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Original Article

Genetic variants in hypothalamic-pituitary-adrenal axis genes and breast cancer risk in Caucasians and African Americans

Hongmei Nan[1,2], Joanne F Dorgan[3], Timothy R Rebbeck[4]

[1]Department of Epidemiology, Richard M Fairbanks School of Public Health, Indiana University, Indianapolis, IN, USA; [2]Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN, USA; [3]Department of Epidemiology and Public Health, Division of Cancer Epidemiology, University of Maryland School of Medicine, Baltimore, MD, USA; [4]Department of Biostatistics and Epidemiology, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

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Abstract: Elevated circulating levels of the adrenal androgen dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are associated with increased breast cancer risk in prospective studies. Genetic variants in hypothalamic-pituitary-adrenal (HPA) axis genes may contribute to these circulating hormone levels, and consequently to breast cancer risk. No previous studies have examined the effects of genetic variants in HPA axis genes on breast cancer risk. We evaluated the associations of 49 single nucleotide polymorphisms (SNPs) in five HPA axis genes (*NR3C1*, *NR3C2*, *CRH*, *CRHR1*, and *CRHBP*) with the risk of breast cancer in the Women's Insights and Shared Experiences (WISE) Study of Caucasians (346 cases and 442 controls), as well as African Americans (149 cases and 246 controls). Of the 49 SNPs evaluated, one showed a nominal significant association (*P* for trend < 0.05) with breast cancer risk among Caucasians, and another two among African Americans. The age-adjusted additive odds ratio (OR) (95% confidence interval (95% CI)) of the SNP rs11747190[A] in the *CRHBP* gene for the risk of breast cancer among Caucasian women was 1.45 (1.09-1.94). The age-adjusted additive ORs (95% CIs) of two SNPs (*CRHBP* rs1700688[T] and *CRHR1* rs17689471[C]) for the risk of breast cancer among African American women were 1.84 (1.13-2.98) and 2.48 (1.20-5.13), respectively. However, these SNPs did not show significant associations after correction for multiple testing. Our findings do not provide strong supportive evidence for the contribution of genetic variants in these HPA axis genes to the risk of developing breast cancer in either Caucasians or African Americans.

Keywords: Single nucleotide polymorphism, HPA axis genes, breast cancer

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is a complex set of direct influences and feedback interactions among the hypothalamus, the pituitary gland, and the adrenal glands. HPA axis plays a fundamental role in regulating hormonal, metabolic and immunologic response to stressors. Previous epidemiologic and histological studies have provided compelling evidence that the androgen, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), that are almost exclusively produced by the adrenals [1], play a role in breast cancer etiology. In our prospective study [2, 3], postmenopausal women in the highest quartiles of DHEA and DHEAS were at a significant 3-4 fold excess risk of breast cancer. Results were unchanged when restricted to women whose bloods were collected further in time from diagnosis, suggesting that elevated adrenal androgens were not due to preclinical disease. Adrenal androgens have been associated with breast cancer in numerous prospective studies with risk estimates for the highest vs. lowest categories ranging from approximately 2-4 [2-7]. In the pooled analysis of these studies, postmenopausal women with the highest levels of DHEA were at a significant 2-fold excess risk of developing breast cancer [8]. Notably, the magnitude of the excess risk was comparable to that observed for estradiol. DHEA from plasma is found at high levels in cancerous human breast tissue [9]. Moreover, DHEA exhibits estradiol-

like activity in aromatase transfected MCF-7 breast cancer cells [10], indicating that DHEA could potentially stimulate breast tumor growth via conversion to estrogens. This is supported by our finding that the association of DHEAS with breast cancer is attenuated after adjusting for estradiol [11]. Overall, studies suggest that DHEA/S increases breast cancer risk in postmenopausal women by acting as substrate for conversion to more active hormones like estradiol.

