Phytoestrogen Exposure, Polymorphisms in COMT, CYP19, ESR1, and SHBG Genes, and Their Associations With Prostate Cancer Risk


Abstract: Prospective phytoestrogen exposure was assessed using both biomarkers and estimates of intake in 89 British men recruited into the Norfolk arm of the European Prospective Investigation into Cancer and Nutrition study, men who subsequently developed prostate cancer. Results were compared with those from 178 healthy men matched by age and date of recruitment. Levels of seven phytoestrogens (daidzein, genistein, glycitein, O-desmethylangolensin, equol, enterodiol, and enterolactone) were measured in spot urine and serum samples. Five single-nucleotide polymorphisms in COMT, CYP19, ESR1, and SHBG genes were genotyped. Urinary levels of all phytoestrogens correlated strongly with serum levels. Correlation coefficients ranged from 0.63 (glycitein) to 0.88 (daidzein) (P < 0.001). Urinary and serum levels correlated significantly with isoflavone intake assessed from food diaries (R = 0.15–0.20; P < 0.05) but not with that from a food-frequency questionnaire. Odds ratios for phytoestrogen exposure, as assessed using the four methods, were not significantly associated with prostate cancer risk (P = 0.15–0.94). Men with the CC genotype for the ESRI PvuII polymorphism had significantly higher risk for prostate cancer compared with men with the TT genotype [adjusted odds ratio = 4.65 (1.60–13.49); P = 0.005]. Our results utilizing a combined prospective exposure provide no evidence that phytoestrogens alter prostate cancer risk in British men, whereas the C allele for the PvuII polymorphism may be associated with increased risk.

Introduction

Prostate cancer incidence varies widely in different regions of the world, with a 10- to 80-fold difference in age-standardized rates between men in North America and men in Japan and China, respectively (1). Migrant studies indicate that the variation in international prostate cancer rates is partly due to lifestyle and environmental factors in addition to differences in prostate cancer detection (2,3). Phytoestrogens are one of the several dietary compounds under active investigation as a possible explanatory factor. Phytoestrogens are naturally occurring plant compounds that are structurally similar to the hormone 17β-estradiol. Phytoestrogens in the human diet can be divided into two main groups, the isoflavones and the lignans. The isoflavones include glycitein, daidzein, and genistein found in legumes and especially soy and their metabolites equol and O-desmethylangolensin (O-DMA) (4). The lignans include enterolactone and enterodiol, derived from colonic microbial fermentation of plant lignans such as matairesinol and secoisolariciresinol that are found in a wide variety of plant foods.

Phytoestrogens are thought to protect against prostate cancer through a variety of mechanisms. Phytoestrogens have been shown to bind competitively to the prostate-dominant estrogen receptor β (5), inhibit steroid metabolism enzymes (for example, 5α-reductase, aromatase, and 17β-hydroxysteroid dehydrogenase) (6–12), stimulate apoptosis (13), act as antioxidants (14,15), and inhibit key enzymes involved in carcinogenesis such as tyrosine kinase (16) and DNA topoisomerase (17). Animal studies have provided supportive evidence that phytoestrogens suppress prostate carcinogenesis in rodents with tumor implants or chemically induced tumors (18).

Despite plausible cancer-protective mechanisms supported by in vitro studies and encouraging results from animal studies, epidemiological data have been inconsistent. A major challenge in conducting epidemiological studies of phytoestrogen and cancer risk lies in quantifying phytoestrogen exposure accurately. Phytoestrogens are absorbed from food, circulate in the bloodstream, and are excreted in
urine. Phytoestrogen exposure can be assessed by food intakes from food-frequency questionnaires (FFQ) or food diaries and translated to dietary phytoestrogen intake using food composition databases or using phytoestrogen concentrations in blood and urine as biomarkers of exposure. Little is known about the agreement between the different methods in quantifying phytoestrogen exposure and whether the choice of method will affect risk estimates.

