REVIEW

Genetic polymorphisms of human cytosol glutathione *S*-transferases and prostate cancer

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Glutathione *S*-transferases (GSTs) are involved in the metabolism of a wide range of carcinogenic chemicals. In humans, cytosol GSTs are divided into eight classes: α (GSTA), μ (GSTM), π (GSTP), θ (GSTT), τ (GSTZ), σ (GSTS), ο (GSTO) and κ (GSTK). The allelic polymorphism of these enzymes is associated with variations in enzyme activity; hence, it may affect the concentration of activated carcinogenic chemicals in the body. In addition to the metabolism of chemical carcinogens, GSTs metabolize steroid hormones, compounds in the diet and other agents potentially involved in prostate carcinogenesis. Three genetic polymorphisms of GSTs, *GSTM1*0* (null), *GSTT1*0* (null) and *GSTP1* A313G, have been well documented. No consistent associations between *GSTM1*, *GSTT1* or *GSTP1* genotypes and prostate cancer have been observed. Recent meta-analysis reports show that these polymorphisms of *GSTM1*, *GSTT1* and *GSTP1* are unlikely to be major determinants of susceptibility to prostate cancer.

Keywords: cancer, glutathione transferase, GST, methylation, polymorphism, prostate, SNP

It is generally accepted that cancer risk is determined by the interaction of environmental and genetic factors. Except for hereditary tumors, external carcinogenic exposure is involved in human tumorigenesis. Carcinogenic chemicals, however, undergo a complicated process of metabolism in the human body. Typically, these chemicals are activated by the so-called Phase I enzymes, resulting in the formation of electrophilic, reactive compounds [1]. The amount of active carcinogens is in good correlation with the risk of DNA damage and cancer formation. Detoxifying enzymes, Phase II enzymes, help in the removal of carcinogens from the body [2]. Most of these enzymes conjugate the carcinogenic chemical with a small molecule, making it less toxic and more water-soluble. Therefore, it seems logical to assume that the detoxifying capacity determines, to a certain extent, individual susceptibility to cancer. Among the Phase II enzymes, the glutathione *S*-transferase (GST) superfamily is suspected to have an influence on cancer susceptibility [3]. GSTs are likely to contribute to interindividual differences in responses to xenobiotics. This review describes the relationship between genetic polymorphisms of human cytosol GSTs and prostate cancer.

Prostate cancer

Carcinoma of the prostate is most common in Western countries and second only to lung cancer as a cause of death due to cancer [4]. As well as other malignancies, prostate carcinoma develops through the accumulation of somatic genetic and epigenetic alterations, resulting in the activation of tumor suppressor genes, caretaker genes and oncogenes. The overall lifetime probability of developing clinically evident prostate cancer by the age of 80 years is much lower than its prevalence at autopsy. The estimated lifetime risk for diagnosis of prostate cancer is 16%, and out of that 3–4% die due to this disease [5]. There is a large variation in prostate cancer incidence rates between racial/ethnic groups, being highest among Africans, intermediate among Caucasians and lowest among Asians [6]. This international variation suggests that both genetic and environmental factors play important roles in the etiology of prostate cancer.

Recently, a new hypothesis has been proposed, in which exposure to environmental factors, such as infectious agents and dietary carcinogens, and hormonal imbalances lead to injury of the prostate, resulting in carcinoma of the prostate [7,8]. Approximately 20% of all human malignancies in adults result from chronic inflammation. Although the cause of prostatic inflammation is unclear, there are various potential sources such as direct infection, urine reflux inducing chemical and physical trauma, dietary factors, estrogens and combinations of these. Epidemiological studies show significant associations between infection, inflammation and prostatic carcinoma, however, these are inconclusive. Many noninfectious mechanisms appear to lead to prostatic epithelial cell and stromal damage; for example, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) induces

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prostate inflammation and atrophy. These phenomena are thought to be related to cellular detoxification. Such environmental factors interact with a variable genetic predisposition related, in part, to differences in the metabolism of carcinogen xenobiotics. Genetic polymorphisms that affect the expression of genes regulating the activity of specific enzymes may modulate susceptibility to prostate cancers. Among the candidate genes to be studied in relation to these interindividual differences, cytosol GST may play a relevant role.

GST & genetic polymorphism

The glutathione transferases (EC 2.5.1.18) have historically also been called glutathione *S*-transferases, and it is this latter name that gave rise to the widely used abbreviation, GST. GSTs are prominent contributors to the cellular biotransformation of electrophilic compounds. They provide protection against the genotoxic and carcinogenic effects of numerous xenobiotics and compounds of endogenous origins. Originally, GSTs were found in the soluble cell fraction, and the enzymes occurring in the cytoplasm are usually referred to as cytosol GSTs.

The cytosol GST enzymes have a relatively wide range of substrates, for example, polycyclic aromatic hydrocarbon, monohalomethanes, heterocyclic amines, ethylene oxide and different pesticide solvents. In addition to the metabolism of chemical carcinogens, GST enzymes are involved in the intracellular transport of steroid hormones [9] and the isomerization of androst-5-ene-3,17-dione to androst-4-ene-3,17-dione, the immediate precursor of testosterone [10].

In humans, cytosol GSTs are divided into eight classes: α (GSTA), μ (GSTM), π (GSTP), θ (GSTT), τ (GSTZ), σ (GSTS), ο (GSTO) and κ (GSTK), each of which contains one or more of the homodimeric or heterodimeric isoforms [11,12]. There have been many reports of genetic polymorphisms for *GSTM1*, *GSTM3*, *GSTT1*, *GSTP1* and *GSTA1*, resulting in either decreased or altered enzyme activity. Owing to their role in metabolism, these polymorphisms may play an important role in prostate cancer susceptibility.

