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PRINCIPAL INVESTIGATOR: Sonia M. Boyapati, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University
Nashville, TN 37232-2103

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Sonia M. Boyapati, Ph.D.

E-mail: sonia.boyapati@vanderbilt.edu

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14. ABSTRACT

There is a strong biological rationale suggesting that soy foods may be important to breast cancer survival. However, this hypothesis has not been adequately tested in epidemiological studies. Soy foods contain isoflavones, a group of phytoestrogens that compete with endogenous estrogens to selectively bind to the estrogen receptor. Tamoxifen, a commonly administered adjuvant therapy for breast cancer survivors with a known benefit to survival, also competes with estrogens to selectively bind to the estrogen receptor. There is some concern that soy foods may compete with tamoxifen. Laboratory data have been conflicting – whereas some studies indicate a competition between soy and tamoxifen, other studies indicate a synergistic effect. Given the common use tamoxifen in breast cancer survivors and the rapid increase of soy containing foods and products in the U.S. market, there is an urgent need to understand the joint effect of soy foods and tamoxifen in human studies. Using existing DNA samples from a large cohort of 1,459 breast cancer survivors living in Shanghai, China, we proposed to examine the joint effects of soy food intake and tamoxifen use on breast cancer survival, and to determine whether this association differs according to polymorphisms in ER-α or ER-ß.

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Gene-environment interaction, complementary and alternative medicine, epidemiology, nutrition

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INTRODUCTION

Due to advances in breast cancer diagnosis and treatment, the breast cancer survivor population has increased dramatically. However, research on environmental modifiable prognostic factors for breast cancer survival has been limited. In addition, genetic susceptibility to environmental modifiers has not been well studied. There is a strong biological rationale suggesting that soy foods may be important to breast cancer survival. However, this hypothesis has not been adequately tested in epidemiological studies. Soy foods contain isoflavones, a group of phytoestrogens that compete with endogenous estrogens to selectively bind to the estrogen receptor. Tamoxifen, a commonly administered adjuvant therapy for breast cancer survivors with a known benefit to survival, also competes with estrogens to selectively bind to the estrogen receptor. There is some concern that soy foods may compete with tamoxifen. In vitro data are conflicting – whereas some studies indicate a competition between soy and tamoxifen, other studies indicate a synergistic effect. Animal studies, however, have been overwhelmingly supportive of a synergistic effect. Given the common use tamoxifen in breast cancer survivors and the rapid increase of soy containing foods and products in the U.S. market, there is an urgent need to understand the joint effect of soy foods and tamoxifen in human studies. *Purpose*: The purpose of this postdoctoral training grant was to provide further training in research concerning breast cancer survival using both observational and molecular epidemiological approaches with the eventual goal of establishing an independent investigator. *Subject*: Using existing data and DNA samples from a large cohort of 1,459 breast cancer survivors living in Shanghai, China, we proposed to examine the joint effects of soy food intake and tamoxifen use on breast cancer survival, and to determine whether this association differs according to polymorphisms in ER-α or ER-ß. *Scope*: Results from this training grant will improve the understanding of the effects of soy food intake on breast cancer survival.

BODY

The body of this report is broken down into specific tasks (in bold print) as stated in the approved statement of work. Beneath each task of the approved statement of work, a summary of research accomplishments achieved during months 12 – 24 is provided.

Task 1. Undergo course work in genetics, cancer biology, molecular cancer research, and molecular epidemiology (Months 4 – 24):

a. **Take 1 course in the Vanderbilt Department of Microbiology and Immunology: Cancer Biology, Fall Semester, 2003, (Months 4 – 8)**
b. **Take 1 course in the Vanderbilt Department of Biochemistry: Molecular Aspects of Cancer Research, Spring Semester, 2004, (Months 9 – 12)**

c. **Take 1 course in the Vanderbilt Department of Biochemistry: Advanced Genetics: Biochemistry and Cell Biology, Fall Semester, 2004, (Months 16-24)**
d. **Take 1 course at the National Cancer Institute, Summer Session: Molecular Epidemiology, 2004, (Month 14).**

1c and 1d. These topics were covered by two workshops I attended in July, 2003 and June, 2004. The biochemistry course was substituted with Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop. The workshop was completed in July, 2003. The Molecular Epidemiology course was substituted with attendance to an international conference on cancer research ("International Course on Molecular Epidemiology") sponsored by the International Agency for Research on Cancer, Vanderbilt University, and the International Epidemiology Institute.

Task 2. Undergo training in the methodology of cohort studies and related analytical techniques, such as survival analysis, by investigating the associations between soy food intake, tamoxifen use, and breast cancer survival using existing data from the Shanghai Breast Cancer Study involving 1459 breast cancer patients (Months 1-18):

a. **Analyze the association between soy food intake and breast cancer survival and prepare a manuscript to report the findings, (Months 1 – 10)**
b. **Analyze the joint effect of soy food intake and tamoxifen use on breast cancer survival and prepare a manuscript to report the findings (Months 10 – 18)**

2a. This manuscript was published in July, 2005 (Boyapati SM, Shu XO, Ruan ZX, Dai Q, Cai Q, Gao YT, Zheng W. Soyfood intake and breast cancer survival: a followup of the Shanghai Breast Cancer Study. *Breast Cancer Res Treat.* 2005 Jul;92(1):11-7).
2b. This task is ongoing. Data have been collected and the next step is to develop an analysis plan.

Task 3. Undergo further training in the analysis of gene-environment interaction by investigating whether the association between soy food intake, tamoxifen use, and breast cancer survival differs according to polymorphisms in the estrogen receptor using existing data from the Shanghai Breast Cancer Study involving 1459 breast cancer patients (Months 1-36):

a. **Learn more about genotyping techniques by observing procedures related to genotyping the ER-ß polymorphism in a molecular epidemiology lab (Months 1 – 18)**
b. **Analyze the association between estrogen receptor gene polymorphisms and breast cancer survival and prepare a manuscript to report the findings (Months 19 – 30)**

c. **Analyze and publish the joint association between soy food intake, tamoxifen use, polymorphisms in the ER, and breast cancer survival (Months 19 – 36)**

3a. As part of a molecular epidemiology conference completed in June, 2004, observed procedures related to genotyping techniques in a state-of-the-art molecular epidemiology laboratory.
3b. The manuscript has been completed and published in *Clinical Cancer Research* in February, 2005 (Boyapati SM, Shu XO, Ruan ZX, Cai Q, Smith JR, Wen W, Gao YT, Zheng W.Polymorphisms in ER-alpha gene interact with estrogen receptor status in breast cancer survival. *Clin Cancer Res.* 2005 Feb 1;11(3):1093-8).
3c. This task is ongoing. Data have been collected and the next step is to develop an analysis plan.

Task 4. Undergo training in breast cancer clinical trial methodology by participating in the field work and implementation of a pilot study investigating the effects of ginseng supplementation on fatigue, sleep disturbance, and immunity in post-menopausal breast cancer survivors (Months 1-12):

a. **Assist in the development and refinement of study materials (Months 1 – 3)**
b. **Participate in subject identification and recruitment (Months 4-8)**
c. **Aid in preparing manuscripts for publication (Months 9 – 12)**

4c. This manuscript was submitted to *Clinical Nurse Specialist* in July, 2005. Please see appendix for a rough draft of the manuscript.

Task 5. Prepare a grant proposal to further investigate molecular markers and to study gene-environment interactions on breast cancer survival (Months 30 – 36).

KEY RESEARCH ACCOMPLISHMENTS

In partial fulfillment of the above tasks, we have published two manuscripts and submitted another for publication. Key research accomplishments from this project and the research to date are provided below.

Major Findings:

In this study population, ER/PR status was obtained from 1001 of the 1459 breast cancer cases (Table 1). Among them, approximately 52.8% were ER+/PR+, 10.8% were ER+/PR-, 10.5% were ER-/PR+, and 25.9% were ER-/PR-. Approximately 27% of cases were missing information on ER/PR status. ER/PR negative breast cancer was related to a reduced, but not statistically significant, disease-free 5-year survival rate. Stage at diagnosis was an important predictor for overall survival. Those participants who received radiotherapy had lower disease-free 5-year survival rates compared to those who did not receive radiotherapy. Participants who received radiotherapy also

had a more advanced cancer, which explains the difference in survival rates. The vast majority (> 90%) of participants received surgery and chemotherapy. Middle age at diagnosis was related to a higher, but not statistically significant (p=0.09), disease-free 5-year survival.

In this comprehensive evaluation of a large cohort of breast cancer survivors, we found that soy food intake was unrelated to disease free survival after diagnosis of breast cancer (Table 2), and the association remains unchanged when traditional breast cancer prognostic factors, factors related to estrogen levels, and polymorphisms in ER-α and ER-ß were taken into consideration (Table 3 and Table 4).

No overall association was observed between *Pvu*II or *Xba*I polymorphisms and breast cancer survival (Table 5). For the $(GT)_n$ polymorphism, analyses were focused on the five most common alleles: $(GT)_{15}$, $(GT)_{16}$, $(GT)_{17}$, $(GT)_{18}$, and $(GT)_{23}$. A significant increased risk for death was observed among participants carrying one $(GT)_{18}$ allele [multiple-adjusted HR was 1.61 (95% CI, 1.15 – 2.27)]. The risk, however, was not elevated among subjects homozygous for this allele (HR = 1.03, 95% CI 0.33 – 3.27) (Table 5).

We found a multiplicative interaction between *PvuII* and $(GT)_{23}$ polymorphisms in the *ER-α* gene and ER status of the breast cancer on breast cancer survival (Table 6 and Table 7).

Although ER status is known to modify the outcome of hormonal treatment for breast cancer, the biological mechanism behind its interaction with ER-α genotypes remains to be clarified. To our knowledge, no previous study has investigated the association between polymorphisms in the *ER-α* gene and breast cancer survival alone or with ER status. In summary, our findings of an interaction between ER status and ER-α polymorphisms on breast cancer survival are new and need to be confirmed in future studies. If confirmed, these findings could have important clinical implications in breast cancer treatment.