In this study we used a combination of food intake assessment and biomarkers to assess the risk of developing prostate cancer in a nested prospective case-control design among men in the Norfolk arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) study. We also assessed prostate cancer risk in relation to five single-nucleotide polymorphisms (SNPs) in four genes (CYP19, ESR1, SHBG, and COMT) involved in sex hormone metabolism and thus possibly phytoestrogen action. Aromatase (encoded by CYP19) is expressed in the stroma and converts androgens to estrogens in the prostate. Estrogens may influence prostate cancer risk through direct signaling via estrogen receptor-alpha (encoded by ESR1) and affect androgen bioavailability through competitive binding to sex hormone-binding globulin (encoded by SHBG) or through generating mutagenic metabolites that can be detoxified by catechoh-O-methyltransferase (encoded by COMT) (19). Five SNPs in these genes were genotyped with the intention of studying phytoestrogen–gene interactions. However, due to the relatively small number of cases and the small number of rare homozygotes for some SNPs, it was not viable to study phytoestrogen–gene interactions in this study. Nonetheless, we have presented the results of the genetic associations with prostate cancer risk.

**Subjects and Methods**

**Study Subjects**

In EPIC-Norfolk, men and women aged 45–75 yr residing in Norfolk, UK, were recruited in 1993–1997 using general practice age-sex registers. A total of 30,452 men and women completed a health questionnaire and gave written informed consent. Permission for the study was obtained from The Norfolk and Norwich Hospital Ethics Committee. FFQs were mailed to the subjects for completion prior to the medical examination. The completed questionnaires were checked for completeness at the medical examination. The 7-day food diaries were given to the subjects at the medical examination after instruction. These were completed and returned by mail (93% compliance). A total of 86 cases and 171 controls completed both the FFQ and food diaries. Information from the 7-day dietary diaries was used to calculate total energy intake using a custom-designed dietary assessment software program, DIER (Data Into Nutrients for Epidemiological Research) (22). Data entries were blinded to case or control status. Dietary isoflavone intakes were determined using a food composition database based on daidzein and genistein concentrations measured in 300 commonly eaten foods. Details on the sampling of foods and analysis of daidzein and genistein and their contents in different foods have been reported elsewhere (23–26). Isoflavone content of foods gathered from a literature search of published values was also incorporated into the food composition database for use in the analysis. The food composition database of isoflavones used in this study represents the United Kingdom’s contribution to the Vegetal Estrogens in Nutrition and the Skeleton database, a regional food composition database established to facilitate the estimation of exposure levels to phytoestrogens in four European countries: Italy, the Netherlands, Ireland, and the United Kingdom (27,28).

**Biomarker Analysis**

Spot urine and plasma samples were analyzed for three isoflavones (daidzein, genistein, and glycitein), two metabolites of daidzein (O-DMA and equol), and two lignans (enterodiol and enterolactone), blinded for case-control status. Triply 13C-labeled standards in methanol were added to 200 µl sample, and conjugates were hydrolyzed to the aglycones, extracted on Strata C18-E SPE cartridges (Phenomenex, Macclesfield, UK). Urine was derivatized to trimethylsilyl derivatives for analysis using isotope dilution gas chromatography/mass spectrometry. Details and information on quality assurance and methodology have been reported elsewhere (29). Limits of detection range from 1.2 ng/ml (enterodiol) to 5.3 ng/ml (enterolactone). The average intra-assay coefficient of variation (CV) ranged from 1.8% (equol) to 6.5% (glycitein). The average interassay CV for all analytes were below 9% except for O-DMA (20.2%) and glycitein (26.5%), both of which did not have a corresponding triply 13C-labeled standard at the time of analysis. Urinary creatinine concentrations were measured based on a ki-
netic modification of the Jaffe reaction using Roche Reagent for creatinine on a Roche Cobas Mira Plus chemistry analyzer (Roche Products, Hertfordshire, UK).

Serum samples were analyzed using isotope dilution liquid chromatography/tandem mass spectrometry. Details and information on quality assurance and methodology have been reported elsewhere (30). Statistically calculated limits of detection range from 82 pg/ml (daidzein) to 222 pg/ml (equol). The average intra-assay CV ranged from 2.8% (enterolactone) to 5.7% (glycitein). The average interassay CV ranged from 3.0% (genistein) to 4.4% (O-DMA).