GSTM1

A total of five μ class genes (*M1–M5*) situated in tandem on chromosome 1p13.3 have been identified. *GSTM1* is expressed in human liver, stomach, brain and other tissues, while *GSTM2–M5* subunits have been detected in extrahepatic tissues. *GSTM1*, located on chromosome 1p13.3, detoxifies a number of electrophillic substances, including carcinogens such as polycyclic aromatic hydrocarbons, ethylene oxide, epoxides and styrene. *GSTM1* expression is hormonally controlled and induced by phenobarbital or by propylthiouracil [13]. Some lipid peroxidation products may be common substrates of both GSTM1 and GSTT1 [14].

Three polymorphisms of the *GSTM1* gene, namely *GSTM1*0*, *GSTM1*A* and *GSTM1*B*, have been identified (Table 1). *GSTM1*0* is a deleted allele, and the homozygous allele (*GSTM1*-null genotype) expresses no GSTM1 protein. Most studies of *GSTM1* polymorphism and cancer have compared the homozygous deletion genotype with the genotypes containing at least one functional allele. *GSTM1A* and *GSTM1B* differ by a C→G substitution at base position 534. This C→G substitution results in the substitution of Lys→Asn at amino acid 172 [35]. The Lys→Asn substitution results in no functional difference between the two alleles [36]. As a result, *GSTM1A* and *GSTM1B* are categorized together as non-null conjugator phenotypes.

Table 2 summarizes the frequency of homozygous deletion polymorphisms in *GSTM1*. The homozygous deletion genotype frequency in *GSTM1* ranges from 22.7 to 54.2% in Caucasians, from 27.1 to 46.7% in African–Americans and from 42.6 to 54.5% in Asians. We have to be cautious when interpreting the results since the frequencies of the polymorphisms were from controls of molecular epidemiological studies concerning prostate cancer. These prostate cancer studies used different study designs; hospital-based (for males with benign prostatic hyperplasia) or population-based.

GSTM3 is also located on chromosome 1p13.3. Alleles have been identified in *GSTM3*, with *GSTM3*A* and *GSTM3*B* differing in intron 6 by a three base pair (bp) deletion in *GSTM3*B*. Although the difference between *GSTM3*A* and *GSTM3*B* is intronic, its importance is suggested by the presence of a recognition motif for the versatile transcription factor YY1 in *GSTM3*B* but not in *GSTM3*A* [37]. Importantly, *GSTM3*B* and *GSTM3*A* are in linkage disequilibrium with *GSTM1*0* [10]. These results suggest that, in some cases, links between clinical phenotypes and *GSTM1* genotypes may reflect polymorphisms in *GSTM3* or, indeed, other μ class GST genes.

Table 1. Human soluble glutathione *S*-transferase polymorphisms relevant to prostate cancer.

Enzyme designation	Class	Gene	Chromosome location	Known alleles	Nucleotide change
GSTA1–1	α	*GSTA1*	6p12	*GSTA1*A*	T567, C69 and G52
				*GSTA1*B*	G567, T69 and A52
GSTM1–1	μ	*GSTM1*	1p13.3	*GSTM1*0*	Null
				*GSTM1*A*	C534
				*GSTM1*B*	G534
GSTM3–3	μ	*GSTM3*	1p13.3	*GSTM3*A*	Wild-type
				*GSTM3*B*	Three base pair deletion intron 6
GSTT1–1	θ	*GSTT1*	22q11.2	*GSTT1*0*	Null
				*GSTT1*1*	Present
GSTP1–1	π	*GSTP1*	11q13	*GSTP1*A*	A313 and C341
				*GSTP1*B*	G313 and C341
				*GSTP1*C*	G313 and T341

GSTT1

GSTT1 encoding for θ class is located on chromosome 22q11.2, and helps in catalyzing the conjugation of halomethanes in human erythrocytes [36]. Substrates of GSTT1 include industrial chemicals, such as methyl chloride, methyl bromide, dichloromethane, ethylene oxide and diepoxy butane, a reactive metabolite of 1,3-butadiene [39,40]. Two alleles have been identified at the *GSTT1* locus: one functional (*GSTT1*1*) and the other nonfunctional (*GSTT1*0*) [36]. Individuals with a homozygous deletion genotype are categorized as having the negative conjugator phenotype, while those who carry either one or both functional alleles are grouped as having the positive conjugator phenotype. The prevalence rates of the null genotype were 26.7 and 41.9–48.3% of the controls of African–American and Asian descent, respectively (Table 2). Among Caucasians, the frequency ranged from 13–58.7% (Table 2).

GSTP1

GSTP1, a member of the π gene family, is located at 11q13, expressed predominantly in the basal layer of the normal prostate epithelium [41]. GSTP1 metabolizes a variety of potential carcinogens, including cigarette smoke-derived chemicals such as benzo[a]pyrene diol epoxides and acrolein [10]. By contrast with most cancers, prostate carcinogenesis is associated with marked downregulation of *GSTP1*. The events leading up to the inactivation of GSTP1 during prostate carcinogenesis remain unclear. Several investigators have speculated that the early loss of GSTP1 function leads to increased vulnerability to oxidant and heterocyclic amine carcinogens, both implicated in prostate carcinogenesis [42,43]. Hence, heritable differences in GSTP1 function may also be associated with prostate cancer development. Two genetic polymorphisms, in exons 5 and 6, both resulting in an amino acid substitution, have been found. However, only the change in exon 5, that is the A→G polymorphism at nucleotide 313, results in an amino acid substitution (Ile105Val) in the substrate-binding site of GSTP1 [44]. The substitution was linked to a change in enzymatic activity [44]. The prevalence rates of the Val/Val homozygosity was 9–12.6% in Caucasian subjects, 13.3–19% in African–Americans and 0–5.4% in Asians, respectively (Table 2).