Heritability of adrenal DHEAS levels is estimated to be 0.58 [12], suggesting that genetics contributes importantly to circulating levels, and by extension to breast cancer risk. However, none of the previous studies have evaluated associations of genetic variants in HPA axis genes with breast cancer risk. In this study, we examined the associations of 49 single nucleotide polymorphisms (SNPs) in five HPA axis genes, including glucocorticoid receptor (*NR3C1*), mineralcorticoid receptor (*NR3C2*), corticotropin releasing hormone (*CRH*), corticotropin releasing hormone receptor 1 (*CRHR1*) and corticotropin releasing hormone binding protein (*CRHBP*), with the risk of breast cancer in the Women's Insights and Shared Experiences (WISE) study of Caucasians (346 cases and 442 controls), as well as African Americans (149 cases and 246 controls).

Materials and methods

Study population and data collection

The WISE study is a population-based retrospective case-control study. Incident primary breast cancer cases were identified through hospitals and the Pennsylvania State Cancer Registry, and frequency-matched controls were identified from the community using random digit dialing. The source population for this study was from the three counties of Philadelphia (Pennsylvania), Delaware (Pennsylvania), and Camden (New Jersey). Details of the study have been reported previously [13-15].

Potentially eligible cases were women residing in these counties at the time of diagnosis who were aged 50-79 years and newly diagnosed with breast cancer between July 1, 1999 and June 30, 2002. The cases were identified through active surveillance at hospitals in these counties. Pennsylvania Cancer Registry lists were reviewed quarterly to validate completeness of case ascertainment. Breast cancer diagnoses were validated by review of pathology reports and medical records. Breast cancer was confirmed if a pathology report was compatible with a first primary, invasive breast cancer. Controls were selected from the same geographic region as the cases and were frequency matched to the cases on race, age (in 5-year age groups) and calendar date of interview (within 3 months). Eligible controls had no history of breast cancer. Both cases and controls were required to live in a noninstitutional setting, to have a household telephone, to speak English, and to have no severe cognitive, language, or speech impairment.

Telephone interviews were used to collect data on demographic characteristics, anthropometry, family history of breast cancer, menstrual and menopausal history, reproductive history, medical history, oral contraceptive (OC) and hormone replacement therapy (HRT) use, smoking and alcohol ingestion. Participants collected buccal swabs according to standard directions and mailed them to the University of Pennsylvania. A total of 346 cases and 442 controls for Caucasians, as well as 149 cases and 246 controls for African Americans were included in this study.

Participants provided verbal informed consent for the interview and written informed consent for the buccal samples. The University of Pennsylvania Committee on Studies Involving Human Beings, the institutional review board at University of Maryland School of Medicine, and the institutional review boards of all the participating hospitals approved this study.

Laboratory assays

We selected six putative functional single nucleotide polymorphisms (SNPs) for two HPA axis genes, *NR3C1* (rs6190, rs6195, rs10052957, and rs41423247) and *NR3C2* (rs5522 and rs2070951). Using the International HapMap project, we identified SNPs that effectively cover the other three HPA axis genes of interest (*CRH*, *CRHR1*, and *CRHBP*). Some of these SNPs are in linkage disequilibrium; therefore, a more efficient set of tagging SNPs can be used to capture the same genetic variation [16]. Using Haploview program and a minimum

Table 1. Characteristics of breast cancer cases and controls in WISE case-control study