Genotype Analyses

All genotyping was carried out using end-point Taqman assays (Applied Biosystems, Warrington, UK) in 384-well arrays that included blank wells as negative controls. Assays were run on MJ Tetrad thermal cyclers (Genetics Research Instrumentation, Braintree, UK), and genotypes were subsequently read on a 7900 Sequence Detector (Applied Biosystems, Warrington, UK) according to manufacturers’ instructions. An automated robotic high-throughput system in a low-volume 384-well format was used, thereby reducing the chance of errors. The quality of each assay was tested on a specific test set of 96 DNA samples (80 unique, 14 duplicates, and 2 no-template controls). The assays were found to be of good quality with clear clustering and showed 100% concordance in the duplicates. Genotype data were obtained on 233 men for ESR1 PvuII (rs2077647) and SHBG 5′ untranslated region (UTR) g-a polymorphisms, 232 men for COMT V156M (rs4680) and CYP19 3′UTR t-c (rs10046) polymorphisms, and 230 men for SHBG D356N (rs6259) polymorphism. The genotype distributions of the five polymorphisms analyzed were found to be consistent with Hardy-Weinberg equilibrium.

Data Analysis

The basic statistical analyses were performed using SPSS software version 11.0 (SPSS UK, Surrey, UK). Urinary excretion of phytoestrogens was expressed as µg/mmol of urinary creatinine. All dietary, urinary, and serum phytoestrogen data were skewed so data were log transformed for all statistical tests. One-way analysis of variance was used to test for significant differences in weight, height, body mass index (BMI), and dietary, urinary, and serum phytoestrogen levels between cases and controls. Pearson product moment correlations were used to assess the degree of association among urinary, serum, and dietary phytoestrogens. All P values were two sided, and P < 0.05 was considered statistically significant. For the calculation of odds ratio for prostate cancer risk, the statistical analyses were performed using conditional logistic regression using Stata version 8.0 (Stata, College Station, TX), matched by age, gender, and date of recruitment. All data for phytoestrogens were transformed to log2 so that the risk estimates would represent a doubling in phytoestrogen exposure (31). Models were adjusted for family history of prostate cancer, weight, height, and energy intake (as assessed from 7-day food diaries), except in the genotype association models where family history was excluded. Weight, height, and energy intake were included in the model because these variables were found to be significantly different between cases and controls.

Results

Table 1 shows the characteristics of the cases versus the controls. There was no significant difference in dietary isoflavone intake, urinary phytoestrogen excretion, and serum phytoestrogen levels between cases and controls (Table 1). Dietary isoflavone intake was low, with an average intake of about 0.5 mg/day. Even subjects at the 95th percentile consumed only 1.3 mg of isoflavones per day. Of 267 subjects, 221 subjects (82.8%) had detectable levels of equol in their serum (≥0.11 ng/ml) and 163 subjects (61.0%) had detectable levels of urinary equol (≥1.90 ng/ml). Serum equol was detected in all of the 163 subjects with detectable urinary equol (data not shown).

Table 2 shows that, for each phytoestrogen measured, the urinary concentration (adjusted for creatinine concentration) correlated strongly with serum concentration. Correlation coefficients ranged from 0.63 (glycitein) to 0.88 (daidzein) with P values of <0.001. The isoflavones generally showed significant intercorrelation, for example, serum glycitein was significantly correlated with urinary daidzein, genistein, glycitein, and O-DMA, except for equol where the correlation to other isoflavones was much weaker.

Table 3 shows that dietary daidzein and genistein intakes as assessed using 7-day food diaries correlated significantly with their respective urinary and serum measurements. Correlation was weak, with correlation coefficients ranging from 0.13 (serum daidzein vs. dietary daidzein) to 0.21 (urinary genistein vs. dietary genistein). In contrast, dietary daidzein and genistein intakes as assessed using FFQs showed no correlation with their corresponding urinary and serum levels (Table 3). Dietary daidzein and genistein intakes assessed using the two methods were significantly correlated, with r = 0.53 (P < 0.001) for daidzein and r = 0.52 (P < 0.001) for genistein (data not shown).

Table 4 shows the adjusted odds ratios of prostate cancer risk according to phytoestrogen exposure as measured using the four methods, with data transformed to log2. None of the phytoestrogens, as assessed using the different methods, was significantly associated with prostate cancer risk. Adjustment for family history of prostate cancer, weight, height, and energy intake lowered the odds ratio estimates for dietary daidzein and genistein (as assessed using food diaries) but not for phytoestrogen exposures assessed by the other methods. Dietary daidzein and genistein as assessed using food diaries showed a trend toward lower prostate cancer risk with a doubling of intakes, but the odds ratios did not reach statistical significance. Doubling of urinary and serum equol levels appeared to be associated with higher prostate cancer risk,
but this was not statistically significant possibly due to lack of power (Table 4).