GSTA1

The α class GSTs, in other words GSTA1–5, are encoded by genes clustered within 6p12. Of all the GSTs, the α class isoenzymes are the most abundant GSTs found in the human liver, and are, notably, responsible for metabolizing the nitrogen mustard group of some anticancer drugs, and for binding endogenous compounds such as bilirubin in the liver [8]. Especially, *GSTA1*, *GSTA2* and *GSTA3* are widely expressed in human tissues, predominantly in the liver, while *GSTA4* is rarely expressed and *GSTA5* is normally undetectable [45,46]. Members of the α class possess high glutathione peroxidise activity, and play an important role in protecting cells against reactive oxygen species and the products of peroxidation. GSTA1 is also active towards several carcinogenic substrates, including polycyclic aromatic hydrocarbon epoxides [10], and the meat-derived carcinogen *N*-acetoxy-2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (*N*-acetoxy-PhIP) [47]. A polymorphism that

Table 2. Frequency of *GSTM1*, *GSTT1*, *GSTP1* (Ile105Val) and *GSTA2* genotypes in three major ethnic groups

First author	Country	Racial descent	*GSTM1*-null (%)	*GSTT1*-null (%)	*GSTP1* (Ile/Val) (%)			*GSTA1*B/*B*			Ref.
					Ile/Ile **A/*A*	Ile/Val **A/*B* or **C*	Val/Val **B* or **C/*B* or **C*	**A/*A*	**A/*B*	**B/*B*	
Autrup *et al.* (1999)	Denmark	Caucasian	53.5	15.3	42	58*					[15]
Rebbeck *et al.* (1999)	USA	Caucasian	47.6	31.2							[16]
Shepard *et al.* (2000)	USA	Caucasian			45.4	44.1	10.5				[17]
Steinhoff *et al.* (2000)	Germany	Caucasian	45	13	55	36	9				[18]
Gsur *et al.* (2001)	Austria	Caucasian	48.8	19.9	39.2	48.2	12.6				[19]
Kote-Jarai *et al.* (2001)	UK	Caucasian	50.0	23.7	51.2	38.5	10.3				[20]
Jeronimo *et al.* (2002)	Portugal	Caucasian			43.3	47.5	9.2				[21]
Kidd *et al.* (2003)	USA	Caucasian	53	15	57	43*					[22]
Acevedo *et al.* (2003)	Chile	Caucasian	22.7								[23]
Debes *et al.* (2004)	USA	Caucasian			38.2	49.0	12.9				[24]
Medeiros *et al.* (2004)	Portugal	Caucasian	54.2	58.7							[25]
Shankar *et al.* (2005)	India	Caucasian	35.4	20.1	57.6	38.9	3.5				[26]
Agalliu *et al.* (2006)	USA	Caucasian	47.5	16.9	43.2	45.7	11.1				[27]
Watson *et al.* (1998)	USA	Caucasian			42	51	7				[28]

*GSTP1 *Val allele (Ile/Val and Val/Val)*

Table 2. Frequency of *GSTM1*, *GSTT1*, *GSTP1* (Ile105Val) and *GSTA2* genotypes in three major ethnic groups

First author	Country	Racial descent	*GSTM1*- null (%)	*GSTT1*-null (%)	*GSTP1* (Ile/Val) (%)			*GSTA1*B/*B*			Ref.
					Ile/Ile **A/*A*	Ile/Val **A/*B* or **C*	Val/Val **B* or **C/*B* or **C*	**A/*A*	**A/*B*	**B/*B*	
Sweeney *et al.* (2003)	USA	Caucasian						35.8	47.5	16.7	[29]
Ning *et al.* (2004)	USA	Caucasian						29.6	54.3	16.1	[30]
London *et al.* (1995)	USA	African–American	27.1								[31]
Watson *et al.* (1998)	USA	African–American			35	46	19				[28]
Agalliu *et al.* (2006)	USA	African–American	46.7	26.7	6.7	80.0	13.3				[27]
Sweeney *et al.* (2003)	USA	African–American						29.8	57.4	12.8	[29]
Ning *et al.* (2004)	USA	African–American						39.7	49.2	11.1	[30]
Murata *et al.* (1998)	Japan	Asian	42.6								[32]
Watson *et al.* (1998)	USA	Asian			67	30	3				[28]
Nakazato *et al.* (2003)	Japan	Asian	50.5	41.9	72.4	27.6	0				[33]
Komiya *et al.* (2005)	Japan	Asian	54.5	48.3	72.9	23.7	5.4	81.0	17.0	2.0	[34]

*GSTP1 *Val allele (Ile/Val and Val/Val)*

influences the hepatic expression of *GSTA1* has recently been described [45,48]. Liver cytosols from individuals who carried the variant *GSTA1*B* allele, which consists of several linked SNPs in the proximal promoter region of *GSTA1*, had reduced levels of GSTA1 enzyme [49]. The gene has a polymorphic SP1-binding site that results in fourfold higher mean hepatic expression of *GSTA1*A* compared with *GSTA1*B* [45,48].

There are a few reports regarding GST α expression in normal and malignant prostate tissues, demonstrating low levels of GST α expression in high-grade prostatic intraepithelial neoplasia and prostate cancers. Increased expression of *GSTA1* was also detected in proliferative inflammatory atrophy (PIA), suggesting the involvement of localized increases in oxidative stress. Recently, using antibodies for Ya, Yc and Yk-type subunits, an immunohistochemical study was conducted in normal and malignant prostate tissues, showing low levels of GST α expression in prostate cancers and heterogeneous staining patterns. These suggest that a lack of detoxification activity plays an important role in carcinogenesis of the prostate.

Association of GST polymorphism with prostate cancer susceptibility

The genetic polymorphism of GSTs has been investigated in many molecular epidemiological studies as a susceptibility factor for prostate cancer development. For each study, we abstracted the publication date, country where the study was conducted, control source and the numbers of cases and controls (Table 3). Healthy subjects recruited from hospitals as controls were categorized as 'hospital-healthy'.

