogens, such as 17-beta estradiol (E2), ethynyl estradiol and diethylstilbestrol in human plasma for both premenopausal and postmenopausal women in our study. Cells were cultured in phenol red Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) until 1 week prior to plasma addition. Phenol red media can act as a weak estrogen (30) and interfere with the bioassay. In order to remove all external sources of estrogen mimics, cells were treated with 'stripped' phenol red free DMEM supplemented with 10% charcoal–dextran stripped FBS for 1 week. After 1 week in stripped medium, cells were seeded at a density of 27 000 cells per well and 200 µl final volume in white, 96-well microtiter plates (Thermo Scientific, Waltham, MA) and incubated at 37°C for 24 hours. The 8 µl of plasma used per sample was diluted in phenol red free medium and then added in quadruplicate directly onto the cells. This step was followed by a final incubation period of 24 hours at 37°C before cell lysis with 5× passive lysis buffer (Promega, Madison, WI). Luciferase gene expression was measured using a microplate luminometer (Berthold Technologies, Centro XS3 LB 960 Instrument). Reporter activity was measured per well by the fluorescence emitted from the chemiluminescent reaction when the enzyme is activated by the substrate. Readings for each well were expressed in RLUs. RLUs from quadruplicate wells were averaged to get one measure per individual. The intra-assay and inter-assay coefficients of variation (CVs) of this assay are 7–23%. The minimum detection limit for E2 is 1.0 pM.

Cell culture and treatments for the GR bioassay

Similar to the ER bioassay, the GR bioassay also used methods previously described by Wilson *et al.* (47). However, the transfected breast cancer cell line MDA-Kb2 expresses two receptors. This cell line was used to measure androgens such as testosterone and dihydrotestosterone present in human plasma for premenopausal and postmenopausal women in our study. Because the androgen receptor and the GR have homologous DNA binding domains and act on the mouse mammary tumor virus promoter, this cell line also has the ability to measure glucocorticoids such as corticosterone and aldosterone using the GR. To distinguish between A and G activities, the use of a potent androgen receptor inhibitor, hydroxyflutamide (OHF), was needed. Cells were cultured in Leibovitz's-15 (L-15) medium with 10% FBS until 1 week before plasma addition. External sources of androgens and glucocorticoids were removed by treating the cells for 1 week with 'stripped' medium composed of L-15 medium and 10% charcoal–dextran serum. The cells were seeded at 27°000 cells per well at 200 µl final volume in white, 96-well plates and incubated at 37°C for 24 hours. The 165 µl of plasma used per female sample was diluted in stripped medium and then added in quadruplicate directly onto cells, both in the presence and absence of 0.5 µM OHF. After 24 hours of incubation at 37°C, the cells were lysed and the microplate was read using the luminometer to obtain RLU readings. To get G activity measurements, RLUs from quadruplicate wells in the presence of OHF were averaged to get one measure per individual. The intra-assay and inter-assay CVs of this assay are 5–10%. The minimum detection limit for cortisol is 4.4 nM.

Statistical analysis

Differences in means and proportions for all analyzed variables between US-born and foreign-born Mexican American women were assessed using two-sided *t*-tests and Fisher's exact tests, respectively. E and G activity measures were ln-transformed in order to approximate the normal distribution. We used linear regression models with the receptor activity as the outcome and demographic and lifestyle factors as predictors. We also included a batch or plate variable to account for experimental variation. To facilitate interpretation of regression results, we calculated the percent change in RLUs per unit change of predictor variables using the formula $[e^{\beta} - 1] * 100$. Analyses were conducted in R (48) or STATA (49).