Table 5 shows that the five polymorphisms in COMT, CYP19, ESR1, and SHBG genes were not associated with prostate cancer risk, except for the ESR1 PvuII polymorphism. Men with CC and CT genotypes for the ESR1 PvuII polymorphism had significantly higher risk of prostate cancer compared with those with the TT genotype. Adjusted odds ratios for CC and CT genotypes versus TT genotype were 4.65 (95% CI = 1.60–13.49; \( P = 0.005 \)) and 2.25 (95% CI = 1.01–5.02; \( P = 0.047 \)), respectively, with \( P_{\text{trend}} = 0.004 \) (Table 5). We did not study any interactions between phytoestrogen exposure and the genetic polymorphisms due to the small number of cases.

### Discussion

In our previous study in 333 women in EPIC-Norfolk, we found that phytoestrogen levels in spot urine (adjusted for

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**Table 1. Characteristics of Cases and Controls\(^a\)**

<table>
<thead>
<tr>
<th></th>
<th>Cases (( n = 89 ))</th>
<th>Controls (( n = 178 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>Mean(^b)</td>
<td>67.7</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>Mean(^b)</td>
<td>77.0*</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>Mean(^b)</td>
<td>171.3*</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m(^2))</strong></td>
<td>Mean(^b)</td>
<td>26.2</td>
</tr>
<tr>
<td><strong>Dietary intake from food diaries</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/day)</td>
<td>9,295.8*</td>
<td>8,857.5–9,734.0</td>
</tr>
<tr>
<td>Daidzein (µg/day)</td>
<td>224.4</td>
<td>198.1–254.2</td>
</tr>
<tr>
<td>Genistein (µg/day)</td>
<td>287.7</td>
<td>255.5–323.9</td>
</tr>
<tr>
<td><strong>Dietary intake from FFQs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein (µg/day)</td>
<td>206.2</td>
<td>174.2–244.1</td>
</tr>
<tr>
<td>Genistein (µg/day)</td>
<td>258.8</td>
<td>219.9–304.6</td>
</tr>
<tr>
<td><strong>Urinary excretion (µg/mmol Cr)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>14.6</td>
<td>11.3–18.9</td>
</tr>
<tr>
<td>Genistein</td>
<td>9.0</td>
<td>7.1–11.4</td>
</tr>
<tr>
<td>Glycitein</td>
<td>3.8</td>
<td>3.0–5.0</td>
</tr>
<tr>
<td>O-DMA</td>
<td>2.7</td>
<td>1.8–4.0</td>
</tr>
<tr>
<td>Equol</td>
<td>1.3</td>
<td>0.9–1.9</td>
</tr>
<tr>
<td>Enterodiol</td>
<td>5.5</td>
<td>4.3–7.1</td>
</tr>
<tr>
<td>Enterolactone</td>
<td>64.6</td>
<td>50.7–82.3</td>
</tr>
<tr>
<td><strong>Serum levels (ng/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>2.4</td>
<td>1.8–3.1</td>
</tr>
<tr>
<td>Genistein</td>
<td>4.8</td>
<td>3.6–6.4</td>
</tr>
<tr>
<td>Glycitein</td>
<td>0.1</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>O-DMA</td>
<td>0.2</td>
<td>0.1–0.3</td>
</tr>
<tr>
<td>Equol</td>
<td>0.3</td>
<td>0.2–0.4</td>
</tr>
<tr>
<td>Enterodiol</td>
<td>0.3</td>
<td>0.2–0.4</td>
</tr>
<tr>
<td>Enterolactone</td>
<td>3.7</td>
<td>2.8–4.9</td>
</tr>
</tbody>
</table>

\( a \): Abbreviations are as follows: CI, confidence interval; FFQs, food-frequency questionnaires; Cr, creatinine; O-DMA, O-desmethylangolensin. *Significantly different (\( P < 0.05 \)) between cases and controls.

\( b \): Arithmetic means and corresponding 95% CIs are displayed for age, weight, height, body mass index, energy intake, and fiber intake. For all other variables, geometric means and corresponding 95% CIs are displayed.