Characteristic	Caucasians		African Americans	
	Cases (n = 346)	Controls (n = 442)	Cases (n = 149)	Controls (n = 246)
Age (yrs, mean)	63.4	62.5	62.2	61.0
Body-mass index (kg/m^2, mean)	24.0	24.0	25.7	26.0
Age at menarche (yrs, mean)	12.5	12.7	12.8	12.7
Age at menopause (yrs, mean)	48.2	48.4	48.2	47.5
Age at first full term pregnancy among parous women (yrs, mean)	24.3	24.6	21.2	20.9
Number of full term pregnancies (%)				
0	19.9	11.5	16.8	6.9
1~2	32.7	39.6	43.0	41.5
≥ 3	47.4	48.9	40.3	51.6
Duration of breast feeding (%)				
Never	69.1	56.1	66.9	67.1
< 12 months	22.3	28.1	19.6	19.8
≥ 12 months	8.7	15.8	13.5	13.2
Menopausal status (%)				
Premenopausal	7.2	7.7	7.4	8.1
Postmenopausal	76.0	80.1	80.5	72.8
Induced (e.g., surgical)/unknown	16.8	12.2	12.1	19.1
Family history of breast cancer in 1st degree relative (%)				
Yes	18.8	19.0	18.1	11.4
No	81.2	81.0	81.9	88.6
Duration of combined estrogen and progestin (CHRT) use (%)				
Never/other HRT use	75.1	70.6	92.6	87.8
< 3 years	8.4	13.6	1.3	6.9
≥ 3 years	16.5	15.8	6.0	5.3
Duration of oral contraceptive (OC) use (%)				
Never	53.8	47.7	55.7	46.8
< 3 years	22.0	26.6	19.5	22.4
≥ 3 years	24.3	25.7	24.8	30.9

r^2 threshold of 0.8, we identified a set of 47 parsimonious tagging SNPs with minor allele frequency greater than 5% to capture genetic variation in each locus (introns and exons, as well as 20 kb upstream of the start of transcription and 10 kb downstream of the end of transcription) of three genes, including *CRH* (11 SNPs), *CRHR1* (21 SNPs), and *CRHBP* (15 SNPs), in a race specific manner for Caucasians and African Americans separately. Information on these 47 SNPs and 6 putative functional SNPs in *NR3C1* and *NR3C2* genes is presented in Supplementary Table 1.

We genotyped these SNPs using the Sequenom platform with 10 ng of all DNA samples in 384-well format. Laboratory personnel were blinded to case-control status, and 3% blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded quality control samples was 100%.

Statistical methods

We used the x^2 test to assess whether the genotypes for all 53 SNPs were in Hardy-Weinberg equilibrium (HWE) among the controls. We evaluated the association between each SNP and breast cancer risk using unconditional logistic regression. An additive model was used to calculate the *P*-value for trend on breast cancer risk according to an ordinal coding for genotype (0, 1 or 2 copies of SNP minor allele). All statistical analyses were two-sided and carried out using SAS V9.2 (SAS Institute, Cary, NC).

Table 2. SNPs significantly associated with breast cancer risk in WISE case-control study

SNP (gene)	Caucasians				African Americans			
rs11747190 (CRHBP)	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]
CC	245 (72.9)	344 (79.4)	1.00	1.00	108 (75.0)	155 (66.8)	1.00	1.00
CA	78 (23.2)	83 (19.2)	1.32 (0.93-1.87)	1.33 (0.93-1.90)	35 (24.3)	74 (31.9)	0.69 (0.43-1.10)	0.70 (0.42-1.16)
AA	13 (3.9)	6 (1.4)	3.10 (1.16-8.30)	3.23 (1.18-8.83)	1 (0.7)	3 (1.3)	0.50 (0.05-4.85)	0.44 (0.03-5.70)
Additive OR			1.45 (1.09-1.94)	1.47 (1.09-1.98)			0.69 (0.44-1.08)	0.69 (0.43-1.12)
P for trend			0.01	0.01			0.10	0.13
rs1700688 (CRHBP)	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]
CC	336 (99.7)	418 (99.8)	1.00	1.00	103 (74.6)	199 (84.0)	1.00	1.00
CT	1 (0.3)	1 (0.2)	1.25 (0.08-20.2)	1.72 (0.10-28.3)	32 (23.2)	37 (15.6)	1.73 (1.01-2.94)	1.66 (0.94-2.92)
TT	0 (0.0)	0 (0.0)	-	1.00 (0.98-1.02)	3 (2.2)	1 (0.4)	5.94 (0.60-58.4)	7.77 (0.73-83.1)
Additive OR			1.25 (0.08-20.2)	1.72 (0.10-28.3)			1.84 (1.13-2.98)	1.83 (1.10-3.05)
P for trend			0.87	0.71			0.01	0.02
rs17689471 (CRHR1)	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]
TT	203 (61.5)	273 (63.5)	1.00	1.00	122 (86.5)	226 (94.2)	1.00	1.00
TC	106 (32.1)	126 (29.3)	1.13 (0.82-1.55)	1.15 (0.83-1.59)	19 (13.5)	14 (5.8)	2.48 (1.20-5.13)	2.72 (1.24-5.98)
CC	21 (6.4)	31 (7.2)	0.88 (0.49-1.58)	0.90 (0.49-1.65)	0 (0.0)	0 (0.0)	-	-
Additive OR			1.02 (0.81-1.29)	1.03 (0.81-1.31)			2.48 (1.20-5.13)	2.72 (1.24-5.98)
P for trend			0.88	0.79			0.01	0.01