The 13 available case-control studies investigated the association between *GSTM1* polymorphism and prostate cancer (Table 3). Three case-control studies reported associations between the *GSTM1*-null genotype and prostate cancer risks [23,26,27]. In a large, case-control study (559 cases, 523 controls), Agalliu *et al.* demonstrated that the *GSTM1*-null genotype was associated with an increased risk of prostate cancer (OR: 1.54; 95% CI: 1.19–2.01) [27]. Among *GSTM1*-null individuals, the relative risk increased linearly with increasing pack-years of smoking (p-value for trend = 0.007), with the highest OR observed for heavy smokers (>30 pack-years). Two other studies in Chile [23] and India [26], which are consistent with Agalliu's data, showed a statistically significant OR of 1.94 and 2.24 for prostate cancer among *GSTM1*-null subjects, respectively. However, ten other studies did not recognize an increased risk associated with a lack of *GSTM1* activity. In addition, Kelada *et al.* observed no interaction between smoking and the *GSTM1* genotype [50]. Moreover, the *GSTM1*-null genotype did not have a familial prostate cancer risk [33].

To our knowledge, there are 11 reports investigating the associations between *GSTT1* polymorphism and prostate cancer (Table 3). Eight case-control studies reported no associations between the *GSTT1*-null genotype and prostate cancer risk. However, two studies reported significant associations between the *GSTT1*-null genotype and prostate cancer risk [18,26]. Intriguingly, in one of those reporting American patients with carcinoma of the prostate, a significantly increased frequency of *GSTT1* non-null genotype was found [50]. Furthermore, a Japanese study yielded similar results of a significantly higher frequency of the *GSTT1* non-null genotype among smokers [32].

There are 12 case-control studies that investigated the association between *GSTP1* polymorphisms and prostate cancer. The results of these studies are inconsistent. Three of them reported that *GSTP1* Ile/Val or Val/Val genotypes might be associated with prostate cancer risk [19,20,26]; the Ile/Val or Val/Val genotypes have been associated with a significant increase in the risk of prostate cancer in North Indian populations [26]. Kote-Jarai *et al.* found that patients in the UK with the *GSTP1* Val/Val genotype were at a higher risk of early-onset prostate cancer [20]. On the contrary, Gsur *et al.* reported that there was a significant decrease in the Val allele (Ile/Val and Val/Val) among cases compared with controls [19]. This result suggests that the Val allele is associated with a decreased risk of prostate cancer in Austrians. However, in nine other studies the *GSTP1* Ile105Val polymorphism was not associated with a statistically significant increase in the risk of prostate cancer.

Thus, it is evident that the association of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms with prostate cancer risk differs widely among different populations, suggesting the significance of ethnic differences and environmental factors on prostate cancer susceptibility.

Recently, Ntais *et al.* reported a meta-analysis that included data from 11 studies with *GSTM1* genotyping (2063 prostate cancer cases and 2625 controls), ten studies with *GSTT1* genotyping (1965 cases and 2554 controls) and

Table 3. Summary of case–control studies on *GSTM1*, *GSTT1*, *GSTP1* (Ile105Val) and *GSTA1* genotypes and prostate cancer incidence

Study	Country	Control source	Racial descent	Cases (n)	Controls (n)	*GSTM1*-null, OR (95% CI)	*GSTT1*-null, OR (95% CI)	*GSTP1*(any Val), OR (95% CI)	*GSTA1*B/*B*, OR (95% CI)	Ref.
Murata *et al.* (1998)	Japan	Hospital	Asian	115	204	1.3 (0.82–2.04)				[32]
Autrup *et al.* (1999)	Denmark	Healthy	Caucasian	153	288	1.27 (0.85–1.90)	1.31 (0.77–2.19)	0.80 (0.54–1.19)		[15]
Kelada *et al.* (2000)	USA	Hospital	Caucasian	276	499	1.00 (0.69–1.29)	1.61 (1.14–2.28)			[50]
Shepard *et al.* (2000)	USA	Hospital	Caucasian	590	803			0.81 (0.63–1.06)		[17]
Steinhoff *et al.* (2000)	Germany	Hospital	Caucasian	91	127	1.20 (0.71–2.05)	2.31 (1.17–4.59)	1.09 (0.66–1.77)		[18]
Gsur *et al.* (2001)	Austria	Hospital (BPH)	Caucasian	166	166	0.86 (0.55–1.36)	0.78 (0.43–1.42)	0.24 (0.09–0.61)		[19]
Kote-Jarai *et al.* (2001)	UK	Healthy	Caucasian	275	280	1.29 (0.92–1.81)	1.05 (0.71–1.55)	Ile/Val 1.30 (0.99–1.69) Val/Val 1.80 (1.11–2.91)		[20]
Jeronimo *et al.* (2002)	Portugal	Healthy + BPH	Caucasian	105	141			1.02 (0.59–1.75)		[21]
Kidd *et al.* (2003)	USA	Participants of the ATBC cancer prevention study	Caucasian	206	194	0.64 (0.43–0.95)	0.74 (0.42–1.33)	1.10 (0.72–1.69)		[22]
Acevedo *et al.* (2003)	Chile	BPH	Caucasian	102	128	1.94 (1.04–3.63)				[23]
Nakazato *et al.* (2003)	Japan	Hospital	Asian	81 (familial)	105	0.98 (0.58–1.66)	0.87 (0.49–1.55)	Ile/Val 0.97 (0.50–1.36) Val/Val 9.31 (0.47–184)		[33]

**GSTT1* non-null, *OR (95% CI).*

*§GSTA1*A/*B* or **B/*B, OR: 1.49, 95% CI: 0.96-2.32.*

ATBC: Finnish male paticpants of the Alpha Tocophenol Beta Carotene (ATBC) cancer prevention study; BPH: Benign prostatic hypertrophy.