**Table 2. Correlation Matrix of Urinary Phytoestrogen Excretion and Serum Phytoestrogen Levels (\( n = 267 \))\(^a\)**

<table>
<thead>
<tr>
<th></th>
<th>Daidzein</th>
<th>Genistein</th>
<th>Glycitein</th>
<th>O-DMA</th>
<th>Equol</th>
<th>Enterodiol</th>
<th>Enterolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum levels (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>0.88***</td>
<td>0.70***</td>
<td>0.65***</td>
<td>0.45***</td>
<td>0.15*</td>
<td>–0.06</td>
<td>–0.10</td>
</tr>
<tr>
<td>Genistein</td>
<td>0.77***</td>
<td>0.86***</td>
<td>0.68***</td>
<td>0.38***</td>
<td>0.14*</td>
<td>–0.10</td>
<td>–0.20**</td>
</tr>
<tr>
<td>Glycitein</td>
<td>0.61***</td>
<td>0.53***</td>
<td>0.63***</td>
<td>0.39***</td>
<td>0.14*</td>
<td>–0.06</td>
<td>–0.15*</td>
</tr>
<tr>
<td>O-DMA</td>
<td>0.50***</td>
<td>0.30***</td>
<td>0.42***</td>
<td>0.77***</td>
<td>0.04</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Equol</td>
<td>0.12*</td>
<td>0.13*</td>
<td>0.11</td>
<td>–0.01</td>
<td>0.77***</td>
<td>–0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Enterodiol</td>
<td>–0.03</td>
<td>–0.10</td>
<td>–0.03</td>
<td>0.05</td>
<td>–0.04</td>
<td>0.78***</td>
<td>0.11</td>
</tr>
<tr>
<td>Enterolactone</td>
<td>–0.01</td>
<td>–0.17*</td>
<td>–0.11</td>
<td>0.14*</td>
<td>0.08</td>
<td>0.21**</td>
<td>0.84***</td>
</tr>
</tbody>
</table>

\( a \): Abbreviation is as follows: O-DMA, O-desmethylangolensin. Pearson correlation coefficients on log-transformed data. *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \).
creatinine) correlated strongly with serum phytoestrogen levels ($r = 0.81–0.91$; $P < 0.001$) (32). Similarly, in this study of 267 men, we observed strong correlations between phytoestrogen levels in spot urine and serum ($r = 0.63–0.88$; $P < 0.001$), providing further support for the suitability of phytoestrogen concentrations in spot urine samples as biomarkers for phytoestrogen exposure. We also found that elevated levels of biomarkers and intake of isoflavones were associated with increased breast cancer risk, significantly so for the biomarkers equol and daidzein in serum and equol in urine samples (32). However, although equol levels in plasma and urine were elevated, no significant effect of phytoestrogens on prostate cancer risk was evident in the present study.

We found significant correlations ($r = 0.13–0.20$; $P < 0.05$) between dietary isoflavone intake as assessed from food diaries and urinary and serum phytoestrogen levels. Studies elsewhere comparing intake with urinary or serum isoflavone concentrations (33–42) have mostly been done in women or a mixed group of men and women. No study has reported findings for men alone. In studies that have included both men and women, reported correlation coefficients ranged from 0.25 ($P = 0.014$) for urinary isoflavones versus FFQ soy food intake in 98 American men and women (36) to 0.59 ($P < 0.01$) for urinary isoflavones versus soy food intake from 3-day food records in 19 Japanese men and women (33). The correlation between intake and urine or serum isoflavones is expected to be higher in populations with greater variation in

<table>
<thead>
<tr>
<th>Table 3. Correlation Matrix of Dietary Isoflavone Intake From 7-Day Food Diaries and Food-Frequency Questionnaires and Urinary and Serum Phytoestrogen Levels ($n = 267$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Intake From 7-Day Food Diaries (µg/day)</td>
</tr>
<tr>
<td>Urinary levels (µg/ mmol Cr)</td>
</tr>
<tr>
<td>Daidzein</td>
</tr>
<tr>
<td>Genistein</td>
</tr>
<tr>
<td>Glycitein</td>
</tr>
<tr>
<td>O-DMA</td>
</tr>
<tr>
<td>Equol</td>
</tr>
<tr>
<td>Enterodiol</td>
</tr>
<tr>
<td>Enterolactone</td>
</tr>
<tr>
<td>Serum levels (ng/ml)</td>
</tr>
<tr>
<td>Daidzein</td>
</tr>
<tr>
<td>Genistein</td>
</tr>
<tr>
<td>Glycitein</td>
</tr>
<tr>
<td>O-DMA</td>
</tr>
<tr>
<td>Equol</td>
</tr>
<tr>
<td>Enterodiol</td>
</tr>
<tr>
<td>Enterolactone</td>
</tr>
</tbody>
</table>