The multivariable regression models included E or G activity (continuous, ln-transformed RLUs) as the outcome and height (continuous, in meters; m), BMI (continuous and ln-transformed, in kg/m^2), proportion of Indigenous American ancestry (continuous, 10% ancestry unit), level of education (less than high school versus high school or more), neighborhood SES (continuous score, based on first component of principal components analysis), age at blood draw (continuous, 10 years unit), alcohol intake at first interview (yes, no), nativity (US-born, foreign-born) and menopausal status (premenopausal, postmenopausal) as predictors. We also conducted two additional analyses, one stratifying by menopausal status, which included use of HT (never, former or current), and the other by nativity, in which we were able to assess the association between E or G activity and years of residence in the USA (continuous, 10 years unit). Models included all predictors (based on the *a priori* hypothesis that they could all influence E and G activity). We did not include use of OCs because only one woman in the study was taking them at the time of blood draw and was therefore excluded from the analysis. African genetic ancestry is relatively low in women of Mexican origin and, therefore, estimates obtained are less reliable than for the major components (Indigenous American and European). As a result, the present analyses only included Indigenous American ancestry (which is the complement of the European ancestry and therefore collinear). Seventeen women had discordant alcohol intake answers during the calendar year prior to selection into the parent study versus during the 6 months prior to the blood draw. All 17 reported to drink some alcohol in the first interview (ranging from half a drink per week to approximately two drinks a day) and no alcohol at blood draw. The E activity analysis included education level, but not SES as predictor, and the G activity analysis included SES but not education level. Since SES and education were highly correlated, for each model we kept the variable that had the largest effect on the adjusted R^2. The multivariable regression analyses excluded individuals with missing data on genetic ancestry (one case from Puerto Rico and three Mexican American cases) and education (three controls), a breastfeeding woman and a woman currently using OCs. The final

sample set included 11 breast cancer cases and 70 controls for E activity and 9 cases and 50 controls for G activity.

Ethical statement

All participants provided written informed consent and the study was approved by the Institutional Review Boards at the University of California San Francisco and the Cancer Prevention Institute of California.

Results

In the present study, we tested if first-generation Mexican immigrants had different E and/or G activity in plasma, as measured using a CALUX cell-based assay, compared with US-born women of Mexican origin, and if those levels were associated with other known breast cancer risk factors. Median E activity of transfected cells after addition of plasma was 2925 RLUs [interquartile range (IQR): 8226], and G was 178 232 RLUs (IQR: 72 207). Table 1 describes the levels of all considered variables by place of birth (US-born versus foreign-born). There were no differences in E or G activity, age at blood draw, height, BMI and menopausal status (the latter due to selection of similar number of postmenopausal and premenopausal women from the two migration groups during the study design). There was a statistically significant difference in the level of education and neighborhood SES, with US-born Latinas having higher levels for both variables, and suggestive differences in alcohol intake during the calendar year prior to selection into the study (higher intake among US-born

Table 1. Sample characteristics by migration status in Mexican American women (*N* = 90)

	US-born	Foreign-born	*P* value[a]
Estrogenic activity level (RLUs), median (IQR)	2403 (9833)	3437 (7000)	0.546
Glucocorticogenic activity level (RLUs), median (IQR)[b]	177 443 (73 473)	183 735 (68 784)	0.813
Age at blood draw (years), mean (SD)	54 (11)	53 (11)	0.624
% Indigenous American ancestry, mean (SD)	44 (12)	42 (14)	0.53
History of breast cancer, *n* (%)			
No	23 (77)	52 (87)	0.245
Yes	7 (23)	8 (13)	
Education, *n* (%)			
<High school	7 (23)	40 (67)	<0.001
High school+	21 (70)	15 (25)	
Unknown	2 (7)	5 (8)	
Neighborhood SES (statewide quintiles), *n* (%)			
1st quintile (lowest)	0	4 (7)	0.011
2nd quintile	2 (7)	19 (32)	
3rd quintile	10 (33)	16 (27)	
4th quintile	9 (30)	15 (25)	
5th quintile (highest)	7 (23)	4 (7)	
Unknown	2 (7)	2 (3)	
Menopausal status at blood draw[d], *n* (%)			
Premenopausal	11 (37)	21 (35)	1.000
Postmenopausal	19 (63)	38 (63)	
Unknown	0	1 (2)	
Height (cm), mean (SD)	157 (6)	156 (6)	0.632
Body mass index (kg/m^2), mean (SD)	29 (6)	30 (6)	0.358
OC use at blood draw[d], *n* (%)			
Never	8 (27)	26 (43)	0.157
Former	22 (73)	32 (53)	
Current	0 (0)	1 (2)	
Unknown	0	1 (2)	
HT use at blood draw[c,d], *n* (%)			
Never	8 (42)	21 (55)	0.186
Former	8 (42)	16 (42)	
Current	3 (16)	1 (3)	
Alcohol intake during calendar year before selection into parent study[d], *n* (%)			
None	16 (53)	44 (73)	0.086
Some	12 (40)	14 (23)	
Unknown	2 (7)	2 (3)	
Alcohol intake during the 6 months prior to blood draw[d], *n* (%)			0.154
None	25 (83)	56 (93)	
Some	5 (17)	4 (7)	
Unknown	0	0	