Table 3. Summary of case–control studies on *GSTM1*, *GSTT1*, *GSTP1* (Ile105Val) and *GSTA1* genotypes and prostate cancer incidence

Study	Country	Control source	Racial descent	Cases (n)	Controls (n)	*GSTM1*-null, OR (95% CI)	*GSTT1*-null, OR (95% CI)	*GSTP1*(anyVal), OR (95% CI)	*GSTA1*B/*B*, OR (95% CI)	Ref.
Debes *et al.* (2004)	USA	Healthy	Caucasian	438 (familial) 499 (sporadic)	510			1.00 (0.74–1.37) 0.84 (0.65–1.09)		[24]
Medeiros (2004)	Portugal	Healthy	Caucasian	150	185	1.20 (0.75–1.90)	0.87 (0.51–1.51)			[25]
Ning *et al.* 2004	USA	Healthy	Caucasian	254	81				0.80 (0.36–1.78)	[30]
			African-American	93	63			0.81 (0.25–2.61)		
Shankar *et al.* (2005)	India	No information	Caucasian	127	144	2.24 (1.37–3.65)	1.89 (1.09–3.28)	2.48 (1.51–4.08)		[26]
Komiya *et al.* (2005)	Japan	Healthy	Asian	190	294	0.76 (0.52–1.12)	1.39 (0.95–2.03)*	0.87 (0.57–1.35)	1.33 (0.39–4.51)§	[34]
Agalliu *et al.* (2006)	USA	Healthy	Caucasian	559	523	1.54 (1.19–2.01)	1.04 (0.73–1.47)	Ile/Val 0.95 (0.72–1.25), Val/Val 1.10 (0.71–1.69)		[27]

**GSTT1* non-null, OR (95% CI).*

*§GSTA1*A/*B or *B/*B, OR: 1.49, 95% CI: 0.96-2.32.*

ATBC: Finnish male paticpants of the Alpha Tocophenol Beta Carotene (ATBC) cancer prevention study; BPH: Benign prostatic hypertrophy.

12 studies with *GSTP1* genotyping (2528 cases and 3076 controls) [51]. The random effects OR was 1.08 (95% CI: 0.95–1.25) for the *GSTM1*-null genotype versus the *GSTM1*-nondeleted genotype, and 0.90 (95% CI: 0.73–1.12) for the *GSTT1*-null genotype versus the *GSTT1*-nondeleted genotype. There was no evidence that the *GSTP1* Val allele modified the risk of prostate cancer. The summary of OR was 1.05 (95% CI: 0.90–1.21). No association was observed in subjects of Caucasian descent (OR = 1.02), and there was only one study subject of Asian descent (OR = 1.25).

There have been only two case-control studies of *GSTA1*. Ning *et al.* reported that the OR associated with risk of prostate cancer according to *GSTA1* genotype was not significant within race and after adjustment for age [30]. Komiya *et al.* stated that the frequency of *GSTA1*A/*B* or **B/*B* genotype individuals among cases increased to 26.3% compared with the control groups (19.0%) [34]; however, this difference did not reach statistical significance (OR 1.49; 95% CI: 0.96–2.32) after adjustment for age and smoking status. The apparent lack of interaction between the *GSTA1* genotype and prostate cancer risk suggests a low impact of hepatic GST expression on the risk of the disease. For example, this may be due to the importance of 2-amino-1-methyl-6-phenylimidazo [4,5] PhIP activation locally in the target tissue, activation of *N*-hydroxy-PhIP by paths other than O-acetylation [52], and the low importance of PhIP in the etiology of prostate cancer.

Agalliu *et al.* found evidence suggesting that carriers of the *GSTM3*B/*B* genotype have a fivefold increased risk of onset of prostate cancer (OR: 5.5; 95% CI: 1.2–25.8) [27]. However, their report is only one study that is relevant to prostate cancer, and no other reports regarding the *GSTM3* genotype and prostate cancer have been found.

Although some substrates are metabolized by specific GST isoenzymes [10], they have overlapping substrate specificities; therefore, a combination of unfavorable genotypes could theoretically confer a high risk. In a few studies, an increased risk was observed when a combination of the variant GST genotypes was present [20,26,34]. Kote-Jarai *et al.* [20] and Shanker *et al.* [26] demonstrated that the combination of GST genotypes might define a specific high-risk genotype (*GSTM1*, *GSTT1*-null and *GSTP1* Val allele) in the UK and India. On the other hand, Komiya *et al.* observed that the OR of carrying the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion was 2.08 (95% CI: 1.14–3.80), with the combined genotyping of *GSTA1*A/*A* and *GSTT1*-null as a reference in Japanese [34].

Future perspective

A number of independent studies have demonstrated the importance of polymorphisms in xenobiotic metabolism as risk factors in the development of cancer associated with chemical exposure. According to these results, many case-control studies have been undertaken regarding *GST* genes and the risk of prostate cancer. However, the evidence from recent studies has not been consistent. The reasons for this disparity in risk are mostly unclear; however, we can speculate on three possibilities.

The first possible reason for these discrepancies, the biochemical evidence for the putative relationship of GST polymorphisms with prostate cancer, is equivocal. It is unlikely that specific environmental carcinogens whose effect might also be modifiable by GST genotype have a high attributable risk for prostate cancer. The second reason is that, in addition to the metabolism of chemical carcinogens, GSTs are involved in the intracellular transport and the synthesis of steroid hormones [12], and compounds found in the diet are potentially involved in prostate carcinogenesis. Population differences in androgen levels and dietary factors have been implicated as a possible explanation. The third reason for these discrepancies may be insufficient study power or bad study design, or a bias against publishing the absence of correlations. Bartsch *et al.* provided state-of-the-art reviews of the application of biomarkers and the design and analysis of molecular epidemiological studies [53]. The prerequisites for proper study design and conduct include:

- A clear definition of representative study populations and controls
- A sample size adequate to provide enough statistical power
- Proper documentation (or measurement) of exposure
- Avoidance of confounding data because of the use of mixed ethnicity study subjects
- Study of only the gene polymorphisms that have been demonstrated to lead to altered phenotypic expression

The rigor and size of study designs will need to increase, as multiple comparisons and the power issue dictate. In parallel with these studies, a clearer understanding of the genetic bias of the polymorphisms has emerged, together with more accurate and less invasive methods for the screening of populations.