*a: Abbreviations are as follows: FFQs, food-frequency questionnaires; Cr, creatinine; O-DMA, O-desmethylangolensin. Pearson correlation coefficients were computed using log-transformed data. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

<table>
<thead>
<tr>
<th>Table 4. Adjusted Odds Ratio (associated with a doubling of exposure) for Dietary, Urine, and Serum Phytoestrogen Levels: Conditional Logistic Regression (matched for age, gender, and date of first health check) Adjusting for Family History of Prostate Cancer, Weight, Height, and Energy Intake*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds Ratio for Doubling of Dietary, Urine, and Serum Phytoestrogen Levels (95% CI)</td>
</tr>
<tr>
<td>Dietary Intake From Food Diaries</td>
</tr>
<tr>
<td>Daidzein</td>
</tr>
<tr>
<td>Genistein</td>
</tr>
<tr>
<td>Glycitein</td>
</tr>
<tr>
<td>O-DMA</td>
</tr>
<tr>
<td>Equol</td>
</tr>
</tbody>
</table>

*a: Abbreviations are as follows: FFQs, food-frequency questionnaires; O-DMA, O-desmethylangolensin. For dietary phytoestrogens from food diaries and urinary and serum phytoestrogens, after the model has excluded missing values, 71 cases have 2 matched controls and 14 cases have 1 matched control; total observations = 241. For dietary phytoestrogens from FFQs, after the model has excluded missing values, 64 cases have 2 matched controls and 19 cases have 1 matched control; total observations = 230. We found significant correlations ($r = 0.13–0.20$; $P < 0.05$) between dietary isoflavone intake as assessed from food diaries and urinary and serum isoflavone levels. Studies elsewhere comparing intake with urinary or serum isoflavone concentrations (33–42) have mostly been done in women or a mixed group of men and women. No study has reported findings for men alone. In studies that have included both men and women, reported correlation coefficients ranged from 0.25 ($P = 0.014$) for urinary isoflavones versus FFQ soy food intake in 98 American men and women (36) to 0.59 ($P < 0.01$) for urinary isoflavones versus soy food intake from 3-day food records in 19 Japanese men and women (33). The correlation between intake and urine or serum isoflavones is expected to be higher in populations with greater variation in
Table 5. Crude and Adjusted Odds Ratio for Five Polymorphisms in COMT, CYP19, ESR1, and SHBG Genes: Conditional Logistic Regression (matched for age, gender, and date of first health check)\(^a\)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Cases (%)</th>
<th>No. of Controls (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)(^b)</th>
<th>(P_{\text{trend}}) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT V158M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>16 (21.3)</td>
<td>34 (21.7)</td>
<td>0.93 (0.40–2.17; (P = 0.862))</td>
<td>0.90 (0.34–2.41; (P = 0.840))</td>
<td>0.878</td>
</tr>
<tr>
<td>CT</td>
<td>40 (53.3)</td>
<td>89 (56.7)</td>
<td>0.83 (0.39–1.74; (P = 0.614))</td>
<td>0.81 (0.35–1.89; (P = 0.629))</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>19 (25.3)</td>
<td>34 (21.7)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>CYP19 3'UTR t-c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19 (25.3)</td>