Table 1. Median values of total soy protein and isoflavones by select genetic and prognostic factors, Shanghai Breast Cancer Study

Subject Characteristic	All Participants			
	N	Number of Deaths	5-year Survival Rate (%)	P-value
Age at diagnosis				
<42	268	69	80	
42-46	258	35	88	
47-52	266	56	81	
53-64	263	56	80	0.09
Education				
Less than high school	595	118	82	
High school or above	460	98	82	0.71
TNM				
I - II	867	140	87	
III – IV	114	53	57	
Unknown	74	23	69	<0.0001
ER/PR status				
ER-/PR-	142	33	79	
ER+/PR+	383	79	83	
Mixed	196	36	85	
Missing	320	64	82	0.76
Surgery				
Yes	1048	214	82	
No	1	0	-	
Unknown	6	2	67	0.19
Chemotherapy				
Yes	985	204	83	
No	58	9	86	
Unknown	12	3	73	0.50
Radiotherapy				
Yes	403	134	73	
No	500	58	88	
Unknown	152	24	87	<0.0001

Table 2. Adjusted associations of total soy protein, total isoflavones, and disease-free survival, Shanghai Breast Cancer Study

	N	Number of Deaths	Unadjusted Hazard Ratio (95% CI)	Adjusted[a] Hazard Ratio (95% CI)
Total Soy Protein				
T1	482	97	1.00 (ref)	1.00 (ref)
T2	495	104	1.04 (0.79 – 1.37)	1.04 (0.79 – 1.37)
T3	481	96	0.97 (0.73 – 1.29)	0.99 (0.73 – 1.33)
Trend Test				*0.98*
Total Isoflavones				
T1	480	96	1.00 (ref)	1.00 (ref)
T2	495	100	1.01 (0.76 – 1.33)	1.02 (0.77 – 1.25)
T3	480	100	1.03 (0.78 – 1.36)	1.06 (0.79 – 1.42)
Trend Test				*0.64*
Among Those with No Recent Dietary Change				
Total Soy Protein				
T1	239	49	1.00 (ref)	1.00 (ref)
T2	246	39	0.76 (0.50 – 1.16)	0.86 (0.56 – 1.32)
T3	239	50	1.00 (0.67 – 1.48)	1.19 (0.78 – 1.80)
Trend Test				*0.45*
Total Isoflavones				
T1	239	51	1.00 (ref)	1.00 (ref)
T2	245	42	0.80 (0.53 – 1.20)	0.83 (0.55 – 1.25)
T3	239	45	0.86 (0.58 – 1.29)	0.95 (0.62 – 1.45)
Trend Test				*0.78*

Table 3. Association between total soy protein and disease-free survival, stratified by selected prognostic and lifestyle factors, Shanghai Breast Cancer Study

Genotype	Total Soy Protein						P for trend
	T1		T2		T3		
	Deaths/ Total	Adjusted HR (95% CI)[a]	Deaths/ Total	Adjusted HR (95% CI)[a]	Deaths/ Total	Adjusted HR (95% CI)[a]	
ER/PR Status							
ER+/PR+	36/178	1.00 (ref)	35/178	0.91 (0.53 – 1.58)	36/172	1.09 (0.62 – 1.91)	*0.78*
ER-/PR-	19/84	1.00 (ref)	20/97	0.81 (0.37 – 1.77)	14/78	0.75(0.29 – 1.89)	*0.52*
Mixed	9/74	1.00 (ref)	16/66	2.48 (0.95 – 6.51)	17/72	1.91 (0.71 – 5.10)	*0.23*
P for interaction						*0.65*	
TNM							
Stages 0-I	17/151	1.00 (ref)	22/144	1.18 (0.61 – 2.28)	20/168	0.85 (0.42 – 1.68)	*0.56*
Stages ≥ 2	80/330	1.00 (ref)	82/351	0.97 (0.71 – 1.33)	76/311	1.04 (0.75 – 1.46)	*0.78*
P for interaction						*0.50*	
Age at Diagnosis							
< Median	47/263	1.00 (ref)	50/242	1.08 (0.72 – 1.61)	43/221	0.98 (0.64 – 1.51)	*0.86*
≥ Median	49/219	1.00 (ref)	53/253	0.97 (0.65 – 1.43)	52/260	0.92 (0.61 – 1.39)	*0.75*
P for interaction						*0.97*	
BMI							
< 23.2	49/256	1.00 (ref)	44/260	0.93 (0.61 – 1.40)	39/209	1.07 (0.68 – 1.69)	*0.72*
≥ 23.2	47/223	1.00 (ref)	60/233	1.23 (0.84 – 1.81)	57/270	0.94 (0.63 – 1.41)	*0.91*
P for interaction						*0.09*	
WHR							
< 0.81	52/262	1.00 (ref)	48/259	0.98 (0.66 – 1.46)	42/238	0.91 (0.59 – 1.42)	*0.73*
≥ 0.81	43/218	1.00 (ref)	55/235	1.20 (0.81 – 1.80)	53/243	1.02 (0.68 – 1.54)	*0.68*
P for interaction						*0.15*	
Menopausal Status							
Premenopausal	58/339	1.00 (ref)	61/310	1.11 (0.77 – 1.59)	58/302	1.09 (0.74 – 1.60)	*0.60*
Postmenopausal	39/143	1.00 (ref)	43/185	0.86 (0.55 – 1.34)	38/129	0.79 (0.49 – 1.28)	*0.34*
P for interaction							

Table 4. Association between total soy protein and disease-free survival, stratified by genetic factors, Shanghai Breast Cancer Study

Genotype	Total Soy Protein						
	T1		T2		T3		
	Deaths/ Total	Adjusted HR (95% CI)[a]	Deaths/ Total	Adjusted HR (95% CI)[a]	Deaths/ Total	Adjusted HR (95% CI)[a]	P for trend
ER-alpha, *PvuII*							
PP	9/53	1.00 (ref)	7/40	1.21 (0.42 – 3.44)	10/40	2.68 (0.96 – 7.51)	*0.08*
Pp	31/171	1.00 (ref)	35/158	1.06 (0.66 – 1.72)	31/162	0.96 (0.57 – 1.61)	*0.97*
Pp	25/121	1.00 (ref)	29/132	1.01 (0.59 – 1.74)	33/145	1.13 (0.65 – 1.98)	*0.66*
P for interaction						*0.68*	
ER-alpha, *XbaI*							
Xx or XX	33/173	1.00 (ref)	30/165	0.97 (0.59 – 1.59)	33/162	1.25 (0.75 – 2.08)	*0.37*
Xx	33/161	1.00 (ref)	40/167	1.16 (0.73 – 1.86)	41/188	1.07 (0.65 – 1.74)	*0.81*
P for interaction						*0.61*	
ER-β, C(14206)T							
CC	59/309	1.00 (ref)	59/292	1.04 (0.72 – 1.49)	63/302	1.13 (0.78 – 1.64)	*0.55*
CT & TT	15/62	1.00 (ref)	16/63	0.92 (0.45 – 1.86)	13/68	0.67 (0.31 – 1.44)	*0.38*
P for interaction						*0.37*	
ER-β, G(25652)A							
GG	33/158	1.00 (ref)	27/150	0.88 (0.52 – 1.47)	42/171	1.26 (0.77 – 2.05)	*0.35*
AG & AA	39/213	1.00 (ref)	51/210	2.73 (0.52 – 1.47)	36/207	2.66 (0.82 – 8.58)	*0.10*
P for interaction						*0.27*	
ER-β, A(50766)G							
AA	22/109	1.00 (ref)	20/110	0.91 (0.49 – 1.69)	30/127	1.23 (0.68 – 2.21)	*0.50*
AG & GG	47/257	1.00 (ref)	55/244	1.14 (0.77 – 1.68)	48/246	1.04 (0.68 – 1.59)	*0.80*
P for interaction						*0.53*	
ER-β, C(33390)G							
CC	69/350	1.00 (ref)	71/333	1.04 (0.75 – 1.45)	70/348	1.11 (0.78 – 1.57)	*0.54*
CG & GG	2/21	1.00 (ref)	6/26	2.10 (0.35 – 12.84)	5/25	0.94 (0.16 – 5.64)	*0.71*

Table 5. Association of breast cancer survival with *PVUII*, *XbaI*, and $(GT)_n$ polymorphisms in the ER-α gene; Shanghai Breast Cancer Study

	N	Number of Deaths	Hazard Ratio (95% CI) Unadjusted	Hazard Ratio (95% CI) Adjusted[a]
ER-α, *PVUII*				
pp	408	73	1.00 (ref)	1.00 (ref)
Pp	509	75	0.83 (0.60 – 1.15)	0.73 (0.51 – 1.04)
PP	135	24	1.01 (0.63 – 1.59)	0.95 (0.57 – 1.56)
ER-α, *XbaI*				
xx	531	93	1.00 (ref)	1.00 (ref)
Xx	493	73	0.87 (0.64 – 1.18)	0.80 (0.56 – 1.12)
XX	36	6	0.97 (0.43 – 2.22)	1.16 (0.50 – 2.68)
ER-α, $(GT)_n$ *repeats*				
No $(GT)_{15}$ alleles	729	122	1.00 (ref)	1.00 (ref)
One $(GT)_{15}$ allele	203	32	0.93 (0.63 – 1.37)	1.00 (0.68 – 1.48)
Two $(GT)_{15}$ alleles	12	0	-	-
No $(GT)_{16}$ alleles	319	51	1.00 (ref)	1.00 (ref)
One $(GT)_{16}$ allele	464	77	1.04 (0.73 – 1.48)	1.07 (0.75 – 1.53)
Two $(GT)_{16}$ alleles	161	26	0.98 (0.61 – 1.58)	1.01 (0.63 – 1.62)
No $(GT)_{17}$ allele	842	139	1.00 (ref)	1.00 (ref)
One $(GT)_{17}$ allele	98	15	0.94 (0.55 – 1.60)	1.03 (0.60 – 1.75)
Two $(GT)_{17}$ alleles	4	0	-	-
No $(GT)_{18}$ allele	704	102	1.00 (ref)	1.00 (ref)
One $(GT)_{18}$ allele	222	49	1.62 (1.15 – 2.28)	1.61 (1.15 – 2.27)
Two $(GT)_{18}$ alleles	18	3	1.13 (0.36 – 3.56)	1.03 (0.33 – 3.27)
No $(GT)_{23}$ allele	757	130	1.00 (ref)	1.00 (ref)
One $(GT)_{23}$ allele	172	21	0.69 (0.44 – 1.10)	0.66 (0.42 – 1.05)
Two $(GT)_{23}$ alleles	16	3	1.08 (0.35 – 3.41)	1.03 (0.32 – 3.24)

[a]Adjusted for: age at diagnosis, stage of disease, and radiotherapy

Table 6. Association of breast cancer survival with *PVUII*, *XbaI*, and $(GT)_n$ polymorphisms in the ER-α gene stratified by ER status; Shanghai Breast Cancer Study

Genotype	Status[a]					
	ER Positive		ER Negative		Unknown	
	Deaths/ Total	Adjusted HR (95% CI)[b]	Deaths/ Total	Adjusted HR (95% CI)[b]	Deaths/ Total	Adjusted HR (95% CI)
ER-alpha, *PvuII*						
pp	41/174	1.00 (ref)	14/120	1.00 (ref)	18/114	1.00 (ref)
Pp	25/221	0.41 (0.25 – 0.67)	18/122	1.27 (0.61 – 2.61)	32/166	1.51 (0.83 – 2.72)
PP	7/60	0.54 (0.24 – 1.23)	11/33	3.30 (1.42 – 7.69)	6/42	1.08 (0.42 – 2.74)
P for interaction						*0.001*
ER-alpha, *XbaI*						
xx	37/236	1.00 (ref)	23/132	1.00 (ref)	33/163	1.00 (ref)
Xx or XX	36/224	1.06 (0.66 – 1.70)	21/145	0.88 (0.47 – 1.62)	22/160	0.73 (0.42 – 1.27)
P for interaction						*0.73*
ER-alpha, $(GT)_n$ *repeats*						
No $(GT)_{15}$ alleles	86/553	1.00 (ref)	53/308	1.00 (ref)	69/380	1.00 (ref)
One or Two $(GT)_{15}$ alleles	13/94	0.93 (0.52 – 1.67)	9/63	0.87 (0.42 – 1.77)	10/58	1.08 (0.55 – 2.11)
P for interaction						*0.87*
No $(GT)_{16}$ alleles	45/372	1.00 (ref)	39/204	1.00 (ref)	53/255	1.00 (ref)
One or Two $(GT)_{16}$ alleles	54/275	1.71 (1.15 – 2.55)	23/167	0.78 (0.46 – 1.32)	26/183	0.66 (0.41 – 1.07)
P for interaction						*0.015*
No $(GT)_{17}$ allele	90/597	1.00 (ref)	60/352	1.00 (ref)	75/405	1.00 (ref)
One or Two $(GT)_{17}$ alleles	9/50	1.17 (0.59 – 2.34)	2/19	0.95 (0.23 – 3.94)	4/33	0.76 (0.28 – 2.12)