[a]We used *t*-test (for continuous variables) or Fisher's exact test (for categorical variables) to assess the significance of the difference in variable distribution between US-born and foreign-born women.

[b]Seven US-born and 20 foreign-born women did not have information on glucocorticogenic activity.

[c]Only among postmenopausal women.

[d]The participants answered two questionnaires, one at first interview and one at blood draw. The one at interview asked about behavior within the year prior to interview. The one at blood draw asked about behavior within the 6 previous months.

women), and menopausal HT (higher use in US-born women). A higher proportion of breast cancer cases were US-born.

Estrogenic activity

We tested the association between E activity of transfected cells after addition of plasma from 86 Mexican American women and multiple anthropometric, lifestyle and demographic factors using univariate and multivariable regression models. In univariable analyses, we found a strong positive association with age at blood draw, where for every 10 years increase in age, E activity decreased 50% ($P = 1 \times 10^{-12}$) (Supplementary Figure S2). Mean E activity level among postmenopausal women was 79% lower than that of premenopausal women ($P < 1 \times 10^{-16}$) (Supplementary Figure S3). Variation in age at blood draw and menopausal status explained ~40% of the variation in E activity (adjusted $R^2 = 0.43$).

We did not find an association between E activity level and nativity (US-born versus foreign-born) in univariate or multivariable models.

The multivariable model suggested a negative association between E activity and proportion of Indigenous American genetic ancestry, where for every 10% increase in ancestry there was a 19% decrease in E activity ($P = 0.014$) (Table 2). In analyses stratified by menopausal status, we did not observe any significant heterogeneity for the described associations, though *P* values increased due to the reduced sample size (Supplementary Table S1). When we stratified the analyses by nativity (US-born, *N* = 28 versus foreign-born, *N* = 57), we observed an important change in the Indigenous American ancestry coefficient, with a strong association among the foreign-born Mexicans (23% change in E activity, $P = 0.009$), but no association among US-born individuals (5% change in E activity, $P = 0.770$) (Table 3). In the model that included foreign-born individuals, we observed a positive association between E activity and years of residence in the USA (For every 10 years of US residence, there was a 28% increase in E activity, $P = 0.035$) (Table 3).

Glucocorticogenic activity

Glucocorticogenic (G) activity was only obtained for 60 of the 86 women due to lack of plasma availability for 26 women. We found no association between G activity, age at blood draw, menopausal status or nativity (Table 4). There was an inverse association with breast cancer status, suggesting that women who had developed breast cancer after recruitment into the study had 21% lower G activity than those who did not ($P = 0.054$). We

Table 2. Association between estrogenic activity, lifestyle and demographic factors in Mexican American women (*N* = 81)