<td>25 (15.9)</td>
<td>1.59 (0.63–4.03; (P = 0.330))</td>
<td>1.17 (0.43–3.23; (P = 0.759))</td>
<td>0.884</td>
</tr>
<tr>
<td>CT</td>
<td>33 (44.0)</td>
<td>92 (58.6)</td>
<td>0.62 (0.31–1.22; (P = 0.165))</td>
<td>0.57 (0.27–1.18; (P = 0.128))</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>23 (30.7)</td>
<td>40 (25.5)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>ESR1 PvuII</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>21 (28.0)</td>
<td>25 (15.8)</td>
<td>3.02 (1.22–7.54; (P = 0.017))</td>
<td>4.65 (1.60–13.49; (P = 0.005))</td>
<td>0.004</td>
</tr>
<tr>
<td>CT</td>
<td>41 (54.7)</td>
<td>84 (53.2)</td>
<td>1.82 (0.90–3.69; (P = 0.098))</td>
<td>2.25 (1.01–5.02; (P = 0.047))</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>13 (17.3)</td>
<td>49 (31.0)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>SHBG 5’ UTR g-a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>6 (8.0)</td>
<td>6 (3.8)</td>
<td>1.87 (0.55–6.38; (P = 0.318))</td>
<td>1.03 (0.27–3.94; (P = 0.970))</td>
<td>0.960</td>
</tr>
<tr>
<td>AG</td>
<td>28 (37.3)</td>
<td>56 (35.4)</td>
<td>1.18 (0.63–2.21; (P = 0.616))</td>
<td>1.01 (0.50–2.06; (P = 0.968))</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>41 (54.7)</td>
<td>96 (60.8)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>SHBG D356N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>2 (2.7)</td>
<td>3 (1.9)</td>
<td>3.19 (0.28–36.9; (P = 0.353))</td>
<td>2.16 (0.16–28.4; (P = 0.557))</td>
<td>0.722</td>
</tr>
<tr>
<td>AG</td>
<td>16 (21.6)</td>
<td>36 (23.1)</td>
<td>0.97 (0.49–1.92; (P = 0.928))</td>
<td>1.04 (0.50–2.19; (P = 0.904))</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>56 (75.7)</td>
<td>117 (75.0)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\): Abbreviations are as follows: OR, odds ratio; CI, confidence interval; UTR, untranslated region. Bold indicates statistically significant results (\(P < 0.05\)). For crude analyses, after the model has excluded missing values, for COMT, 51 cases have 2 matched controls and 20 cases have 1 matched control; total observations = 193. For CYP19, 50 cases have 2 matched controls and 22 cases have 1 matched control; total observations = 194. For ESR1 and SHBG 5’ UTR, 51 cases have 2 matched controls and 21 cases have 1 matched control; total observations = 195. For SHBG D356N, 50 cases have 2 matched controls and 20 cases have 1 matched control; total observations = 190. For adjusted analyses, after the model has excluded missing values, for COMT, 51 cases have 2 matched controls and 20 cases have 1 matched control; total observations = 195. For CYP19, 50 cases have 2 matched controls and 22 cases have 1 matched control; total observations = 194. For adjusted analyses, after the model has excluded missing values, for ESR1 and SHBG 5’ UTR, 51 cases have 2 matched controls and 21 cases have 1 matched control; total observations = 190. For adjusted analyses, after the model has excluded missing values, for SHBG D356N, 50 cases have 2 matched controls and 20 cases have 1 matched control; total observations = 190.