P for interaction						*0.65*
No $(GT)_{18}$ allele	72/541	1.00 (ref)	51/312	1.00 (ref)	65/363	1.00 (ref)
One or Two $(GT)_{18}$ alleles	27/106	2.09 (1.33 – 3.29)	11/59	0.97 (0.49 – 1.94)	14/75	1.09 (0.60 – 1.97)
P for interaction						*0.12*
No $(GT)_{23}$ allele	95/561	1.00 (ref)	50/234	1.00 (ref)	71/384	1.00 (ref)
One or Two $(GT)_{23}$ alleles	4/86	0.25 (0.09 – 0.69)	12/47	1.48 (0.77 – 2.87)	8/54	0.69 (0.33 – 1.44)
P for interaction						*< 0.001*

[a]Adjusted for: age at diagnosis, stage of disease, and radiotherapy

Table 7. Association of breast cancer survival with ER status stratified by *PVUII*, *XbaI*, and $(GT)_n$ polymorphisms in the ER-α gene; Shanghai Breast Cancer Study

Genotype	Status[a]					
	ER Positive		ER Negative		Unknown	
	Deaths/ Total	Adjusted HR (95% CI)[b]	Deaths/ Total	Adjusted HR (95% CI)[b]	Deaths/ Total	Adjusted HR (95% CI)
ER-alpha, *PvuII*						
pp	41/174	1.00 (ref)	14/120	0.61 (0.34 – 1.11)	18/114	1.03 (0.35 – 3.08)
Pp	25/221	1.00 (ref)	18/122	1.08 (0.62 – 1.85)	32/166	0.81 (0.23 – 2.85)
PP	7/60	1.00 (ref)	11/33	3.93 (1.51 – 10.25)	6/42	2.93 (0.73 – 11.81)
P for interaction						*0.001*
ER-alpha, *XbaI*						
xx	37/236	1.00 (ref)	23/132	1.06 (0.66 – 1.72)	33/163	1.27 (0.52 – 3.11)
Xx or XX	36/224	1.00 (ref)	21/145	1.13 (0.85 – 1.52)	22/160	1.32 (0.71 – 2.48)
P for interaction						*0.73*
ER-alpha, $(GT)_n$ repeats						
No $(GT)_{15}$ alleles	86/553	1.00 (ref)	53/308	1.16 (0.84 – 1.59)	69/380	1.01 (0.46 – 2.25)
One or Two $(GT)_{15}$ alleles	13/94	1.00 (ref)	9/63	1.29 (0.65 – 2.56)	10/58	1.85 (0.31 – 10.88)
P for interaction						*0.87*
No $(GT)_{16}$ alleles	45/372	1.00 (ref)	39/204	1.40 (0.96 – 2.05)	53/255	0.86 (0.54 – 1.38)
One or Two $(GT)_{16}$ alleles	54/275	1.00 (ref)	23/167	1.66 (0.78 – 3.55)	26/183	0.83 (0.25 – 2.75)
P for interaction						*0.015*
No $(GT)_{17}$ allele	90/597	1.00 (ref)	60/352	1.15 (0.85 – 1.55)	75/405	1.34 (0.69 – 2.60)
One or Two $(GT)_{17}$ alleles	9/50	1.00 (ref)	2/19	0.59 (0.12 – 2.87)	4/33	1.45 (0.14 – 15.36)
P for interaction						*0.65*

No $(GT)_{18}$ allele	72/541	1.00 (ref)	51/312	1.22 (0.88 – 1.70)	65/363	1.66 (0.83 – 3.32)
One or Two $(GT)_{18}$ alleles	27/106	1.00 (ref)	11/59	0.85 (0.42 – 1.72)	14/75	0.48 (0.11 – 2.23)
P for interaction						*0.12*
No $(GT)_{23}$ allele	95/561	1.00 (ref)	50/234	0.95 (0.69 – 1.31)	71/384	1.08 (0.53 – 2.19)
One or Two $(GT)_{23}$ alleles	4/86	1.00 (ref)	12/47	5.27 (2.08 – 13.36)	8/54	7.33 (1.32 – 40.75)
P for interaction						*< 0.001*

[a]Adjusted for: age at diagnosis, stage of disease, and radiotherapy

REPORTABLE OUTCOMES

- Boyapati SM, Shu XO, Ruan ZX, Dai Q, Cai Q, Gao YT, Zheng W. Soyfood intake and breast cancer survival: a followup of the Shanghai Breast Cancer Study. *Breast Cancer Res Treat.* 2005 Jul;92(1):11-7).
- Boyapati SM, Shu XO, Ruan ZX, Cai Q, Smith JR, Wen W, Gao YT, Zheng W. Polymorphisms in ER-alpha gene interact with estrogen receptor status in breast cancer survival. *Clin Cancer Res.* 2005 Feb 1;11(3):1093-8).

CONCLUSIONS

Results from the soy food intake and breast cancer survival manuscript indicate that there is no association between soy protein intake and breast cancer survival. Other research findings indicate that polymorphisms in ER-α may modify the association between ER/PR status and breast cancer survival.

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2. Boyapati SM, Shu XO, Ruan ZX, Cai Q, Smith JR, Wen W, Gao YT, Zheng W. Polymorphisms in ER-alpha gene interact with estrogen receptor status in breast cancer survival. *Clin Cancer Res.* 2005 Feb 1;11(3):1093-8.

APPENDICES

A1. Boyapati SM, Shu XO, Ruan ZX, Dai Q, Cai Q, Gao YT, Zheng W. Soyfood intake and breast cancer survival: a followup of the Shanghai Breast Cancer Study. *Breast Cancer Res Treat.* 2005 Jul;92(1):11-7.

A2. Boyapati SM, Shu XO, Ruan ZX, Cai Q, Smith JR, Wen W, Gao YT, Zheng W. Polymorphisms in ER-alpha gene interact with estrogen receptor status in breast cancer survival. *Clin Cancer Res.* 2005 Feb 1;11(3):1093-8.

A3. Draft of ginseng manuscript submitted to *Clinical Nurse Specialist*

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Report

Soyfood intake and breast cancer survival: a followup of the Shanghai Breast Cancer Study

Sonia M. Boyapati[1], Xiao-ou Shu[2], Zhi Xian Ruan[1], Qi Dai[1], Qiuyin Cai[1], Yu-tang Gao[1], and Wei Zheng[1]

[1]*Division of General Internal Medicine, Department of Medicine, Center for Health Services Research, Vanderbilt Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232, USA;* [2]*Department of Epidemiology, Shanghai Cancer Institute, Shanghai, 200032, China*

Key words: breast cancer, epidemiology, estrogen receptor, isoflavones, mammary, phytoestrogens, soy, soyfood, study, survival

Summary

Soy and its constituents have been shown in many *in vivo* and *in vitro* studies and in some epidemiological studies to have anti-cancer effects. Some soy constituents, however, also stimulate cell proliferation, which has raised concerns in promoting soy intake among breast cancer survivors. To investigate whether soy intake may be associated with breast cancer survival, we evaluated data from a cohort of 1459 breast cancer patients who participated in the Shanghai Breast Cancer Study between 1996 and 1998. Usual soy food intake was assessed using a validated food frequency questionnaire at baseline. The median follow-up time for this cohort of women was 5.2 years. We found that soy intake prior to cancer diagnosis was unrelated to disease-free breast cancer survival (adjusted hazard ratio [HR] = 0.99, 95% confidence interval [CI], 0.73–1.33 for the highest tertile compared to the lowest tertile). The association between soy protein intake and breast cancer survival did not differ according to ER/PR status, tumor stage, age at diagnosis, body mass index (BMI), waist to hip ratio (WHR), or menopausal status. Additionally, the soy-survival association did not appear to vary according to *Xba*I or *Pvu*II polymorphisms in ER-alpha, or *C(14206)T*, *G(25652)A*, or *A(50766)G* polymorphisms in ER-beta. These data suggest that soyfoods do not have an adverse effect on breast cancer survival.

Abbreviations: CI: confidence interval; HR: hazard ratio; QC: Quality control; SBCS: Shanghai Breast Cancer Study; TNM: tumor-node-metastasis

Introduction

In vivo and *in vitro* data suggest that isoflavones, the major soy phytoestrogen, may compete with endogenous estrogens to bind to the estrogen receptor (ER), promote the synthesis of sex hormone binding globulin, and inhibit 17β-hydroxysteroid dehydrogenases – clear anti-estrogenic effects [1, 2]. On the other hand, soy foods have also been suggested to act as estrogen agonists, binding the estrogen receptor and stimulating cell proliferation [3–5]. Epidemiological studies investigating the effects of soy foods and breast cancer risk, particularly those conducted among Asian populations where soy food consumption is common, have generally shown an inverse association between soy food intake and breast cancer risk (for review see Ref.[6]). However, the relationship between soy food intake and breast cancer prognosis remains unknown. Given the increasing breast cancer survivor population and the fact that soy foods are being consumed more commonly among U.S. women as an alternative medicine for symptoms of estrogen deprivation and for promoting cardiovascular health, it is imperative to investigate whether soy has a beneficial or detrimental effect on survival after breast cancer diagnosis. We report here an in-depth evaluation of the association between soyfood intake and breast cancer survival using data collected from a large cohort of breast cancer patients who were recruited as part of the Shanghai Breast Cancer Study.

Materials and methods

Participants and study design

Through a rapid case-ascertainment system and supplemented by the population-based Shanghai Cancer Registry, we identified 1602 women who were between the ages of 25–64 years and were diagnosed with a primary breast cancer between August 1996 and March 1998. Of them, 1459 (91.1%) completed an in-person interview and were included as cases in the Shanghai

Breast Cancer Study. Reasons for non-participation included: refusal (109 cases, 6.8%), death prior to interview (17 cases, 1.1%), and inability to locate (17 cases, 1.1%). The median time interval between diagnosis and interview was 66 days. The initial institutional cancer diagnoses were confirmed by independent review of pathological slides by two senior pathologists. Blood samples (10 ml from each woman) were obtained from 1193 (82%) cases who participated in the study. These samples were typically processed within 6 h of collection, and were stored at −70°C until the relevant bioassays were conducted. Information on cancer diagnosis, disease stage (TNM stage), cancer treatments, and ER/PR status was abstracted from medical charts using a standard protocol.

Patients were followed up through January 2003 with a combination of active follow-up and record linkage to the death certificates kept by the Vital Statistics Unit of the Shanghai Center for Disease Control and Prevention. Of the 1459 patients included in the original study, 1290 (88.4%) were successfully contacted either by home visit ($N = 1241$, 85.0%) or by telephone ($N = 49$, 3.4%) from March 2000 to December 2002. Among them, 200 patients were deceased. Through interview of patients, or next of kin for deceased patients, we obtained information on disease progress, recurrence, quality of life, and cause of death (if deceased). Survival status for the remaining 169 participants who could not be contacted through a home visit or by telephone call was established in June 2003 by linkage to the mortality registry. Of them, 40 deaths were identified; information on the date of death and cause of death was obtained. In the mortality registry, 126 subjects had no match and were assumed to be still living. Their censoring date was assigned to be December 31, 2002, 6 months prior to our search of the vital statistics registry, in order to allow for a possible delay of entry of the death certificates into the registry. Three subjects had insufficient information for the record linkage and were excluded from the current analysis. This study was approved by the Institutional Review Board of all participating institutes.