Expert opinion

Three genetic polymorphisms of GSTs, that is *GSTM1*0* (null), *GSTT1*0* (null) and *GSTP1 A313G*, have been well documented. However, associations between other polymorphisms of GSTs, that is to say *GSTA*, *GSTZ*, *GSTS*, *GSTO* and *GSTK*, and prostate cancer are not well known. It will be interesting to know whether polymorphisms in these genes influence susceptibility to prostate cancer.

Of particular interest are studies of gene–environment interactions and gene–gene–environment interactions. To date, molecular epidemiology studies of prostate cancer have rarely looked at a variety of potential gene–environment interactions or explored associations and interactions with more than one genetic polymorphism. Future studies that examine the association among several genetic polymorphisms should take into account risk factors for prostate cancer, such as diet and other environmental exposures, as well as possible biological pathways. In addition, some studies had limited power to detect more associations between *GST* polymorphisms and prostate cancer risk; further well-designed and large-scale studies are needed to reveal these associations.

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Executive summary

Prostate cancer

- Carcinoma of the prostate is most common in Western countries, and second only to lung cancer as a cause of death due to cancer.

Glutathione S-transferases families

- In humans, cytosol glutathione *S*-transferases (GSTs) are divided into eight classes: α (GSTA), μ (GSTM), π (GSTP), θ (GSTT), τ (GSTZ), σ (GSTS), ο (GSTO) and κ (GSTK).

Glutathione S-transferase substrates

- GSTs metabolize numerous harmful chemicals produced endogenously and in the environment.

Genetic polymorphisms of glutathione S-transferase

- Cytosol GSTs display polymorphisms in humans, which is likely to contribute to interindividual differences in responses to xenobiotics.

Association of glutathione S-transferase polymorphisms with prostate cancer susceptibility

- Three genetic polymorphisms of GST: *GSTM1*0* (null), *GSTT1*0* (null) and *GSTP1* A313G, have been well documented.
- No consistent associations between *GSTM1*, *GSTT1* or *GSTP1* genotypes and prostate cancer have been observed.

Conclusions

- Further well-designed, large-scale studies are required to detect the precise associations between GST polymorphisms and prostate cancer risk.
- Research is needed on the associations between the polymorphisms of *GSTA*, *GSTZ*, *GSTS*, *GSTO* and *GSTK* and prostate cancer.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Mayer UA: Overview of enzymes of drug metabolism. *J. Pharmacokinet. Biopharm.* 24, 449–459 (1996).
2. Taningher M, Malacne D, Izzotti A, Ugolini D, Parodi S: Drug metabolism polymorphisms as modulators of cancer susceptibility. *Mutat. Res.* 436, 227–261 (1999).
3. Ryberg D, Skaug V, Hewer A *et al.*: Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. *Carcinogenesis* 18, 1285–1289 (1997).
4. Weinmann SJ, Boe RK, Glass GA, Weirs SN: Prostate cancer screening and mortality: a case-control study (US). *Cancer Causes and Control* 14, 133–138 (2004).
5. Merrill RM, Morris MK: Prevalence-corrected prostate cancer incidence rates and trends. *Am. J. Epidemiol.* 155, 148–152 (2002).
6. Hsing AW, Tsao L, Devesa SS: International trends and patterns of prostate cancer incidence and mortality. *Int. J. Cancer* 85, 60–67 (2000).

7. De Marzo AM, Platz EA, Sutcliffe S *et al.*: Inflammation in prostate carcinogenesis. *Nat. Rev. Cancer* 7, 256–269 (2007).
8. Sun J, Turner A, Xu J, Gronberg H, Isaacs W: Genetic variability in inflammation pathways and prostate cancer risk. *Urol. Oncol.* 25, 250–259 (2007).
9. Listowsky I, Abramovitz M, Homma H, Niitsu Y: Intracellular binding and transport of hormones and xenobiotics by glutathione-*S*-transferases. *Drug. Metab. Rev.* 19, 305–318 (1998).
10. Hayes JD, Pulford DJ: The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* 30, 445–600 (1995).
11. Hayes JD, Strange RC: Glutathione-*S*-transferase polymorphisms and their biological consequence. *Pharmacology* 61, 154–166 (2000).
12. Hayes JD, Flanagan JU, Jowsey IR: Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.* 45, 51–88 (2005).

•• **Recent review with extensive discussion on glutathione S-transferase (GST).**

