
Yen-Ling Low, Alison M. Dunning, Mitch Dowsett, Robert N. Luben, Kay-Tee Khaw, Nick J. Wareham, and Sheila A. Bingham

Abstract

Studies to identify common genetic variants contributing to breast cancer risk often yield inconsistent results. Breast cancer is a complex disease involving both genetic and environmental determinants. Dietary isoflavones are thought to reduce breast cancer risk by stimulating circulating sex hormone-binding globulin (SHBG) levels. The SHBG gene contains a D356N polymorphism and the N variant is associated with reduced SHBG clearance compared with the D variant. In this study, we show a significant gene-environment interaction between SHBG D356N polymorphism and dietary isoflavone exposure on circulating SHBG levels in 1,988 postmenopausal women. SHBG levels were positively associated with isoflavones in women carrying the N variant ($\tau_P^2 = 1.9\%$; $P = 0.006$) but not in women carrying only the D variant ($\tau_P^2 = 0.0\%$; $P = 0.999$; $P_{inter} = 0.019$). This finding shows that the subtle effects of some genetic variants may be magnified and only become detectable in the presence of certain exposures. This gene-environment interaction might explain heterogeneity in studies associating SHBG gene variants and soy consumption with breast cancer risk in Far East population exposed to high isoflavone levels compared with populations with lower levels. (Cancer Res 2006; 66(18): 8980-3)

Introduction

Inability to replicate results is a common problem in genetic association studies of complex diseases. Explanations include false-positive results that have occurred by chance, inadequate statistical power, and true variation in the underlying association between genotype and outcome between populations studied (1). However, secular trends in incidence and migrant studies suggest that lifestyle factors have substantial effects on risk (2). Failure to assess exposure to environmental factors in the interplay between genes and environmental factors may be a further explanation for the lack of consistent findings in genetic association studies. Here, we report a significant gene-environment interaction in relation to breast cancer risk and discuss how it may explain heterogeneity in genetic association studies. The environmental exposure examined are dietary phytoestrogens, the gene variant is the SHBG D356N polymorphism, and the intermediate risk marker is circulating sex hormone-binding globulin (SHBG) levels, which are directly associated with breast cancer risk in postmenopausal women (3).

In a previous study, we investigated the associations between polymorphisms in sex hormone metabolism genes and circulating sex hormones in 2,453 breast cancer cases and 1,850 healthy postmenopausal controls in United Kingdom. The N variant of the SHBG D356N (rs6259) polymorphism is thought to create SHBG molecules with reduced clearance (4). This results in higher circulating SHBG levels, which should translate to reduced breast cancer risk. However, the SHBG D356N (rs6259) polymorphism was not significantly associated with breast cancer risk [NN versus DD; odds ratio (OR), 1.53; 95% confidence interval (95% CI), 0.90-2.59] in our previous case control study (5). The SHBG D356N polymorphism also showed no significant association in a separate case control study on familial ($n = 158$) and sporadic breast cancer ($n = 222$) in Nordic and Polish women [NN versus DD; OR, 0.81; 95% CI, 0.58-1.15; ref. 6]. Another study in Italian women found that the N variant was more common in breast cancer patients (21.2%) than the controls (11.6%; ref. 7). Hence, none of the studies conducted in Western populations have found a significant protective effect for the N variant.

Phytoestrogens (mainly classified into isoflavones and lignans) are diphenolic compounds in plants, which subsequently form part of the human diet. They are structurally similar to 17β-estradiol and have been postulated to protect against breast cancer through modulation of endogenous sex hormone levels (8). Soy is a particularly rich source of the isoflavone phytoestrogens, which are therefore consumed in large amounts in Far East populations, such as China. Phytoestrogens have been postulated to stimulate SHBG (encoded by SHBG gene) production and increase circulating SHBG levels. They may also directly modulate concentrations of circulating estradiol by inhibiting enzymes involved in estrogen biosynthesis and metabolism, such as 17β-hydroxysteroid dehydrogenase (encoded by HSD17B1 gene) and aromatase (encoded by CYP19 gene; ref. 8). We therefore investigated whether there were interactions between gene variants in SHBG, HSD17B1, and CYP19 genes and phytoestrogen exposure in affecting circulating sex hormones and SHBG levels.