[a]Multivariate-adjusted ORs are adjusted for age, age at menarche (< 12 yr, 12 yr, or > 12 yr), number of full term pregnancies (0, 1 to 2, or ≥ 3), menopausal status (premenopausal, postmenopausal, or induced/unknown), family history of breast cancer in 1st degree relative (yes or no), body-mass index (< 25, 25 to 30, or ≥ 30), duration of CHRT use (never/other HRT use, < 3 yrs, or ≥ 3 yrs), and duration of OC use (never, < 3 yrs, or ≥ 3 yrs).

Results

Descriptive characteristics of cases and controls in this study are summarized in **Table 1**. The mean age at diagnosis of breast cancer cases was 63.4 years for Caucasians and 62.2 years for African Americans. Compared with controls, breast cancer cases in both Caucasians and African Americans had lower number of full term pregnancies. Among Caucasians, cases were less likely to breast-feed, more likely to have used long-term (≥ 3 years) combined estrogen and progestin hormone replacement therapy (CHRT). Among African Americans, cases were more likely to have a family history of breast cancer in a first degree relative and less likely to have used long-term (≥ 3 years) oral contraceptives (OCs).

The distributions of genotypes for four SNPs (rs6190, rs10052957, rs6999100, and rs-242936) among African Americans were not in Hardy-Weinberg equilibrium among controls (*P* for HWE = 0.000); thus were excluded from the analyses (Supplementary Table 1), and a total of 49 SNPs were included in the analyses. We evaluated the association of each SNP with breast cancer risk among Caucasians and African Americans separately. Of the 49 SNPs evaluated, one showed a nominal significant association with breast cancer risk among Caucasians (*P* for rs11747190, 0.01), and another two among African Americans (*P* for rs1700688, 0.01; *P* for rs17689471, 0.01). The age-adjusted additive odds ratio (OR) (95% confidence interval (95% CI)) of the SNP rs11747190[A] in the *CRHBP* gene for the risk of breast cancer among Caucasian women was 1.45 (1.09-1.94). The age-adjusted additive ORs (95% CIs) of two SNPs (*CRHBP* rs1700688[T] and *CRHR1* rs17689471[C]) for the risk of breast cancer among African American women were 1.84 (1.13-2.98) and 2.48 (1.20-5.13), respectively (**Table 2**). These findings remained consistent after adjusting for breast cancer-related factors (**Table 2**). After correction for multiple testing (Bonferroni correction), these SNPs did not show significant associations with breast cancer risk (all *P*-values > 0.05/49 = 0.001).

Considering that the relationship of genetic variants in HPA axis genes with breast cancer risk could be affected by menopausal status or HRT/OC use, for those three SNPs that showed nominal associations in the main effect analyses, we conducted additional analyses in which premenopausal women or HRT/OC users were excluded. The results did not materially change for each of the three SNPs (data not shown).

Discussion

Overall, our study did not show significant associations between 49 SNPs in five HPA axis genes and breast cancer risk in both Caucasians and African Americans. None of the previous studies have examined the associations of genetic variants in HPA axis genes with breast cancer risk.