13. Mannervik KB, Alin P, Guthenberg C *et al.*: Nomenclature for human glutathione-*S*-transferases. *Biochem. J.* 282, 305–306 (1992).
14. Seidegard J, Pero RW, Miller DG, Beattie EJ: A glutathione-S-transferase in human leukocytes as a marker for the susceptibility to lung cancer. *Carcinogenesis.* 7: 751–753 (1986).
15. Autrup JL, Thomassen LH, Olsen JH, Wolf H, Autrup H: Glutathione *S*-transferases as risk factors in prostate cancer. *Eur. J. Cancer Prev.* 8, 525–32 (1999)
16. Rebbeck TR, Walker AH, Jaffe JM, White DL, Wein AJ, Malkowicz B: Glutathione *S*-transferase-μ (*GSTM1*) and -τ (*GSTT1*) genotypes in the etiology of prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* 8, 283–287 (1999).
17. Shepard TF, Platz EA, Kantoff PW *et al.*: No association between the I105V polymorphism of the glutathione *S*-transferase P1 gene (*GSTP1*) and prostate cancer risk: a prospective study. *Cancer Epidemiol. Biomarkers Prev.* 9, 1267–1268 (2000).
18. Steinhoff C, Frank KH, Golka K *et al.*: Glutathione transferase isozyme genotypes in patients with prostate and bladder carcinoma. *Arch. Toxicol.* 74, 521–526 (2000).
19. Gsur A, Haidinger G, Hinteregger S *et al.*: Polymorphisms of glutathione-*S*-transferase genes (*GSTP1, GSTM1* and *GSTT1*) and prostate-cancer risk. *Int. J. Cancer* 95: 152–155 (2001).
20. Kote-Jarai Z, Easton D, Edwards SM *et al.*: Relationship between glutathione *S*-transferase M1, P1 and T1 polymorphisms and early onset prostate cancer. *Pharmacogenetics* 11, 325–330 (2001).
21. Jerónimo C, Varzim G, Henrique R *et al.*: I105V polymorphism and promoter methylation of the *GSTP1* gene in prostate adenocarcinoma. *Cancer Epidemiol. Biomarkers Prev.* 11, 445–450 (2002).
22. Kidd LC, Woodson K, Taylor PR, Albanes D, Virtamo J, Tangrea JA: Polymorphisms in glutathione-*S*-transferase genes (*GST-M1, GST-T1* and *GST-P1*) and susceptibility to prostate cancer among male smokers of the ATBC cancer prevention study. *Eur. J. Cancer Prev.* 12, 317–320 (2003).
23. Acevedo C, Opazo JL, Huidobro C, Cabezas J, Iturrieta J, Quinones Sepulveda L: Positive correlation between single or combined genotypes of *CYP1A1* and *GSTM1* in relation to prostate cancer in Chilean people. *Prostate* 57, 111–117 (2003).
24. Debes JD, Yokomizo A, McDonnell SK *et al.*: Gluthatione-*S*-transferase P1 polymorphism I105V in familial and sporadic prostate cancer. *Cancer Genet. Cytogenet.* 155, 82–86 (2004).
25. Medeiros R, Vasconcelos A, Costa S *et al.*: Metabolic susceptibility genes and prostate cancer risk in a southern European population: the role of glutathione *S*-transferases *GSTM1, GSTM3,* and *GSTT1* genetic polymorphisms. *Prostate* 58, 414–420 (2004).
26. Shankar D, Srivastava L, Mandhni A, Mittal B, Mittal RD: Genetic polymorphism of glutathione *S*-transferase genes (*GSTM1, GSTT1* and *GSTP1*) and susceptibility to prostate cancer in Northern India. *BJU Int.* 95, 170–173 (2005).
27. Agalliu I, Lin DW, Salinas CA, Feng Z, Stanford JL: Polymorphisms in the glutathione *S*-transferase M1, T1, and P1 genes and prostate cancer prognosis. *Prostate* 66, 1535–1541 (2006).
28. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA: Human glutathione *S*-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 19, 275–280 (1998).
29. Sweeney C, Ambrosone CB, Joseph L *et al.*: Association between a glutathione *S*-transferase A1 promoter polymorphism and survival after breast cancer treatment. *Int. J. Cancer* 103, 810–814 (2003).
30. Ning B, Wang C, Morel F *et al.*: Human glutathione *S*-transferase A2 polymorphisms: variant expression, distribution in prostate cancer cases/controls and a novel form. *Pharmacogenetics* 14, 35–44 (2004).
31. London SJ, Daly AK, Cooper J, Navidi WC, Carpenter CL, Idle JR: Polymorphism of glutathione S-transferase M1 and lung cancer risk among African–Americans and Caucasians in Los Angeles County, California. *J. Natl. Cancer Inst.* 87, 1246–1253 (1995).
32. Murata M, Shiraishi T, Fukutome K *et al.*: Cytochrome P4501A1 and glutathione *S*-transferase M1 genotypes as risk factors for prostate cancer in Japan. *Jpn. J. Clin. Oncol.* 28, 657–660 (1998).
33. Nakazato H, Suzuki K, Matsui H *et al.*: Association of genetic polymorphisms of glutathione-*S*-transferase genes (*GSTM1, GSTT1* and *GSTP1*) with familial prostate cancer risk in a Japanese population. *Anticancer Res.* 23, 2897–2902 (2003).
34. Komiya Y, Tsukino H, Nakao H, Kuroda Y, Imai H, Katoh T: Human glutathione *S*-transferase A1, T1, M1, and P1 polymorphisms and susceptibility to prostate cancer in the Japanese population. *J. Cancer Res. Clin. Oncol.* 131, 238–42 (2005).
35. Seidegard J, Vorachek WR, Pero RW, Pearson WR: Hereditary differences in the expression of the human glutathione transferase active on trans-stilben oxide are due to a gene deletion. *Proc. Natl. Acad. Sci. USA* 85, 7203–7207 (1998).
36. Widersten M, Pearson WR, Engstrom A, Mannervik B: Heterologous expression of the allelic variant μ-class glutathione transferase μ and ψ. *Biochem. J.* 276, 519–524 (1991).
37. Inskip A, Elexperu-Camiruaga J, Buxton N *et al.*: Identification of polymorphism at the glutathione *S*-transferase, *GSTM3* locus: evidence for linkage with *GSTM1*A. Biochem. J.* 312, 713–716 (1995).
38. Pemble S, Schroeder KR, Spencer SR *et al.*: Human glutathione-*S*-transferases τ: cDNA cloning and the characterization of polymorphism. *Biochem. J.* 300, 271–276 (1994).
39. Lof A, Johanson G, Rannug A, Warholm M: Glutathione transferase T1 phenotype affects the toxicokinetics of inhaled methyl chloride in human volunteers. *Pharmacogenetics* 10, 645–653 (2000).