	Univariate analysis			Multivariate analysis		
	Coefficient (95% CI)[a]	% Change in RLUs[b]	*P* value	Coefficient (95% CI)	% Change in RLUs[b]	*P* value
Nativity (ref. US-born)	0.12 (–0.46, 0.71)	13	0.672	–0.07 (–0.57, 0.43)	–7	0.781
Age at blood draw (per 10 years)	–0.69 (–0.89, –0.50)	–50	<0.001	–0.40 (–0.69, –0.12)	–33	0.007
Indigenous American ancestry (per 10%)	–0.16 (–0.37, 0.06)	–15	0.156	–0.21 (–0.37, –0.04)	–19	0.014
Breast cancer (ref. No)	–0.57 (–1.30, 0.16)	–43	0.122	0.13 (–0.50, 0.77)	14	0.68
Education (ref. <High school)	–0.02 (–0.60, 0.55)	–2	0.934	–0.15 (–0.63, 0.33)	–14	0.543
Postmenopausal (ref. Premenopausal)	–1.55 (–2.00, –1.09)	–79	<0.001	–1.17 (–1.79, –0.55)	–69	<0.001
Height (per 10 cm)	0.21 (–0.23, 0.65)	23	0.349	–0.19 (–0.56, 0.18)	–17	0.315
BMI (ln kg/m^2)	–0.04 (–0.19, 0.11)	–4	0.591	0.00 (–0.12, 0.11)	0	0.968
Alcohol intake (ref. None)[c]	0.10 (–0.52, 0.71)	11	0.759	0.04 (–0.44, 0.51)	4	0.877

CI, confidence interval.

[a]The coefficients and 95% CI are based on the ln-transformed RLUs.

[b]Percent change in RLUs (untransformed) per unit change in predictor was estimated using the formula [e^{β} – 1] * 100.

[c]Alcohol intake during calendar year before selection into parent study.

Table 3. Association between estrogenic activity, lifestyle and demographic factors by place of birth (*N* = 81)

	US-born women (*N* = 28)			Foreign-born women (*N* = 53)		
	Coefficient (95% CI)[a]	% Change in RLUs[b]	*P* value	Coefficient (95% CI)	% Change in RLUs[b]	*P* value
Age at blood draw (per 10 years)	–0.59 (–1.19, 0.03)	–45	0.06	–0.77 (–1.25, –0.28)	–54	0.003
Indigenous American ancestry (per 10%)	0.05 (–0.33, 0.44)	5	0.77	–0.26 (–0.46, –0.07)	–23	0.009
Breast cancer (ref. No)	0.62 (–0.58, 1.83)	86	0.291	–0.19 (–1.05, 0.67)	–17	0.659
Education (ref. ≤High school)	–0.58 (–1.54, 0.41)	–44	0.238	–0.07 (–0.69, 0.55)	–7	0.824
Years in the USA (per 10 years)				0.25 (0.02, 0.49)	28	0.035
Postmenopausal (ref. Premenopausal)	–1.27 (–2.51, 0.02)	–72	0.047	–0.75 (–1.54, 0.03)	–53	0.06
Height (per 10 cm)	–0.14 (–1.01, 0.73)	–13	0.735	–0.25 (–0.69, 0.19)	–22	0.254
BMI (ln kg/m^2)	–0.00 (–0.22, 0.22)	0	0.98	–0.08 (–0.24, 0.09)	–8	0.359
Alcohol intake (ref. None)[c]	0.29 (–0.60, 1.19)	34	0.504	–0.30 (–0.95, 0.34)	–26	0.346

[a]The coefficients and 95% CI are based on the ln-transformed RLUs.

[b]Percent change in RLUs (untransformed) per unit change in predictor was estimated using the formula [e^{β} – 1] * 100.

[c]Alcohol intake during calendar year before selection into parent study.