Negative feedback by cortisol on the hypothalamus and pituitary acting via the glucocorticoid receptor (GR) is critical for containing the HPA response to stressors. The GR gene (i.e., *NR3C1*) is highly polymorphic [17] and includes 4 common putative functional SNPs examined in this study. The A1220G SNP (rs6195) in *NR3C1* is associated with higher BMI [18], higher waist-to-hip ratio (WHR) [19], enhanced cortisol suppression and increased insulin response to dexamethasone [20], and carriers have an increased cortisol response to a psychological stress test [21]. The *NR3C1 Bcl*1 restriction fragment length polymorphism (RFLP, rs-41423247) is associated with BMI, WHR, and enhanced cortisol response to a standard lunch [22], but a diminished cortisol response to a psychological stress test was reported for individuals homozygous for this polymorphism [21]. Also, carriers of the ER22/23EK polymorphism (rs6190) are less sensitive to negative feedback by cortisol [23], have lower fasting insulin levels [23], and lower c-reactive protein levels [24] compared to non-carriers. The *TthIII* I polymorphism (rs10052957) in the 5' flanking region of *NR3C1* has been reported to influence basal cortisol [25]. Under basal conditions negative feedback of cortisol on the HPA axis is primarily via the mineralcorticoid receptor (*NR3C2*) in the hypothalamus and anterior pituitary. A previous study reported that the I180V SNP (rs5522) in *NR3C2* is associated with higher plasma cortisol following a stressor [26]. Also, a G-2C SNP (rs2070951) in the promoter region of *NR3C2* decreases transactivational activity *in vitro* [27].

Corticotropin releasing hormone (CRH) is secreted by the hypothalamus and stimulates

adrenocorticotropic hormone (ACTH) secretion by the pituitary. Individuals who are carriers of variant allele of *CRH Xmn* I polymorphism (rs5030875) and *NR3C1 TthIII* I polymorphism (rs10052957) have elevated cortisol levels before and during physiologic stress [28]. CRH stimulates pituitary ACTH secretion by binding to corticotrophin releasing hormone receptor 1 (*CRHR1*). The *CRHR1* gene is located on chromosome 17 and is highly polymorphic. However, to our knowledge, none of the known polymorphisms in *CRHR1* have been evaluated in relation to adrenal androgen or cortisol secretion or to adiposity. Corticotropin releasing hormone binding protein (*CRHBP*) inhibits activity of CRH by binding to it in the portal vascular system that connects the hypothalamus to the pituitary. Several SNPs in *CRHBP* were related to alcohol abuse in Caucasians and to anxiety disorders in Native Americans [29] but we are not aware of studies that evaluated associations with adrenal androgen or cortisol secretion or with obesity.

In summary, we evaluated the associations between 49 SNPs in five HPA axis genes and breast cancer risk in both Caucasians and African Americans. We did not find strong supportive evidence for the contribution of genetic variants in these HPA axis genes to the risk of developing breast cancer, although three SNPs in two genes showed suggestive association. The sample size of this study was modest, and additional larger studies are warranted to confirm the suggestive associations observed in the present study.

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Disclosure of conflict of interest

None.

Address correspondence to: Hongmei Nan, Department of Epidemiology, Richard M Fairbanks School of Public Health, IU Simon Cancer Center, Indiana University, R3-C252, 980 W. Walnut St, Indianapolis, IN 46202, USA. Tel: +317-278-3907; E-mail: hnan@iu.edu

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Supplementary Table 1. 53 SNPs in five HPA axis genes