Dietary assessment

A food frequency questionnaire (FFQ) was administered at the time of enrollment in the Shanghai Breast Cancer Study (parent case-control study). A detailed description of the dietary assessment has been given elsewhere [7]. Briefly, during an in-person interview, usual diet over the past 5 years was assessed using a 76-item FFQ. The FFQ was designed to capture over 85% of foods commonly consumed by Shanghai residents. For soy foods, specific questions on consumption of soymilk, tofu, dry soybeans, soy products other than tofu, fresh soybeans, and soybean sprouts were included. These soy foods are thought to account for over 90% of soy foods consumed in Shanghai [7, 8]. Specific questions in the FFQ assessed frequency of consumption (i.e. daily, weekly, monthly, yearly, or never) and the amount of raw food item typically consumed in lians (where 1 lian is equal to 50 g) or jins (where 1 jin is equal to 500 g). For seasonal food items, participants were asked to describe their consumption based on market availability and total months of consumption per year. Approximately 7 months after initiation of the study, a supplementary survey was added in which all participants were asked whether their intake levels of soyfoods 1 week before interview were similar, increased, or decreased compared to their usual intake levels of these foods during the past 5 years. The supplementary survey was completed for 1104 participants. Approximately 65.8% reported no change in soy food intake, 16.9% reported an increase, and 16.8% reported a decrease.

Soy protein content of each food item was estimated based on the Chinese Food Composition Table 1 [9].

Table 1. Median values of total soy protein and isoflavones by select genetic and prognostic factors, Shanghai Breast Cancer Study

Subject characteristic	All Participants			
	N	Number of deaths	5-year survival rate (%)	*P*-value
Age at diagnosis				
<42	268	69	80	
42–46	258	35	88	
47–52	266	56	81	
53–64	263	56	80	0.09
Education				
Less than high school	595	118	82	
High school or above	460	98	82	0.71
TNM				
I–II	867	140	87	
III–IV	114	53	57	
Unknown	74	23	69	<0.0001
ER/PR status				
ER−/PR−	142	33	79	
ER+/PR+	383	79	83	
Mixed	196	36	85	
Missing	320	64	82	0.76
Surgery				
Yes	1048	214	82	
No	1	0	–	
Unknown	6	2	67	0.19
Chemotherapy				
Yes	985	204	83	
No	58	9	86	
Unknown	12	3	73	0.50
Radiotherapy				
Yes	403	134	73	
No	500	58	88	
Unknown	152	24	87	<0.0001

Total soy protein intake was calculated by summing the product of soy protein content of each food item and amount of food consumption. Total isoflavone intake was also calculated using published data [10]. Soy protein and soy isoflavones are better measurements of total soyfood intake because individuals who rarely eat one type of soyfood might eat other types frequently and soy protein and isoflavone concentrations vary widely across different soyfoods. Because results from our analyses of main effect showed similar patterns using soy protein and soy isoflavone intakes, we present results only on soy protein for the stratified analyses.

Genotyping

Genomic DNA was extracted from buffy coat fractions. *Pvu*II and *Xba*I genotypes were determined with PCR-RFLP method as described previously [11, 12]. Measurement of ER-β genotypes has been reported elsewhere [13]. For the ER-β gene, genotypes were assessed for T(-11891)C, C(14206)T, G(25652)A, C(33390)G, A(50766)G, G(50995)A polymorphisms with a successful genotyping rate ranging from 92.0% to 93.3% among a range of 1097–1149 participants. For ER-α, genotyping data were obtained from 1069 (89.6%) participants who donated a blood sample. The major reasons for incomplete genotyping were insufficient DNA used in the particular assays and unsuccessful PCR amplification.

Statistical analysis

The primary outcome for this study was disease-free survival. Survival time was calculated as the time from cancer diagnosis to death from breast cancer, censoring at the date of last contact. The Cox regression model was applied to evaluate the effect of soy food intake on disease-free survival with adjustments for age and known prognostic factors for breast cancer, including TNM status, cancer treatments, and age at diagnosis. Stratified analyses by traditional breast cancer prognostic factors were performed to examine potential interactive effects of these factors on the association between soyfood intake and disease-free breast cancer survival. All statistical tests are based on two-sided probability.

Results

In this study population, ER/PR status was obtained from 1001 of the 1459 breast cancer cases. Among them, approximately 52.8% were ER+/PR+, 10.8% were ER+/PR−, 10.5% were ER−/PR+, and 25.9% were ER−/PR−. Approximately 27% of cases were missing information on ER/PR status. ER/PR negative breast cancer was related to a reduced, but not statistically significant, disease-free 5-year survival rate. Stage at diagnosis was an important predictor for overall survival. Those participants who received radiotherapy had lower disease-free 5-year survival rates compared to those who did not receive radiotherapy. Participants who received radiotherapy also had a more advanced cancer, which explains the difference in survival rates. The vast majority (>90%) of participants received surgery and chemotherapy. Middle age at diagnosis was related to a higher, but not statistically significant ($p = 0.09$), disease-free 5-year survival. Clinical prognostic factors were found to be similar for subjects who were included in the genotyping analyses (data not shown).

Unadjusted and multiple-adjusted associations of total soy protein and total isoflavones with disease-free breast cancer survival are shown in Table 2. No overall association was observed between total soy protein intake or total isoflavone intake and disease-free breast cancer survival. In a subgroup analysis, we also examined the association between soy protein or isoflavone intake and disease-free survival among those with no recent dietary change to examine whether post-diagnosis change in soy food consumption may alter these associations. Similar null associations for total soy protein intake and isoflavone intake were found among those who reported no recent dietary change.

The association between soy protein intake and disease-free survival did not vary by selected prognostic and lifestyle factors, including ER/PR status, TNM status, age at diagnosis, BMI, and WHR (Table 3). Nor did genetic polymorphisms in ER-α (*Pvu*II and *Xba*I) or ER-β (C(14206)T, G(25652)A, A(50766)G, and C(33390)G genes modify the soy protein intake and disease-free survival association (Table 4).

Discussion

Soy foods have generated considerable interest among breast cancer survivors as an alternative medicine for alleviating postmenopausal symptoms and promoting heart health [14, 20]. However, there is considerable uncertainty as to the effects of soy intake among breast cancer survivors due to conflicting laboratory data. To our knowledge, no previous epidemiological study has investigated the association between soy food intake and breast cancer survival. In this comprehensive evaluation of a large cohort of breast cancer survivors, we found that soy food intake was unrelated to disease free survival after diagnosis of breast cancer, and the association remained unchanged when traditional breast cancer prognostic factors, factors related to estrogen levels, and polymorphisms in ER-α and ER-β were taken into consideration.

It is well-known from cell culture and animal studies that soy isoflavones can compete with 17β-estradiol to bind the estrogen receptors and act as an estrogen antagonist. In addition to the ER-mediated mechanism of effect, there are several other means by which soy may exert cancer inhibitory effects in estrogen sensitive

tissues, including breast. Isoflavones promote the synthesis of sex hormone binding globulin (lowering the biological availability of sex hormones), and inhibit 17β-hydroxysteroid dehydrogenases (reducing estrogen synthesis), More recently, genestein, one of the primary isoflavones found in soybeans, has been shown to increase concentrations of transforming growth factor β (TGFβ), a peptide growth factor important in inhibiting growth of cancer cells, and inhibit DNA topoisomerase II. However, not all laboratory studies have been supportive of the hypothesis that soy is protective against breast cancer. A recent *in vitro* study concluded that large doses of dietary isoflavonoids may affect metabolism of steroid hormones in humans and lead to elevated levels of active estrogen in mammary tissue [15]. Other studies have shown that genestein may facilitate cell proliferative activity, and possibly induce tumor progression *via* ER-ERE binding [16], while still others indicate that soy may stimulate growth of estrogen-dependent human breast cancer cells [17].

Given the vast and growing number of both *in vitro* and *in vivo* studies that have investigated the health effects of soy products, and their conflicting results, it can sometimes be difficult to extrapolate these findings to human populations. The majority of previous studies have focused on one or a few constituents of soy (e.g., genestein), and/or on one or a few markers of effect (e.g. cell proliferation). Very few laboratory or intervention studies have examined the effect of whole soy foods [3, 4]. It is probable that the effect of soy is dependent on the totality of its constituents, many of which may work together synergistically, to exert an effect. Study of the chemopreventive effect of soy without considering these factors could be misleading. Other general limitations to previous laboratory studies include: lack of statistical power/small sample size across studies, differences in soy isolates due to processing techniques, and the fact that laboratory studies typically use much higher amounts of soy than are typically found in human diets [3, 4, 18].

ER-α and *ER-*β are nuclear receptor proteins that act as ligand-inducible transcription factors and mediate the effects of estrogens within breast tissue [21]. Phytoestrogens can bind to both *ER-*α and *ER-*β due to their structural similarity to estradiol. A recent study showed that phytoestrogens induced an increase in ER binding to the estrogen response element (ERE), with a greater binding to ERE for *ER-*β than for *ER-*α [19]. Therefore, it is plausible that the effect of soy on breast cancer may depend on the status and/or function of ER. There are several known polymorphisms in the *ER-*α and *ER-*β genes, some of which have been associated with breast cancer risk and survival [11–13]. We have previously reported that soy appeared to have a stronger effect on the risk of ER/PR positive breast cancer [6]. However, we did not find that the association between soy food intake and prognosis of breast cancer varies by ER status, or polymorphisms of ER genes.

This study has several strengths, including (a) a population-based patient cohort, (b) the relatively long follow-up period (median of 5.2 years), (c) a large

Table 2. Adjusted associations of total soy protein, total isoflavones, and disease-free survival, Shanghai Breast Cancer Study

	N	Number of deaths	Unadjusted hazard ratio (95% CI)	Adjusted[a] hazard ratio (95% CI)
Total soy protein				
T1	482	97	1.00 (ref)	1.00 (ref)
T2	495	104	1.04 (0.79–1.37)	1.04 (0.79–1.37)
T3	481	96	0.97 (0.73–1.29)	0.99 (0.73–1.33)
Trend test				0.98
Total isoflavones				
T1	480	96	1.00 (ref)	1.00 (ref)
T2	495	100	1.01 (0.76–1.33)	1.02 (0.77–1.25)
T3	480	100	1.03 (0.78–1.36)	1.06 (0.79–1.42)
Trend test				0.64
Among those with no recent dietary change				
Total soy protein				
T1	239	49	1.00 (ref)	1.00 (ref)
T2	246	39	0.76 (0.50–1.16)	0.86 (0.56–1.32)
T3	239	50	1.00 (0.67–1.48)	1.19 (0.78–1.80)
Trend test				0.45
Total isoflavones				
T1	239	51	1.00 (ref)	1.00 (ref)
T2	245	42	0.80 (0.53–1.20)	0.83 (0.55–1.25)
T3	239	45	0.86 (0.58–1.29)	0.95 (0.62–1.45)
Trend test				0.78

[a] Adjusted for age at diagnosis, stage of disease, radiotheraphy, ER/PR status, and total energy intake.