Table 4. Association between glucocorticogenic activity, lifestyle and demographic factors (*N* = 59)

	Univariate analysis			Multivariate analysis		
	Coefficient (95% CI)[a]	% Change in RLUs[b]	*P* value	Coefficient (95% CI)	% Change in RLUs	*P* value
Nativity (ref. US-born)	−0.4 (−0.20, 0.12)	−33	0.628	−0.05 (−0.22, 0.13)	−5	0.589
Age at blood draw (per 10 years)	0.04 (−0.03, 0.11)	4	0.238	0.03 (−0.06, 0.13)	3	0.468
Indigenous American ancestry (per 10%)	−0.03 (−0.09, 0.03)	−3	0.305	−0.03 (−0.09, 0.03)	−3	0.338
Breast cancer (ref. No)	−0.09 (−0.29, 0.11)	−9	0.359	−0.23 (−0.45, 0.00)	−21	0.054
Neighborhood SES (continuous score)	−0.07 (−0.17, 0.04)	−7	0.217	−0.09 (−0.20, 0.02)	−9	0.106
Postmenopausal (ref. Premenopausal)	0.15 (−0.01, 0.31)	16	0.073	0.16 (−0.06, 0.37)	17	0.157
Height (per 10 cm)	0.11 (−0.04, 0.26)	12	0.149	0.16 (0.01, 0.32)	17	0.037
BMI (ln kg/m^2)	−0.01 (−0.05, 0.03)	−1	0.604	0.01 (−0.04, 0.05)	1	0.781
Alcohol intake (ref. None)[c]	0.16 (−0.00, 0.33)	17	0.057	0.22 (0.05, 0.40)	25	0.015

[a]The coefficients and 95% CI are based on the ln-transformed RLUs.
[b]Percent change in RLUs (untransformed) per unit change in predictor was estimated using the formula [e^{β} − 1] * 100.
[c]Alcohol intake during calendar year before selection into parent study.

also observed a positive association with alcohol intake during the year prior to the first interview (compared with non-drinkers, women who reported drinking at least some alcohol had a 25% higher G activity, *P* = 0.015) and a positive association with height (per every 10 cm there was a 17% increase in G activity, *P* = 0.037) (Table IV). Stratified analyses did not suggest heterogeneity by menopausal status (Supplementary Table S2) or nativity (Supplementary Table S3) regarding these two variables. The association between G activity and alcohol intake was not observed when alcohol intake at blood draw instead of interview was included in the model (Supplementary Table S4). However, we found a statistically significant association between discordant status for the two alcohol variables and G activity (Supplementary Table S5).

Discussion

We presented the results of a semi-targeted analysis of E and G activity in plasma in Mexican American women from the San Francisco Bay Area. Our results suggest that E activity in plasma is associated with Indigenous American ancestry and length of US residence among foreign-born Mexican American women. We also observed an association between G activity and alcohol intake. Indigenous American ancestry has consistently been associated with breast cancer risk, with lower risk among women with high Indigenous American ancestry (50,51). A genome-wide association study in Latinas reported the existence of a single nucleotide polymorphism, only present in Indigenous American populations, and associated with protection against breast cancer (52). In addition to this protective variant, non-genetic factors are likely to contribute to the decreased breast cancer risk in highly Indigenous American women, given that genetic ancestry is correlated with sociodemographic, reproductive and lifestyle factors such as education, neighborhood SES, use of HT and alcohol intake (53).

Our results suggest that lower levels of E activity among women with high Indigenous American ancestry could partly contribute to the inverse association between Indigenous American ancestry and breast cancer risk. It is unclear from our results if the lower E activity is due to lower levels of endogenous estrogens or lower levels of endocrine disruptors, and further studies will need to be conducted to clarify these results. Targeted studies looking at the association between endogenous estrogens and breast cancer risk have already shown a positive relationship (25,26), and a previous study of E activity in Asian women strongly suggested increased activity among women who developed breast cancer, beyond the effect of endogenous estrogen levels (19). Analyses did not include variables such as age at menarche, number of live births and breastfeeding, which are important breast cancer risk factors related to variation in estrogen levels. We focused on factors that were likely to be acting on hormone levels near the time of sample extraction. Given the average age of women in the study, remote events such as age at menarche or breastfeeding were not thought as likely to be reflected in estrogen levels. In order to confirm our assumption, we ran a model that included these variables, which did not show any meaningful change in estimates compared with those in the model without those variables (data not shown). There were only four women in the study who reported using HT at the time of blood draw. In the model for postmenopausal women, it was clear that the difference in activity was between current users versus former or never users. To make sure that the current use of HT was not affecting the association between years of residence in the USA and E activity, we tested a model that included only the postmenopausal foreign-born women and information on hormone replacement therapy use, which confirmed that the association with years of residence in the USA was independent of HT (*P* value for years in the USA was slightly lower when including HT, *P* = 0.026).