Materials and Methods

Participants in this large cross-sectional study were 1,988 healthy postmenopausal women in Norfolk arm of the European Prospective...
Investigation of Cancer and Nutrition cohort (9) and served as controls for our previous study (5). All subjects were >55 years, had reported no menstruation for at least 1 year, and had not taken hormone replacement therapy for at least 3 months before giving the blood sample and had their postmenopausal status confirmed through hormonal measurement (plasma estradiol <150 pmol/L). Five polymorphisms in HSD17B1, CYP19, and SHBG genes were chosen based on available information on known SNPs in this gene at the time of genotyping. Genotyping was carried out using end point Taqman assays (Applied Biosystems, Warrington, United Kingdom) in 384-well arrays, which included blank wells as negative controls. Estradiol was measured from plasma by RIA after ether extraction (10). Testosterone was measured using a solid-phase RIA kit (Diagnostic Products, Gwynedd, United Kingdom). SHBG was measured using a liquid-phase immunoradiometric kit (Orion Diagnostica, Espoo, Finland). Androstenedione was measured using a solid-phase RIA kit (Diagnostic Systems Laboratories, Oxford, United Kingdom). Estrone was extracted with ether and liquid column chromatography on a Lipidex 5000 (Perkin-Elmer, Boston, MA) with elution using chloroform/hexane/methanol (50:50:1) and then measured using a RIA kit. Details of genotyping analysis and hormone measurements have been described previously (5). Urinary levels of isoflavones (sum of daidzein, genistein, glycitein, equol, and O-desmethylangolensin) and lignans (sum of enterodiol and enterolactone) were measured using liquid chromatography/tandem mass spectrometry (11) and used as biomarkers for dietary isoflavones and lignans intake (12).

Both urinary phytoestrogens and plasma SHBG data were skewed. Therefore, data were logarithmically transformed to obtain approximately normal frequency distributions. All subsequent statistical tests were done on transformed data.

ANCOVA was used to assess the association between phytoestrogen exposure, genetic polymorphisms, and sex hormone and SHBG levels. Analyses were adjusted for the following variables, which are or are potentially associated with sex hormone levels: age, body mass index, physical activity, time of day at venipuncture, and time interval between last meal and venipuncture. Phytoestrogen-gene interactions were formally assessed by including interaction terms in the statistical model. To minimize multiple testing, only two summary measures of phytoestrogens (isoflavones and lignans) were used instead of testing each of the seven phytoestrogens individually. In addition, for polymorphisms in HSD17B1 and CYP19, phytoestrogen-gene interactions were only tested on the ratio of substrate and product sex hormones whose conversions were catalyzed directly by the enzyme. Only where a phytoestrogen-gene interaction term was significant (P < 0.05) was then the analysis of the association between phytoestrogens and the hormone was repeated, stratified according to different genotypes. We chose to use the stringent criteria of P < 0.05 so as to reduce the chance of false positives. Only results for the isoflavone interaction with SHBG D356N variant had P_interaction < 0.05 and are shown. P_values of the other interactions were all above 0.10 and are not shown.

Results and Discussion

We found significant diet-gene interaction between isoflavones and SHBG D356N (rs6259) polymorphism. There was no significant relationship between isoflavones and SHBG levels in women as a whole (β = 0.011; P = 0.149). However, significant differences emerged when the analysis was stratified according to women with different genotypes for SHBG D356N polymorphism. In women carrying either one or two copies of the N variant, isoflavone exposure was positively associated with plasma SHBG levels (β_N = 1.9%; P = 0.006). However, no association was observed in women carrying only the D variant (β_D = 0.0%; P = 0.999; P_interaction = 0.019). Figure 1 shows the interaction between isoflavones and SHBG D356N polymorphism in affecting SHBG levels. There were no significant interactions between isoflavones and the other polymorphisms analyzed and between lignans and any of the polymorphisms analyzed.

The observed interaction between isoflavones and SHBG D356N polymorphism is supported by a biologically plausible mechanism. The N variant is thought to create an anchor site for an additional carbohydrate chain to attach onto the SHBG molecule, resulting in reduced clearance rate of SHBG from the circulation compared with the D variant (4). The N variant has been associated with higher SHBG levels in these postmenopausal women in our previous study with N allele carriers having ~7% (P = 0.005) higher SHBG levels compared with noncarriers (5). In Chinese postmenopausal women, N allele carriers had 11.9% higher SHBG levels compared with noncarriers, although that study was smaller (n = 411) and the difference was not significant (P = 0.06; ref. 13). Neither of these studies included exposure to isoflavones. The results of the present study suggest that, in women carrying the N variant (associated with reduced SHBG clearance), isoflavones stimulate SHBG levels in a dose response manner. However, isoflavones seem to have no effect in women with higher SHBG clearance rate.