Table 3. Association between total soy protein and disease-free survival, stratified by selected prognostic and lifestyle factors, Shanghai Breast Cancer Study

	Total soy protein						*P* for trend
	T1		T2		T3		
	Deaths/total	Adjusted HR (95% CI)[a]	Deaths/total	Adjusted HR (95% CI)[a]	Deaths/total	Adjusted HR (95% CI)[a]	
ER/PR Status							
ER+/PR+	36/178	1.00 (ref)	35/178	0.91 (0.53–1.58)	36/172	1.09 (0.62–1.91)	0.78
ER−/PR−	19/84	1.00 (ref)	20/97	0.81 (0.37–1.77)	14/78	0.75(0.29–1.89)	0.52
Mixed	9/74	1.00 (ref)	16/66	2.48 (0.95–6.51)	17/72	1.91 (0.71–5.10)	0.23
P for interaction				0.65			
TNM							
Stages 0–1	17/151	1.00 (ref)	22/144	1.18 (0.61–2.28)	20/168	0.85 (0.42–1.68)	0.56
Stages ≥ 2	80/330	1.00 (ref)	82/351	0.97 (0.71–1.33)	76/311	1.04 (0.75–1.46)	0.78
P for interaction				0.50			
Age at diagnosis							
< Median	47/263	1.00 (ref)	50/242	1.08 (0.72–1.61)	43/221	0.98 (0.64–1.51)	0.86
≥ Median	49/219	1.00 (ref)	53/253	0.97 (0.65–1.43)	52/260	0.92 (0.61–1.39)	0.75
P for interaction				0.97			
BMI							
< 23.2	49/256	1.00 (ref)	44/260	0.93 (0.61–1.40)	39/209	1.07 (0.68–1.69)	0.72
≥ 23.2	47/223	1.00 (ref)	60/233	1.23 (0.84–1.81)	57/270	0.94 (0.63–1.41)	0.91
P for interaction				0.09			
WHR							
< 0.81	52/262	1.00 (ref)	48/259	0.98 (0.66–1.46)	42/238	0.91 (0.59–1.42)	0.73
≥ 0.81	43/218	1.00 (ref)	55/235	1.20 (0.81–1.80)	53/243	1.02 (0.68–1.54)	0.68
P for interaction				0.15			
Menopausal status							
Premenopausal	58/339	1.00 (ref)	61/310	1.11 (0.77–1.59)	58/302	1.09 (0.74–1.60)	0.60
Postmenopausal	39/143	1.00 (ref)	43/185	0.86 (0.55–1.34)	38/129	0.79 (0.49–1.28)	0.34
P for interaction				0.38			

[a] Adjusted for age at diagnosis, stage of disease, radiotheraphy, ER/PR status, and total energy intake (exception: stratifying variable of interest not included in adjustment).

Table 4. Association between total soy protein and disease-free survival, stratified by genetic factors, Shanghai Breast Cancer Study

Genotype	Total Soy Protein						*P* for trend
	T1		T2		T3		
	Deaths/Total	Adjusted HR (95% CI)[a]	Deaths/Total	Adjusted HR (95% CI)[a]	Deaths/Total	Adjusted HR (95% CI)[a]	
ER-α, PvuII							
PP	9/53	1.00 (ref)	7/40	1.21 (0.42–3.44)	10/40	2.68 (0.96–7.51)	0.08
Pp	31/171	1.00 (ref)	35/158	1.06 (0.66–1.72)	31/162	0.96 (0.57–1.61)	0.97
pp	25/121	1.00 (ref)	29/132	1.01 (0.59–1.74)	33/145	1.13 (0.65–1.98)	0.66
P for interaction				0.68			
ER-α, XbaI							
Xx or XX	33/173	1.00 (ref)	30/165	0.97 (0.59–1.59)	33/162	1.25 (0.75–2.08)	0.37
xx	33/161	1.00 (ref)	40/167	1.16 (0.73–1.86)	41/188	1.07 (0.65–1.74)	0.81
P for interaction				0.61			
ER-β, C(14206)T							
CC	59/309	1.00 (ref)	59/292	1.04 (0.72–1.49)	63/302	1.13 (0.78–1.64)	0.55
CT & TT	15/62	1.00 (ref)	16/63	0.92 (0.45–1.86)	13/68	0.67 (0.31–1.44)	0.38
P for interaction				0.37			
ER-β, G(25652)A							
GG	33/158	1.00 (ref)	27/150	0.88 (0.52–1.47)	42/171	1.26 (0.77–2.05)	0.35
AG & AA	39/213	1.00 (ref)	51/210	2.73 (0.52–1.47)	36/207	2.66 (0.82–8.58)	0.10
P for interaction				0.27			
ER-β, A(50766)G							
AA	22/109	1.00 (ref)	20/110	0.91 (0.49–1.69)	30/127	1.23 (0.68–2.21)	0.50
AG & GG	47/257	1.00 (ref)	55/244	1.14 (0.77–1.68)	48/246	1.04 (0.68–1.59)	0.80
P for interaction				0.53			
ER-β, C(33390)G							
CC	69/350	1.00 (ref)	71/333	1.04 (0.75–1.45)	70/348	1.11 (0.78–1.57)	0.54
CG & GG	2/21	1.00 (ref)	6/26	2.10 (0.35–12.84)	5/25	0.94 (0.16–5.64)	0.71
P for interaction				0.42			

[a] Adjusted for age at diagnosis, stage of disease, radiotheraphy, ER/PR status, and total energy intake.

sample size, (d) high soyfood intake, and (e) detailed information on prognostic factors such as ER/PR status, and disease stage. The major limitation of this study is that soy food information was collected with the reference period being 5 years prior to breast cancer diagnosis. Although soy food consumption constitutes a lifetime habit for most Chinese people, we can not rule out the possibility that breast cancer patients may have altered their soy food intake pattern after cancer diagnosis. However, analysis restricted to women who reported 'no dietary change' in their soy consumption at time of interview indicated a null association, similar to that of all women combined. Another limitation is lack of detailed information on tamoxifen use, an important anti-estrogen widely prescribed for long-term adjuvant treatment for breast cancer. Therefore, the potential associations between soy and tamoxifen use, an interaction that is biologically plausible and has been reported in *in vitro* studies, could not be evaluated.

In summary, we did not find any association between soy food intake and disease-free breast cancer survival among a large population of Chinese women who commonly eat soy foods. At this point, there is no evidence to indicate that soy foods are unsafe for breast cancer patients.

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Address for offprints and correspondence: Dr Xiao Ou Shu, Center for Health Service Research, Vanderbilt University, Medical Center East, Suite 6000, Nashville, TN 37232-8300 USA; *Tel.:* +1-615-936-0713; *Fax:* +1-615-936-1269; *E-mail:* xiao-ou.shu@vanderbilt.edu

Polymorphisms in *ER*-α Gene Interact with Estrogen Receptor Status in Breast Cancer Survival

Sonia M. Boyapati,[1] Xiao Ou Shu,[1] Zhi Xian Ruan,[2] Qiuyin Cai,[1] Jeffrey R. Smith,[1] Wanqing Wen,[1] Yu-Tang Gao,[1] and Wei Zheng[1]

[1]Division of General Internal Medicine, Department of Medicine, Center for Health Services Research, Vanderbilt Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee; and [2]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

ABSTRACT

***Purpose*: The effects of estrogens are mediated primarily through estrogen receptor (ER) in breast tissue, and polymorphisms in the *ER* genes may alter the functions of these receptors. Polymorphisms in the *ER*-α gene have been reported to be associated with breast cancer risk. However, to our knowledge, no study has been published on the relation between *ER*-α gene polymorphisms and breast cancer survival.**

***Experimental Design*: To determine whether three common polymorphisms in the *ER*-α gene, *Pvu*II, *Xba*I, and GT dinucleotide repeats are associated with breast cancer survival, we evaluated data from a cohort of 1,069 breast cancer patients who participated in the Shanghai Breast Cancer Study between 1996 and 1998. The median follow-up time for this cohort of women was 5.2 years.**

***Results*: No overall association was observed between *ER* gene polymorphisms and breast cancer survival. The genotype associations, however, were modified by ER status in breast cancer tissues. Comparing those with the PP genotype to the pp genotype of the *PvuII* polymorphism, the hazard ratios (HR) of dying were 3.30 [95% confidence interval (95% CI), 1.42-7.69] and 0.54 (95% CI, 0.24-1.23), respectively, for participants with ER-negative breast cancer and ER-positive breast cancer. Similarly, compared with those with no $(GT)_{23}$ alleles, carrying one or two $(GT)_{23}$ alleles of the GT repeat polymorphism was related to a HR of 1.48 (95% CI, 0.77-2.87) for ER-negative breast cancer and a HR of 0.25 (95% CI, 0.09-0.69) for ER-positive cancer. The effect of ER on breast cancer survival was also modified by genotypes of *ER*-α gene. Tests for multiplicative interaction were highly significant.**

***Conclusions*: These data suggest that the *ER*-α gene polymorphisms and ER status may have an interactive effect on breast cancer survival.**

INTRODUCTION

Estrogens play a crucial role in the pathogenesis and progression of breast cancer. The effects of estrogens are mediated primarily through intracellular estrogen receptors (ER). To date, there are two known ERs, *ER*-α and *ER*-β. Both are nuclear receptor proteins that act as ligand-inducible transcription factors (1). *ER*-α expression has been used in clinical practice as an indicator for selecting hormone therapy and loss of *ER*-α expression is often associated with poor survival (2).

There are several known polymorphisms in the *ER*-α gene, some of which alter the function of the receptor (1). Of the known polymorphisms, *Pvu*II, *Xba*I, and the recently identified $(GT)_n$ polymorphisms have been reported to be associated with breast cancer risk (3-8). *Pvu*II and *Xba*I are located on intron 1, whereas the $(GT)_n$ repeats polymorphism is located at 2.8 kb 5′ to exon 1D (9). To our knowledge, no study has been published on the relation between *ER*-α polymorphisms and breast cancer survival. The association of ER polymorphisms with breast cancer risk suggests that these polymorphisms may also affect breast cancer survival. However, it is difficult to extrapolate the findings from etiologic research to cancer prognosis for several reasons: (a) there is not enough evidence to conclusively determine the direction of the polymorphism association with breast cancer risk and (b) even if the associations with breast cancer risk were conclusive, it is not certain whether the polymorphisms would play the same role in breast cancer risk as breast cancer survival. In this report, we evaluated the association of three common polymorphisms in the *ER*-α gene, *Pvu*II, *Xba*I, and GT dinucleotide repeats with breast cancer survival in a cohort of breast cancer patients who were recruited as part of the Shanghai Breast Cancer Study, a population-based case-control study.