Gene	rs#	Caucasians			African Americans		
		Major/minor allele	MAF (%)[a]	P for HWE	Major/minor allele	MAF (%)[a]	P for HWE
Glucocorticoid receptor (NR3C1)	rs6190	G/A	2.7	0.569	G/A	0.0	0.000
	rs6195	A/G	2.9	0.537	A/G	0.8	0.897
	rs10052957	G/A	32.9	0.810	G/A	24.2	0.000
	rs41423247	G/C	34.8	0.333	G/C	20.7	0.008
Mineralcorticoid receptor (NR3C2)	rs5522	A/G	11.4	0.503	A/G	8.7	0.059
	rs2070951	C/G	47.4	0.525	C/G	26.5	0.415
Corticotropin releasing hormone (CRH)	rs3176921	T/C	9.1	0.753	C/T	42.05	0.759
	rs5030875	T/G	3.5	0.492	T/G	16.09	0.001
	rs5030877	C/G	13.2	0.886	G/C	39.66	0.496
	rs6984398	T/C	5.7	0.152	T/C	41.85	0.012
	rs6990486	G/A	4.7	0.310	G/A	16.81	0.232
	rs6999100	T/C	12.5	0.117	T/C	48.39	0.000
	rs10090752	G/A	0.8	0.863	G/A	22.80	0.189
	rs10105164	C/T	3.7	0.576	C/T	32.92	0.176
	rs10957368	T/G	3.5	0.490	T/G	18.80	0.243
	rs11990370	G/T	9.0	0.806	G/T	50.00	0.795
	rs16932615	T/C	2.8	0.661	T/C	14.63	0.924
Corticotropin releasing hormone receptor 1 (CRHR1)	rs110402	C/T	44.8	0.357	C/T	31.1	0.809
	rs171440	C/T	47.9	0.656	C/T	38.2	0.760
	rs171441	C/T	8.6	0.933	C/T	20.4	0.163
	rs173365	C/T	43.4	0.385	T/C	46.5	0.940
	rs242920	C/T	0.3	0.942	C/T	7.9	0.652
	rs242924	C/A	44.6	0.411	C/A	30.3	0.432
	rs242936	C/T	45.4	0.001	T/C	43.8	0.000
	rs242939	A/G	7.1	0.172	A/G	29.4	0.225
	rs242942	G/A	11.3	0.220	G/A	22.2	0.484
	rs242944	T/C	43.2	0.399	C/T	43.2	0.532
	rs4792825	A/G	9.6	0.250	A/G	13.9	0.012
	rs4792886	G/A	10.0	0.152	G/A	31.3	0.807
	rs6503448	A/G	41.9	0.352	A/G	23.1	0.604
	rs6503449	A/G	5.0	0.941	A/G	41.2	0.116
	rs12938031	A/G	37.8	0.282	A/G	10.3	0.686
	rs12944712	G/A	43.6	0.281	G/A	23.9	0.921
	rs12953076	C/T	15.7	0.363	C/T	16.1	0.358
	rs17689471	T/C	21.9	0.003	T/C	2.9	0.642
	rs17689966	A/G	42.3	0.074	G/A	49.1	0.944
	rs17763104	G/A	11.5	0.550	G/A	6.6	0.948
	rs17763658	G/A	6.4	0.317	G/A	8.7	0.536
Corticotropin releasing hormone binding protein (CRHBP)	rs1700680	C/T	36.2	0.996	C/T	37.3	0.079
	rs1700688	C/T	0.1	0.980	C/T	8.2	0.603
	rs1715747	A/G	31.2	0.205	G/A	37.7	0.081
	rs1715751	A/G	36.3	0.823	A/G	43.1	0.099
	rs1715752	G/A	36.2	0.856	G/A	37.1	0.068
	rs1715760	G/A	48.9	0.001	G/A	16.9	0.005
	rs2055628	G/T	10.0	0.884	G/T	12.9	0.252
	rs2135078	C/T	34.4	0.386	C/T	41.6	0.918
	rs7728378	T/C	39.4	0.125	C/T	24.9	0.044
	rs10062367	G/A	17.2	0.168	G/A	37.7	0.876
	rs10473984	G/T	3.9	0.397	G/T	29.2	0.168
	rs10474485	C/A	20.1	0.823	C/A	35.9	0.100
	rs10514082	T/C	12.9	0.980	T/C	9.0	0.958
	rs11747190	C/A	11.0	0.698	C/A	17.2	0.073
	rs28365143	G/A	5.2	0.867	G/A	16.3	0.007

[a]Minor allele frequency (MAF) was calculated among controls in this study.