Higher SHBG levels have been associated with lower breast cancer risk in postmenopausal women and the OR for a doubling in SHBG levels was 0.88 (95% CI, 0.76-1.03; ref. 3). At the low levels found in this population, the protective effect of a 7% higher SHBG levels associated with the N allele would have an undetectable effect on breast cancer risk. From Fig. 1B, it can be inferred that, if a study of gene variants was conducted in a Western population at the lower range of isoflavone exposure, very little difference in SHBG levels between women carrying different gene variants would be observed. For instance, the SHBG D356N polymorphism only accounted for 0.6% of the total variance in SHBG levels in British postmenopausal women (5). Consequently, the N variant would be unlikely to be associated with reduced breast cancer risk as observed in previous studies in Western populations (5–7).

However, in Chinese populations of postmenopausal women, SHBG levels are higher than found here (13, 14). In one study, SHBG levels were twice those of this United Kingdom population, and the N variant was associated with a significant reduction in breast cancer risk (NN/ND versus DD; OR, 0.73; 95% CI, 0.53-0.99). The effect was particularly marked in women with above median levels of SHBG (OR, 0.50; 95% CI, 0.28-0.90) with no significant effect in women with lower plasma levels (13).

Isoflavone exposure is 10 times higher in Chinese and Japanese populations consuming soy compared with Western populations (8) and it would be of interest to examine whether the interaction found here is also present in populations at higher phytoestrogen exposure levels. Moreover, in circumstances where there are significant gene-environment interactions, failure to take into account the level of environmental exposure may lead to genetic association studies producing conflicting results. This finding shows how gene-environment interactions could be a further explanation for the lack of consistency in genetic association studies, as results may be dependent on the level of relevant environmental exposures, most of which are not captured in such studies. This hypothesis should be tested further in populations that are sufficiently large to examine interactions at different isoflavone exposure levels and in which information on genotype, isoflavone exposure, SHBG levels, and breast cancer risk are captured in the same study.

This study is the largest cross-sectional study conducted thus far to examine the interaction between phytoestrogen exposure and SHBG gene variants in postmenopausal women (N = 1,988).
Previous cross-sectional studies (15–18) have involved not >500 postmenopausal women and have only investigated the main effects between phytoestrogen exposure and SHBG levels but not the diet-gene interactions. Only one previous study conducted by us has examined phytoestrogen-gene interactions with SHBG variants (19). That study involved only 125 postmenopausal women and did not find any significant interaction between phytoestrogen exposure and \( \text{SHBG} \) gene variants, probably due to limited power.

In contrast, this current study involved 1,988 subjects. Even in the stratified analysis, we have 72% power to detect a significant association between phytoestrogen exposure and SHBG levels at \( P < 0.05 \) for women carrying the \( N \) variant (\( n = 408 \)).

The finding of gene-environment interaction in this study also shows the importance of studying relevant gene variants in studies of environmental factors on cancer risk. From Fig. 1A, there was no significant association between phytoestrogen exposure and SHBG levels at \( P < 0.05 \) for women carrying the \( N \) variant (\( n = 408 \)).

The picture proved to be misleading as distinct differences emerged when the data were stratified according to women with different genotypes. Hence, where gene-environment interaction exists, failure to consider genotype may mask significant associations specific to particular genotypes. This is likely to contribute to the considerable heterogeneity in results of studies investigating isoflavones and breast cancer risk as highlighted in a recent meta-analysis and accompanying editorial on soy intake and breast cancer risk (20, 21).

Genes are not amenable to change, whereas environmental factors are. From the public health perspective, the usefulness of genetic association studies will be greatly enhanced if they could incorporate investigations of relevant environmental exposure and potential gene-environment interactions. Studies of such interactions will not only clarify the inconsistent literature on the low-penetrance gene variants affecting breast cancer risk but also uncover ways by which risk may be modified for those who may derive maximal benefit.

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References

Implications of Gene-Environment Interaction in Studies of
Gene Variants in Breast Cancer: An Example of Dietary
Isoflavones and the D356N Polymorphism in the Sex
Hormone-Binding Globulin Gene

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