MATERIALS AND METHODS

Participants and Study Design. Through a rapid case ascertainment system and supplemented by the population-based Shanghai Cancer Registry, we identified 1,602 women who were between the ages of 25 to 64 years and were diagnosed with a primary breast cancer between August 1996 and March 1998. Of them, 1,459 (91.1%) completed an in-person interview and were included as cases in the Shanghai Breast Cancer Study. Reasons for nonparticipation included refusal (109 cases, 6.8%), death before interview (17 cases, 1.1%), and inability to locate (17 cases, 1.1%). The median time interval between diagnosis and interview was 66 days. The initial institutional cancer diagnoses were confirmed by independent review of pathologic slides by two senior pathologists. Blood samples (10 mL from each woman) were obtained from 1,193 (82%) cases who

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participated in the study. These samples were typically processed within 6 hours of collection and were stored at −70°C until the relevant bioassays were conducted. Information on cancer diagnosis, disease stage (tumor-node-metastasis stage), cancer treatments, and ER/progesterone receptor (PR) status was abstracted from medical charts using a standard protocol.

Patients were followed up through January 2003 with a combination of active follow-up and record linkage to the death certificates kept by the Vital Statistics Unit of the Shanghai Center for Disease Control and Prevention. Of the 1,459 patients included in the original study, 1,290 (88.4%) were successfully contacted either by home visit (n = 1,241, 85.0%) or by telephone (n = 49, 3.4%) from March 2000 to December 2002. Among them, 200 patients were deceased. Through interview of patients, or next of kin for deceased patients, we obtained information on disease progress, recurrence, quality of life, and cause of death (if deceased). Survival status for the remaining 169 participants who could not be contacted through a home visit or by telephone call was established in June 2003 by linkage to the mortality registry. Of them, 40 deaths were identified; information on the date of death and cause of death was obtained. Subjects (n = 126) had no match in the mortality registry and were assumed to be still living. Their censoring date was assigned to be December 31, 2002, 6 months before our search of the vital statistics registry, to allow for a possible delay of entry of the death certificates into the registry. Three subjects had insufficient information for the record linkage and were excluded from the current analysis. This study was approved by the Institutional Review Board of all participating institutes.

Laboratory Protocols. Determination of *Pvu*II and *Xba*I Polymorphisms. Genomic DNA was extracted from buffy coat fractions. *Pvu*II and *Xba*I genotypes were determined with PCR-RFLP method described previously (7). Briefly, the primers for analysis were 5′-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3′ (forward) and5′-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3′ (reverse). These primers generated a 1.3-kb fragment, and contain a part of *intron 1* and *exon 2* of the *ER-* gene. The PCR products were digested by the *Pvu*II and *Xba*I restriction endonucleases, respectively. The DNA fragments were then separated using 1.5% agarose gel and detected by ethidium bromide staining. *PP* and *XX*, signifying the absence of restriction sites, gave one 1.3-kb fragment. *pp*, signifying the presence of *Pvu*II restriction sites on both alleles, was digested into two fragments (0.85 and 0.45 kb). The *xx* genotype was revealed by *Xba*I digestion into two fragments (0.9 and 0.4 kb).

Determination of $(GT)_n$ Polymorphism. Genotyping for the $(GT)_n$ polymorphism was done by detection of fluorescent amplimers on an ABI PRISM 3700 automated DNA analyzer. Details of genotyping method have been described elsewhere (8). Briefly, the primers were forward 5′-gtgtCTGCTCAAATCTCCTCTG-3′ and reverse 5′-GTTAAGAAGGGCCTTTAC-3′. The forward primer was labeled with 6-carboxyfluorescein. Allele fragment size estimation was accomplished using the internal size standard Genescan 400HD ROX and the Local Southern algorithm of GENESCAN software. Allele binding and adjustment of run mobility according to control alleles of Centre d'Etude du Polymorphisme Humain 1347-02 were accomplished by custom software. The number of $(GT)_n$ repeats were confirmed by direct sequencing using BigDye Terminator Chemistry on an ABI PRISM 3700 automated DNA Analyzer.

The laboratory staff was blind to the identity of the subject. QC samples were included in genotyping assays. Each 96-well plate contains one water, two Centre d'Etude du Polymorphisme Humain 1347-02 DNA, two blinded QC DNA, and two unblinded QC DNA samples. The blinded QC samples were taken from the second tube of study samples included in the study.

Genotyping data for the $(GT)_n$ polymorphism were obtained from 947 cases who gave blood samples. For the *Pvu*II and *Xba*I polymorphisms, genotyping data were obtained from 1,069 cases who gave blood samples. The major reasons for incomplete genotyping were insufficient DNA used in the particular assays and unsuccessful PCR amplification.

Statistical Analysis. The primary outcome for this study was overall survival. Survival time was calculated as the time from cancer diagnosis to death, censoring at the date of last contact. The Kaplan-Meier method was used to compute 5-year survival rates, and the log-rank test was applied to test the differences in survival across different genotypes. The proportional hazard assumption was checked by log (−log) plots. The Cox regression model was applied to evaluate the effect of the *ER-α* genotype on overall survival with adjustments for age at diagnosis and known prognostic factors for breast cancer, including tumor-node-metastasis status and cancer treatment. Stratified analyses by ER status and traditional breast cancer prognostic factors were done to examine potential interactive effects of these variables on the association between *ER-α* genotype and breast cancer survival. Test for multiplicative interaction was conducted by including the main term and product of two study variables in the Cox regression model. All statistical tests are based on a two-sided probability.

RESULTS

Among subjects with genotype data (n = 1,063), ER status was obtained from 739 breast cancer cases. Of these, ~62.4% were ER+ and 37.6% were ER−. Approximately 30.5% of cases were missing information on ER status. ER status was not significantly associated with 5-year survival in this study population. As expected, stage at diagnosis was an important predictor for overall survival (Table 1). Having radiotherapy was related to a higher mortality. Patients who received radiotherapy had a more advanced cancer; however, the inverse association persisted even after adjustment for stage of disease. Nearly all subjects received surgery, six subjects with missing surgery information were excluded from the subsequent analyses. The vast majority (95%) of participants received chemotherapy. Older age at diagnosis was related to lower but not statistically significant survival. Clinical prognostic factors were found to be similar for all subjects as well as those who were included in the genotyping analyses (data not shown).

Unadjusted and multiple-adjusted associations of breast cancer survival with *Pvu*II, *Xba*I, and $(GT)_n$ polymorphisms are shown in Table 2. No overall association was observed between *Pvu*II or *Xba*I polymorphisms and breast cancer survival. For the $(GT)_n$ polymorphism, analyses were focused on the five most

Table 1 Overall survival by demographics and known prognostic factor for breast cancer: Shanghai Breast Cancer Study

	Descriptive characteristics*				
Subject characteristic	*n*	No. deaths	5-year survival (%)	*P*	Adjusted† HR
Age at diagnosis					
<40	171	33	77	0.03	1.00
40-49	514	70	86		0.76 (0.50-1.15)
50-59	232	50	76		1.14 (0.74-1.78)
>60	135	19	85		0.73 (0.42-1.29)
Education					
Elementary	124	26	78	0.25	1.00
Middle or high school	800	129	83		0.74 (0.45-1.20)
College or higher	128	17	85		0.58 (0.31-1.11)
Tumor-necrosis-metastasis					
I-II	865	103	88	<0.0001	1.00
III-IV	114	49	57		4.27 (3.02-6.04)
Unknown	73	20	73		2.54 (1.55-4.17)
ER status					
Positive	455	73	82	0.83	1.00
Negative	275	43	82		0.95 (0.65-1.39)
Unknown	322	56	82		0.87 (0.61-1.25)
PR status					
Positive	459	73	83	0.78	1.00
Negative	263	41	83		1.08 (0.73-1.58)
Unknown	330	58	81		0.92 (0.64-1.30)
Surgery					
Yes	1,046	170	84	0.19	1.00
No	0	0	—		—
Unknown	6	2	67		1.41 (0.32-6.14)
Chemotherapy					
Yes	983	161	84	0.50	1.00
No	58	8	86		0.73 (0.35-1.52)
Unknown	11	3	73		1.61 (0.49-5.32)
Radiotherapy					
Yes	402	100	75	<0.0001	1.00
No	499	53	89		0.47 (0.33-0.66)
Unknown	151	19	87		0.58 (0.35-0.96)

*Distribution of data across all three genotypes was similar; therefore, *PVU*II was chosen for comparison purposes only.
†Adjusted for age at diagnosis, stage of disease, and radiotherapy.

common alleles: $(GT)_{15}$, $(GT)_{16}$, $(GT)_{17}$, $(GT)_{18}$, and $(GT)_{23}$. A significant increased risk for death was observed among participants carrying one $(GT)_{18}$ allele [multiple-adjusted hazard ratio (HR), 1.61; 95% confidence interval (95% CI), 1.15-2.27]. The risk, however, was not elevated among subjects homozygous for this allele (HR, 1.03; 95% CI, 0.33-3.27).

Genotype associations were modified by ER status in breast cancer tissues (Table 3). Among participants with an ER+ cancer, the adjusted HRs associated with Pp and PP genotypes compared with a pp genotype were 0.41 (95% CI, 0.25-0.67) and 0.54 (95% CI, 0.24-1.23), respectively. These genotypes were associated with HRs of 1.27 (95% CI, 0.61-2.61) and 3.30 (95% CI, 1.42-7.69) for ER-negative breast cancer. Similarly, among those carrying one or two $(GT)_{23}$ alleles the HR was 0.25 (95% CI, 0.09-0.69) compared with those with no $(GT)_{23}$ alleles for participants with ER-positive breast cancer, whereas the corresponding HR for ER-negative cancer was 1.48 (95% CI, 0.77-2.87). Tests for multiplicative interaction were both highly significant. For the *Xba*I polymorphisms, the genotype-breast cancer survival associations did not vary according to ER status.

The effects of ER status on breast cancer survival by ER-α genotypes are shown in Table 4. Among participants with a PP genotype, ER-negative cancer was associated with a worse prognosis than ER-positive breast cancer (HR, 3.93; 95% CI, 1.51-10.25), whereas ER negativity was not related to survival among those participants with pp or Pp genotypes. ER negativity was related to poor survival among patients who had one or two $(GT)_{23}$ alleles of the GT repeat polymorphism (HR, 5.27; 95% CI, 2.08-13.36) but was unrelated to prognosis among subjects carrying no $(GT)_{23}$ allele. We did not observe any interaction between *Xba*I polymorphism and ER status on breast cancer survival.

Additional analyses were conducted stratified by ER/PR status, disease stage, and age at diagnosis. Results from the analyses stratifying by ER/PR status were similar to those found when considering ER and PR status individually (data not shown). There were no differences in the genotype-survival association according to disease stage or age at diagnosis (data not shown).