\(^b\): Adjusted for weight, height, and dietary energy intake from 7-day food diaries.

intakes. In contrast to the significant correlation between dietary isoflavone intake as assessed from food diaries and urinary and serum levels, we found no correlation between intake assessed from FFQs used in the present study and these biomarkers. FFQs as a dietary assessment tool has been shown to be prone to greater measurement errors compared with 7-day food diaries, although the errors of both dietary assessment methods are substantially correlated (21.43.44). This could explain our observations of the lack of correlation between FFQ intakes and urinary and serum biomarkers but the significant correlation between FFQs and food diaries.

The correlation between dietary markers and biomarkers was generally weak, with correlation coefficients only reaching up to 0.21 (for urinary genistein vs. dietary genistein from food diaries). This is likely due to inadequacies of the food composition database in capturing isoflavone intake fully, especially from soy additives added in an increasing number of processed foods. The poor correlation illustrates the importance of having a comprehensive food composition database for dietary methods to capture phytoestrogen exposure accurately. This is especially vital in Western populations where phytoestrogen intake may be largely derived from soy additives, most of which are not usually captured in common food composition databases.

Although results from in vitro studies and animal studies have largely supported the protective effects of phytoestrogens on prostate cancer, data from epidemiological studies have been less encouraging. Only one (45) of three cohort studies (46,47), one (48) of two nested case-control studies (49), and three (50–52) of nine case-control studies (53–58) have reported significant protective effects of phytoestrogens on prostate cancer. All the investigations that showed significant protective effects had included Japanese or Chinese subjects with high habitual soy consumption, with the exception of the study by Jacobsen et al. (45). Jacobsen et al. reported a protective effect of soy milk consumption in Seventh-day Adventist men in California. The odds ratio for soy milk consumption of more than once daily was 0.3 (0.1–0.9) compared with never consuming soy milk. However, there were only 3 cases who consumed soy milk more than once daily, and 190 of 225 cases never had soy milk. Also, consumption of other soy foods was not investigated. Other studies in Western populations (55,56) had not found significant protective effects of phytoestrogens on prostate cancer risk.

Of the previous investigations, only two (45,50) of 10 studies (46,47,53–58) investigating dietary phytoestrogens found significant protective effects. In contrast, three of four studies
(48,49,51,52) utilizing phytoestrogen levels in blood as biomarkers had reported significant protective effects. The only biomarker study (49) that did not find a protective effect had measured serum enterolactone rather than serum isoflavones, which were measured in the other studies. Although many factors such as differences in study design and study populations could account for the inconsistencies in results, it seems possible that the choice of exposure marker could be one of them.

In contrast to studies elsewhere, we have utilized four different methods to categorize exposure to phytoestrogens and measured seven phytoestrogens in both urine and serum. Despite this, we were unable to show a relation between phytoestrogen exposure and prostate cancer risk. The low level of phytoestrogen consumption in our study population and the homogeneity in exposure may have limited our ability to detect a protective effect for phytoestrogens. The lack of power in this relatively small study may have also accounted for the failure to detect statistically significant associations. In addition, we cannot exclude residual confounding from other, as-yet unknown factors. There was a suggestion of an enhanced risk in equol excreters, but this resulted from other, as-yet unknown factors. There was a suggestion of an enhanced risk in equol excreters, but this could be one of them.

In this study, we also investigated the associations between five SNPs in CYP19, ESR1, SHBG, and COMT genes and prostate cancer risk. Only limited SNPs were chosen due to the scant information on SNPs in these genes at the time of this study. The limited SNP coverage would have hampered our ability to study the associations between genetic variation in these genes and prostate cancer risk. Nonetheless, we found significantly higher risk of prostate cancer in men with the CC genotype for the ESR1 PvuII polymorphism compared with men with the TT genotype. Statistically significant results may arise from multiple testing, which increases the chance of detecting false-positive results. In this study, there were two separate sets of tests, one set for phytoestrogens and another set for the SNPs to avoid multiple testing. None of the phytoestrogen associations were positive. We have only tested for five SNPs, and the only positive result was for the ESR1 SNP. Hence, it is unlikely to have arisen from multiple testing, especially because the \( P \) value was 0.005. The ESR1 PvuII polymorphism lies in an intron and is probably a neutral base change in linkage disequilibrium with another, as-yet unknown variant in the gene, which has a functional effect. However, it has been suggested that the \( C \) allele might generate a B-myb transcription factor binding site that could alter the transcription, stability, or structure of the estrogen receptor-alpha transcript and the subsequent estrogen receptor-alpha protein (59). Hence, the PvuII polymorphism could affect estrogen signaling, which in turn could affect prostate cancer risk. In the case-control study by Modugno et al. (60) in which 88 Caucasian prostate cancer cases were compared with 241 male Caucasian controls, they reported an odds ratio of 1.85 (95% CI = 1.97–5.99; \( P = 0.003 \)) compared with men with the CC genotype (63), in direct contrast to our findings. In our previous study in 125 postmenopausal women in EPIC-Norfolk, we observed an interaction between phytoestrogens and the ESR1 PvuII polymorphism in affecting plasma estradiol levels (64). However, due to the relatively small number of cases and the small number of rare homozygotes for some SNPs, it was not viable to investigate phytoestrogen–gene interactions on cancer end points in that or the present study. Phytoestrogen intake varies widely across populations and is typically approximately 100 times higher in Japanese compared with Caucasians (4). If the PvuII polymorphism does indeed affect cancer susceptibility and its effects could be modified by phytoestrogen exposure, this could potentially account for the divergent findings between Caucasian and Japanese populations. Nonetheless, given that all the studies that had been done so far were based on relatively small numbers of subjects, this hypothesis remains speculative. However, this interesting hypothesis deserves further investigation on a larger scale.

Acknowledgments and Notes

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References


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