The analysis of G activity, even though limited by a small sample size, also provided interesting results that warrant replication. Despite there being only nine breast cancer cases in the sample, we observed a negative association with G activity (the average G activity was lower among the women who developed breast cancer compared with those who did not). This is consistent with the observation that GR stimulation decreases the risk of relapse in breast tumors that are ER positive (54) due to cross talk between ER and GR (54,55). We also observed an association between G activity and alcohol intake as reported during the first interview (which for some of the individuals was 4 years before the time of blood draw). Due to low levels of alcohol intake among Mexican American women, we decided to compare individuals who responded that they do not drink at all to those who responded that they drink some alcohol. Women who reported drinking at least some alcohol had higher G activity than those

who responded that they never drank. Stress-induced glucocorticoid secretion triggers changes in gene expression through activation of the GR, which might alleviate immediate negative feelings associated with stress but lead to behavioral pathologies such as addiction, anxiety and depression (56). Studies have shown that inactivation of GR decreases motivation to take alcohol or other drugs (57,58). Our finding that G activity is higher among drinkers is consistent with these results and suggests the possibility that stress-induced activation of the GR might lead to increased levels of alcohol intake among some women in this overall low alcohol consumption group.

The study had some limitations. One limitation was that the data analyzed only included 11 women who had developed breast cancer. Ideally, we would have analyzed a larger number of cases to test if the associations observed between E or G activity and breast cancer risk factors mediated the association between those factors and breast cancer risk. Due to the small number of cases, we focused our analysis on the relationship between E and G activity and other factors that have been associated with breast cancer risk and were likely to be correlated with this activity. Another limitation was the relatively small overall sample size. However, we were able to discover some interesting associations that warrant replication and further investigation in future studies including a larger number of Latina women as well as women from other race/ethnicities. In addition, we lacked measures of endogenous estrogen, which would have allowed us to estimate what proportion of the variability in E activity might be due to differences in the level of estrogen-like compounds of exogenous origin versus differences in endogenous levels of estrogen. Finally, endogenous hormone levels as well as exposure to other receptor antagonists and agonists vary on a daily basis and therefore a measure of E and G activity obtained from a single plasma sample might not represent the average level of exposure for the individual. However, we believe that, at the population level, observed associations are informative and should be further explored, while absence of association cannot be taken as conclusive given that it is possible that for certain exposures the time at which the biospecimen was collected could be crucial.

In summary, we have investigated the levels of plasma estrogenic and glucocorticogenic activity in Mexican American women born in and outside the USA and tested their association with lifestyle, demographic and anthropometric breast cancer risk factors. Despite the null association with the main predictor (nativity), the cell-based measure of E and G activity reflected the expected differences by age at blood draw and menopausal status and also suggested previously unknown associations with genetic ancestry, years of US residence and alcohol intake. Future research will use cutting edge mass spectrometry-based technology to further identify the specific chemicals, and their precursors, that contribute to the observed associations and possibly to breast cancer risk. If modifiable, these agents could be targets of prevention programs, which would eventually reduce breast cancer incidence.

Supplementary material

Supplementary Tables 1–5 and Figures 1–3 can be found at http://carcin.oxfordjournals.org/

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