DISCUSSION

In this study, we found a multiplicative interaction between *PvuII* and $(GT)_{23}$ polymorphisms in the *ER-α* gene and ER status of the breast cancer on breast cancer survival. Although ER status is known to modify the outcome of hormonal treatment for breast cancer (10), the biological mechanism behind its interaction with ER-α genotypes remains to be

Table 2 Association of breast cancer survival with *PVUII*, *Xba*I, and $(GT)_n$ polymorphisms in the *ER*-α gene: Shanghai Breast Cancer Study

	n	No. deaths	HR (95% CI) unadjusted	HR (95% CI) adjusted*
ER-α, *PVUII*				
pp	408	73	1.00 (reference)	1.00 (reference)
Pp	509	75	0.83 (0.60-1.15)	0.73 (0.51-1.04)
PP	135	24	1.01 (0.63-1.59)	0.95 (0.57-1.56)
ER-α, *Xba*I				
xx	531	93	1.00 (reference)	1.00 (reference)
Xx	493	73	0.87 (0.64-1.18)	0.80 (0.56-1.12)
XX	36	6	0.97 (0.43-2.22)	1.16 (0.50-2.68)
ER-α, $(GT)_n$ repeats				
No $(GT)_{15}$ alleles	729	122	1.00 (reference)	1.00 (reference)
One $(GT)_{15}$ allele	203	32	0.93 (0.63-1.37)	1.00 (0.68-1.48)
Two $(GT)_{15}$ alleles	12	0	—	—
No $(GT)_{16}$ alleles	319	51	1.00 (reference)	1.00 (reference)
One $(GT)_{16}$ allele	464	77	1.04 (0.73-1.48)	1.07 (0.75-1.53)
Two $(GT)_{16}$ alleles	161	26	0.98 (0.61-1.58)	1.01 (0.63-1.62)
No $(GT)_{17}$ allele	842	139	1.00 (reference)	1.00 (reference)
One $(GT)_{17}$ allele	98	15	0.94 (0.55-1.60)	1.03 (0.60-1.75)
Two $(GT)_{17}$ alleles	4	0	—	—
No $(GT)_{18}$ allele	704	102	1.00 (reference)	1.00 (reference)
One $(GT)_{18}$ allele	222	49	1.62 (1.15-2.28)	1.61 (1.15-2.27)
Two $(GT)_{18}$ alleles	18	3	1.13 (0.36-3.56)	1.03 (0.33-3.27)
No $(GT)_{23}$ allele	757	130	1.00 (reference)	1.00 (reference)
One $(GT)_{23}$ allele	172	21	0.69 (0.44-1.10)	0.66 (0.42-1.05)
Two $(GT)_{23}$ alleles	16	3	1.08 (0.35-3.41)	1.03 (0.32-3.24)

*Adjusted for age at diagnosis, stage of disease, and radiotherapy.

clarified. To our knowledge, no previous study has investigated the association between polymorphisms in the *ER*-α gene and breast cancer survival alone or with ER status.

The three polymorphisms investigated in this study are located in different areas of the *ER*-α gene. The GT repeat polymorphism is located at 2.8 kb 5′ to exon 1D, and *Pvu*II and *Xba*I are located on intron 1. These polymorphisms are not in close linkage disequilibrium in the Chinese population (7, 8).

Although polymorphisms in the *ER* gene have been linked to altered tissue responsiveness to estrogens, the functional

Table 3 Association of breast cancer survival with *PVUII*, *Xba*I, and $(GT)_n$ polymorphisms in the *ER*-α gene stratified by ER status: Shanghai Breast Cancer Study

	Status*					
	ER positive		ER negative		Unknown	
Genotype	Deaths/total	Adjusted HR (95% CI)†	Deaths/total	Adjusted HR (95% CI)†	Deaths/total	Adjusted HR (95% CI)
ER-α, *PvuII*						
pp	41/174	1.00 (ref)	14/120	1.00 (ref)	18/114	1.00 (ref)
Pp	25/221	0.41 (0.25-0.67)	18/122	1.27 (0.61-2.61)	32/166	1.51 (0.83-2.72)
PP	7/60	0.54 (0.24-1.23)	11/33	3.30 (1.42-7.69)	6/42	1.08 (0.42-2.74)
P for interaction						0.001
ER-ER-α, *Xba*I						
xx	37/236	1.00 (reference)	23/132	1.00 (reference)	33/163	1.00 (reference)
Xx or XX	36/224	1.06 (0.66-1.70)	21/145	0.88 (0.47-1.62)	22/160	0.73 (0.42-1.27)
P for interaction						0.73
ER-α, $(GT)_n$ repeats						
No $(GT)_{15}$ alleles	86/553	1.00 (reference)	53/308	1.00 (reference)	69/380	1.00 (reference)
One or two $(GT)_{15}$ alleles	13/94	0.93 (0.52-1.67)	9/63	0.87 (0.42-1.77)	10/58	1.08 (0.55-2.11)
P for interaction						0.87
No $(GT)_{16}$ alleles	45/372	1.00 (reference)	39/204	1.00 (reference)	53/255	1.00 (reference)
One or two $(GT)_{16}$ alleles	54/275	1.71 (1.15-2.55)	23/167	0.78 (0.46-1.32)	26/183	0.66 (0.41-1.07)
P for interaction						0.015
No $(GT)_{17}$ allele	90/597	1.00 (reference)	60/352	1.00 (reference)	75/405	1.00 (reference)
One or two $(GT)_{17}$ alleles	9/50	1.17 (0.59-2.34)	2/19	0.95 (0.23-3.94)	4/33	0.76 (0.28-2.12)
P for interaction						0.65
No $(GT)_{18}$ allele	72/541	1.00 (reference)	51/312	1.00 (reference)	65/363	1.00 (reference)
One or two $(GT)_{18}$ alleles	27/106	2.09 (1.33-3.29)	11/59	0.97 (0.49-1.94)	14/75	1.09 (0.60-1.97)
P for interaction						0.12
No $(GT)_{23}$ allele	95/561	1.00 (reference)	50/234	1.00 (reference)	71/384	1.00 (reference)
One or two $(GT)_{23}$ alleles	4/86	0.25 (0.09-0.69)	12/47	1.48 (0.77-2.87)	8/54	0.69 (0.33-1.44)
P for interaction						<0.001

*Adjusted for age at diagnosis, stage of disease, and radiotherapy.

Table 4 Association of breast cancer survival with ER status stratified by *PVUII*, *Xba*I, and $(GT)_n$ polymorphisms in the *ER-α* gene: Shanghai Breast Cancer Study

	Status*					
	ER positive		ER negative		Unknown	
Genotype	Deaths/total	Adjusted HR (95% CI)[b]	Deaths/total	Adjusted HR (95% CI)[b]	Deaths/total	Adjusted HR (95% CI)
ER-α, *PvuII*						
pp	41/174	1.00 (reference)	14/120	0.61 (0.34-1.11)	18/114	1.03 (0.35-3.08)
Pp	25/221	1.00 (reference)	18/122	1.08 (0.62-1.85)	32/166	0.81 (0.23-2.85)
PP	7/60	1.00 (reference)	11/33	3.93 (1.51-10.25)	6/42	2.93 (0.73-11.81)
P for interaction						0.001
ER-α, *Xba*I						
xx	37/236	1.00 (reference)	23/132	1.06 (0.66-1.72)	33/163	1.27 (0.52-3.11)
Xx or XX	36/224	1.00 (reference)	21/145	1.13 (0.85-1.52)	22/160	1.32 (0.71-2.48)
P for interaction						0.73
ER-α, $(GT)_n$ repeats						
No $(GT)_{15}$ alleles	86/553	1.00 (reference)	53/308	1.16 (0.84-1.59)	69/380	1.01 (0.46-2.25)
One or two $(GT)_{15}$ alleles	13/94	1.00 (reference)	9/63	1.29 (0.65-2.56)	10/58	1.85 (0.31-10.88)
P for interaction						0.87
No $(GT)_{16}$ alleles	45/372	1.00 (reference)	39/204	1.40 (0.96-2.05)	53/255	0.86 (0.54-1.38)
One or two $(GT)_{16}$ alleles	54/275	1.00 (reference)	23/167	1.66 (0.78-3.55)	26/183	0.83 (0.25-2.75)
P for interaction						0.015
No $(GT)_{17}$ allele	90/597	1.00 (reference)	60/352	1.15 (0.85-1.55)	75/405	1.34 (0.69-2.60)
One or two $(GT)_{17}$ alleles	9/50	1.00 (reference)	2/19	0.59 (0.12-2.87)	4/33	1.45 (0.14-15.36)
P for interaction						0.65
No $(GT)_{18}$ allele	72/541	1.00 (reference)	51/312	1.22 (0.88-1.70)	65/363	1.66 (0.83-3.32)
One or two $(GT)_{18}$ alleles	27/106	1.00 (reference)	11/59	0.85 (0.42-1.72)	14/75	0.48 (0.11-2.23)
P for interaction						0.12
No $(GT)_{23}$ allele	95/561	1.00 (reference)	50/234	0.95 (0.69-1.31)	71/384	1.08 (0.53-2.19)
One or two $(GT)_{23}$ alleles	4/86	1.00 (reference)	12/47	5.27 (2.08-13.36)	8/54	7.33 (1.32-40.75)
P for interaction						<0.001

*Adjusted for age at diagnosis, stage of disease, and radiotherapy.

impact of these *ER-α* polymorphisms is not well understood (11). Noncoding short tandem repeats may act as protein binding sites. It has been reported that protein binding may depend on the ability of short tandem repeats to form specific DNA structures and an increasing number of short tandem repeats in the promoter region or introns may interfere with transcription processes by their effect on secondary DNA structure (9). Previous studies investigating ER polymorphisms and breast cancer risk have produced mixed results, which may be explained by ethnicity of the populations under study (3-8). We have linked presence of the $(GT)_{17}$ or $(GT)_{18}$ allele of GT polymorphism, or PP allele of *PvuII* polymorphism to an reduced risk of breast cancer (7, 8) in Chinese population. We did not find that the associations between ER-α genotypes and breast cancer risk vary according to ER/PR status (7, 8).

Previous studies have shown that common *ER-α* polymorphisms, including the one found at the *Pvu*II restriction site, are associated with cardiovascular disease risk (13) and possibly modify the effects of estrogens on HDL cholesterol level (14) and changes in bone mineral density and vertebral fractures (15). The latter two studies showed that women with the pp genotype who received hormone replacement therapy had a significantly more pronounced response in outcome than women with other genotypes (i.e., a greater increase in HDL cholesterol levels and bone mineral density; refs. 14, 15), suggesting that this polymorphism may have an interactive effect with hormones.

ER status is the main determinant for tamoxifen use and modulates its effect. According to an ongoing study of Chinese breast cancer patients conducted by our group, we found that 83% of ER-positive breast cancer versus 28% of ER-negative breast cancer patients used tamoxifen up to 18 months post-diagnosis. The interaction between genotype and ER status on breast cancer prognosis found in the current study, therefore, could reflect a differential effect of tamoxifen by genotype. Unfortunately, information regarding tamoxifen use in the current study was collected ~3 to 4 years after cancer diagnosis. This information was missing for most of the deceased cases and subjects whose survival status was established via linkage to the death registry. Therefore, direct evaluation of the interaction between *ER-α* gene polymorphism and tamoxifen use was not possible. Nevertheless, we did include tamoxifen use in the model that evaluated the interaction between genotype and ER status, treating those with missing tamoxifen information as a subgroup. We found that patterns of interactive effect remained but the point estimates attenuated (data not shown), suggesting tamoxifen use may play a role in the ER genotype and breast cancer survival association. Future studies are needed to evaluate the possible interactive effect between ER genotype and tamoxifen use on breast cancer survival.

Our study has several strengths that should be considered in interpreting its results, including (a) a population-based patient cohort, (b) the relatively long follow-up period of 5.2 years, (c) a large sample size, (d) relatively homogeneous genetic background (~98% of the study population are Han Chinese), and (e) detailed information on ER/PR status, disease stage, and treatment information.

There are also a few limitations that must be considered in evaluating these results. As mentioned above, information regarding tamoxifen use was only collected for a subset of the women participating in this study, making the evaluation

of the interaction of ER genotype and tamoxifen impossible. In addition, ER status was abstracted from hospital record and missing for ~30% of the study participants, thus compromising the statistical power of this study to evaluate interactions between genotype and ER status.

In summary, our findings of an interaction between ER status and ER-α polymorphisms on breast cancer survival are new and need to be confirmed in future studies. If confirmed, these findings could have important clinical implications in breast cancer treatment.

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Identification Page

Julie L. Elam, MSN, RN
Doctoral Student, Indiana University School of Nursing
Ph (W): 317-274-4529
Fax (W): 317-278-2021
jlelam@iupui.edu

Xiao-Ou Shu MD, PhD
Professor, Vanderbilt University Center for Health Services Research
Ph (W): 615-936-0713
Fax (W): 615-936-1269
Xiao-ou.shu@vanderbilt.edu

Janet S. Carpenter, PhD, RN
Associate Professor, Indiana University School of Nursing
Ph (W): 317-274-4529
Fax (W): 317-278-2021
carpentj@iupui.edu

Sonia Boyapati, PhD
Post-Docotoral Research Fellow, Vanderbilt University Center for Health Services Research
Ph (W): 615-936-0713
Fax (W): 615-936-1269
Sonia.boyapati@vanderbilt.edu

Janet Friedmann-Gilchrist, PhD, RD
Clinical Nutrition Investigator, Arkansas Children's Nutrition Center
Ph (W): 501-364-2805
Fax (W): 501-364-3947
gilchristjanetm@uams.edu

Address for correspondence: Julie L. Elam, 1111 Middle Drive, NU338, Indianapolis, IN, 46202,

jlelam@iupui.edu

Author Biographies

Ms. Julie L. Elam is a doctoral student at the Indiana University School of Nursing. She was a Research Clinical Nurse Specialist at Vanderbilt University at the time this project was implemented.

Dr. Xiao-Ou Shu is a Professor at Vanderbilt University. She was the lead investigator on this project.

Dr. Janet S. Carpenter is an Associate Professor in the Indiana University School of Nursing. She was an Assistant Professor at Vanderbilt University during this project.

Dr. Sonia Boyapati was a post-doctoral research fellow at Vanderbilt University and served as the Project Coordinator for this study.

Dr. Janet Gilchrist is a Research Scientist at the Arkansas Children's Nutrition Center and was a Research Assistant Professor at Vanderbilt University during this project period.

Title Page

Problems with Ginseng for the Treatment of Breast Cancer Fatigue

Abstract

1. **Abstract and Key Words:** An abstract of no more than 150 words, which includes the central theme and major subdivisions of the paper. A research paper should include the general purpose, methodology, results, and conclusions/application of the study. The abstract should not contain abbreviations, footnotes, or references. Include 3 key word descriptors for indexing purposes. Use terms from Medical Subject Headings (MeSH) list of Index Medicus; if suitable MeSH terms are not yet available use present terms.

Background: Although literature suggests fatigue is commonly reported by women during and following breast cancer treatment, treatment options are limited. While ginseng is widely used among cancer survivors in Asian countries to increase energy, its efficacy for treating cancer related fatigue has not been carefully studied. The purpose of this study was to generate pilot data concerning the use and efficacy of ginseng for treating breast cancer related fatigue.

Patients/Methods: Breast cancer survivors seeking treatment for fatigue participated in an eight week randomized, double blind, placebo controlled trial.

Results: A variety of problems in evaluating this intervention were noted including large numbers of survivors with contraindications to ginseng use, our lack of success in double blinding the intervention due to strong odor from the ginseng, and measurement device failure.

Discussion: Evaluating ginseng for breast cancer treatment related fatigue or implementing this intervention in a clinical setting may not be feasible.

Key words: Complementary Therapy, Breast Cancer, Symptoms

Background/Introduction

Literature suggests that fatigue is commonly reported by women during and following breast cancer treatment. Fatigue has been defined as the feeling of tiredness influenced by circadian patterns that varies in terms of duration, intensity and unpleasantness and has been reported as the most distressing complaint of cancer treatment (Corless, Bunch, Kemppainen, Holzemer, Nokes, Eller, Portillo, Butensky, Nicholas, Bain, Davis, Kirksey, & Chou, 2002; Piper, Lindsey, & Dodd, 1987). It is generally believed that fatigue persists for years after treatment ends (Berger, 1998; Berger, 2003; Berger & Higginbotham, 2000; Berger & Walker, 2001), suggesting that even survivors of breast cancer are in need of appropriate treatment. However, because mechanisms and etiologies for fatigue are poorly understood, treatment options can be limited.

Although ginseng is widely used among cancer survivors in Asian countries to increase energy, efficacy for treating cancer related fatigue has not been carefully studied (Miller, 1998; Sato & Miyata, 2000; Shin, Kim, Yun, Morgan, & Vainio, 2000). Most studies investigating ginseng for fatigue have been related to physical activity outcomes, rather than fatigue per se, and have been limited by lack of control groups and small sample sizes (Bentler, Hartz, & Kuhn, 2005; Block & Mead, 2003; Cardinal & Engels, 2001). Therefore, the purpose of this study was to generate pilot data concerning the use and efficacy of ginseng for treating breast cancer related fatigue.

There are three major types of ginseng: Asian ginseng (Panax or red ginseng), Siberian ginseng (Eleutherococcus senticosus), and American ginseng (Panax quinquefolius). All ginseng types contain saponins and have different molecular structures and, thus, different effects. Red ginseng, the most common Asian ginseng, is processed and considered a "hot" agent in

traditional Chinese medicine (Attele, Wu, & Yuan, 1999; Medical Economics Company, 1998; Miller, 1998). Red ginseng possesses a strong stimulating and restoring effect for vitality and energy. This type of ginseng can only be used for brief periods to facilitate disease recovery and is typically used in winter seasons to increase energy and increase immune activity. Toxicity can ensue if this hot agent is overdosed or used improperly. The result is "ginseng syndromes" that cause increased blood pressure, dry eyes and throat. Siberian or Russian ginseng is derived from the plant Eleutherococcus senticosus and has similar stimulant and toxic effects as other ginseng products (Attele et al., 1999; Bahrke & Morgan, 2000). For example, Siberian ginseng has been associated with false elevations of digoxin levels in absence of true digoxin toxicity (Miller, 1998). Due to the noted toxicities from both Asian and Siberian ginseng, this pilot study used unprocessed ginseng (American ginseng) for the intervention due to its mild overall effects. American ginseng can be used for longer time periods to promote health and increase disease resistance without the adverse effects of the "hot" agents (Attele et al., 1999; Bahrke & Morgan, 2000; Medical Economics Company, 1998).

Patients and Methods

Pre- and postmenopausal breast cancer survivors who had completed chemotherapy and/or radiotherapy and who were seeking treatment for fatigue were eligible for this study. The recruitment goal for this pilot study was 20 participants. Subjects were excluded if they met the following criteria: currently taking chemotherapy or radiotherapy; had never received chemotherapy nor radiotherapy; history of chronic liver/kidney diseases; taking insulin or oral hypoglycemic drugs for diabetes; diagnosed with depression or hypertension; taking antidepressants for any reason; pregnant as verified by urine pregnancy testing; or taking any anticoagulants including antidepressants, warfarin, heparin, aspirin, coumadin, or other NSAIDS

daily (Hu, Yang, Ho, Chan, Heng, Chan, Duan, Koh, & Zhou, 2005; Medical Economics Company, 1998). These exclusion criteria were based on clinical pharmacology publications and review articles regarding the pharmacological effects of ginseng (Bahrke & Morgan, 2000; Coon & Ernst, 2002; Hu et al., 2005; Medical Economics Company, 1998; Miller, 1998).

Potential participants were identified from two recruitment databases maintained at a southeastern university who had agreed to be contacted for other studies received a screening telephone call. Detailed information about the study was given over the phone using a standardized script to women who were interested and eligible in participating in the study. After receiving verbal consent, written informational packets were mailed to the participant including study pamphlet, consent, and study team contact information. One week after packets were mailed, follow-up phone calls were performed to assess interest in participation and to schedule the first study visit. Written informed consent was obtained via mail or at the first study visit.

Eligible and interested subjects were randomized into two treatment groups, group A or group B. Group A took part in one week of baseline assessment, two weeks of placebo followed by four weeks of ginseng supplement. Group B took part in one week baseline assessment, two weeks of ginseng followed by four weeks of placebo. Subjects were instructed to take three capsules per day of placebo sugar pill or ginseng (500mg/capsule) (provided by Root to Health Inc., Wausau, WI) depending on randomization.

Outcomes were assessed using subjective and objective measures. Self-reported fatigue was assessed using the fatigue and vigor subscales of the Profile of Mood States. In addition, objective activity and sleep were measured using wrist actigraphy (Octagonal Basic Motionlogger ®, Ambulatory Monitoring, Inc., Ardsley, NY) and a waist accelerometer (RT3 Tri-Axial Research Tracker, Stayhealthy Inc., Monrovia, CA).

Procedures for the study were as follows. On the first day of the baseline assessment, the study nurse traveled to participants' homes or worksites and completed the following: (1) review of study procedures (2) blood pressure measure, and (3) instruction on the use and wear of the wrist actigraphy and waist accelerometer. Once placed, the objective measures were worn for 7 days. The wrist actigraph was worn for 24 hours per day for each of the seven days while the waist accelerometer was worn during the daytime while awake. The two objective measures were placed during the baseline, third, fifth, and seventh week of the study. Blood samples were also drawn for natural killer cell activity (NK cell) consisting of a 10 ml peripheral blood draw at baseline, week 3 and week 7.

Results

Recruitment results revealed low feasibility of implementing this intervention on a large scale. Of 166 women who were screened, 90% were found not to be eligible for the following reasons: 22% were not fatigued or did not feel fatigued enough to want treatment; 21% reported having a medical contraindication to taking ginseng; and 47% were taking medications for which no data on the safety of taking ginseng in combination with those medications were known. During an 8 month recruitment period, only 5 eligible patients were identified.

Difficulties in double blinding the study were also encountered. Efforts were made to mask the odor of the ginseng but these were unsuccessful. The capsules were stored with mint leaves but this did not succeed in masking the odor of the ginseng tablets. Upon opening the pill containers, patients and study nurses could readily distinguish between placebo and ginseng capsules.

Equipment failures were also encountered when the wrist and waist accelerometers were used to assess activity levels. Problems in initializing and downloading these devices were

routinely encountered and resulted in none of the 5 eligible patients having complete data for all time points.

No subjects withdrew due to undesirable side effects of the ginseng/placebo. No adverse events were reported.

Discussion

Our pilot study results suggest that evaluating the use of ginseng for breast cancer treatment related fatigue or implementing this intervention in a clinical setting may not be feasible. Although ginseng is sold over the counter at health food stores, we identified very few women who did not have medical contraindications to taking this herb. These recruitment results point to the importance of assessing ginseng use in patients and counseling as necessary regarding the potential for drug-herb interactions or other adverse effects. Study results also point to the need to consider feasibility of double blinding when using ginseng or other herbal interventions. The inability to successful blind study participants and nurses could seriously undermine future studies. Finally, our results confirm the need for careful pre-testing, staff training, and patient instruction on the use of physiological monitoring devices. Future studies using these devices should allow for attrition related to equipment failure when determining the sample size needed.

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