40. Their R, Lewalten J, Kempkes M, Selinski S, Bruning T, Bolt HM: Haemoglobin adducts of acrylonitrile and ethylene oxide in acrylonitrile workers dependent on polymorphisms of the glutathione transferases GSTT1 and GSTM1. *Arch. Toxicol.* 73, 197–202 (1999).
41. Terrier P, Townsend AJ, Coindre JM, Triche TJ, Cowan KH: An immunohistochemical study of pi class glutathione S-transferase expression in normal human tissue. *Am. J. Pathol.* 137, 845–853 (1990).
42. De Weese TL, Hruszkewycz AM, Marnett LJ: Oxidative stress in chemoprevention trials. *Urology* 57, 137–140 (2001).
43. Nelson WG, De Marzo AM, De Weese TL: The molecular pathogenesis of prostate cancer: implications for prostate cancer prevention. *Urology* 57, 39–45 (2001).
44. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J: Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione *S*-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *J. Biol. Chem.* 272, 10004–10012 (1997).
45. Coles BF, Morel F, Rauch C *et al.*: Effect of polymorphism in the human glutathione *S*-transferase A1 promoter on hepatic *GSTA1* and *GSTA2* expression. *Pharmacogenetics* 11, 663–669 (2001).
46. Parsons JK, Nelson CP, Gage WR, Nelson WG, Kensler TW, De Marzo AM: *GSTA1* expression in normal, preneoplastic, and neoplastic human prostate tissue. *Prostate* 49, 30–37 (2001).
47. Coles B, Nowell SA, MacLeod SL, Sweeney C, Lang NP, Kadlubar FF: The role of human glutathione *S*-transferases (hGSTs) in the detoxification of the food-derived carcinogen metabolite *N*-acetoxy-PhIP, and the effect of a polymorphism in *hGSTA1* on colorectal cancer risk. *Mutat. Res.* 482, 3–10 (2001).
48. Morel F, Rauch C, Coles B, Le Ferrec E, Guillouzo A: The human glutathione transferase α locus: genomic organization of the gene cluster and functional characterization of the genetic polymorphism in the *hGSTA1* promoter. *Pharmacogenetics* 12, 277–286 (2002).
49. Board P: Ligandin revisited: resolution of the α class glutathione transferase gene family. *Pharmacogenetics* 12, 275–276 (2002).
50. Kelada SN, Kardia SL, Walker AH, Wein AJ, Malkowicz SB, Rebbeck TR: The glutathione *S*-transferase-μ and -τ genotypes in the etiology of prostate cancer: genotype-environment interactions with smoking. *Cancer Epidemiol. Biomarkers Prev.* 9, 1329–1334 (2000).
51. Ntais C, Polycarpou A, Ioannidis JP: Association of *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms with the risk of prostate cancer: a meta-analysis. *Cancer Epidemiol. Biomarkers Prev.* 14, 176–181 (2005).

•• **Meta-analyses in studies of the relationship between *GSTM1*, *GSTT1* and *GSTP1*, and prostate cancer.**

52. Nelson CP, Kidd LC, Sauvageot J *et al.*: Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione S-transferase P1. *Cancer Res.* 61, 103–109 (2001).
53. Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K: Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol Biomarkers Prev.* 9, 3–28 (2000).

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Plasma Isoflavone Level and Subsequent Risk of Breast Cancer Among Japanese Women: A Nested Case-Control Study From the Japan Public Health Center-Based Prospective Study Group

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ABSTRACT

Purpose
Because they have large variations in consumption, Asian countries are suitable settings for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Nevertheless, no prospective study from Asia has assessed blood or urine levels as biomarkers of isoflavone intake.

Patients and Methods
A total of 24,226 women ages 40 to 69 years in the Japan Public Health Center–based prospective study who responded to the baseline questionnaire and provided blood in 1990 to 1995 were observed to December 2002. During a mean 10.6 years of follow-up, 144 patients newly diagnosed with breast cancer were identified. Two matched controls for each patient were selected from the cohort. Isoflavone levels were assessed by plasma level and food frequency questionnaire, and the odds ratio of breast cancer according to isoflavone level was estimated using a conditional logistic regression model.

Results
We found a statistically significant inverse association between plasma genistein and risk of breast cancer, but no association for plasma daidzein. Adjusted odds ratios for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; *P* for trend, .02) and 0.71 for daidzein (95% CI, 0.35 to 1.44; *P* for trend, .54). Median plasma genistein values in the control group were 31.9 ng/mL for the lowest and 353.9 ng/mL for the highest quartile groups. Regarding dietary intake of isoflavones, nonsignificant inverse associations were observed for both genistein and daidzein.

Conclusion
This nested case-control study found an inverse association between plasma genistein and the risk of breast cancer in Japan.

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INTRODUCTION

Soy foods, a traditional staple dish in Asian countries, are a primary source of isoflavones, such as genistein and daidzein, which are classified as phytoestrogens. Because breast cancer risk is substantially lower in Asian than Western countries,[1] the contribution of a high isoflavone intake to low breast cancer risk has been hypothesized.[2] This hypothesis has been supported by in vitro studies at high genistein concentrations and in the majority of animal studies, which together have demonstrated various anticancer effects of isoflavones acting via both estrogen-dependent and -independent mechanisms.[3,4] Estrogen-dependent mechanisms arise through the mediation of estrogen receptor α and β, owing to the similar chemical structure of isoflavones to the human estrogen hormone and their binding affinity to estrogen receptors.[4,5] For this reason, they have been hypothesized to behave like selective estrogen receptor modulators. In contradiction to potential protective effects, however, genistein exhibits estrogenic properties at low concentrations, which could theoretically enhance breast cancer risk.[3,4] In fact, some animal studies have reported that genistein stimulates tumor development and growth.[6,7] Although a recent meta-analysis found that soy intake was associated with a

small reduction in breast cancer risk, the authors concluded that in view of these risk-enhancing effects, recommendations for high-dose isoflavone supplementation to prevent breast cancer or its recurrence were premature.[8] Phytoestrogen supplements, however, are commercially marketed for use by postmenopausal women as natural and safe alternatives to hormone replacement therapy. The effect of relatively high-dose isoflavone on breast cancer risk is now of concern.

Because they have large variations in consumption among individuals, Asian countries serve as suitable venues for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Despite this advantage, only a few epidemiological studies on soy or isoflavone intake and breast cancer risk from Asia have been reported.[9] In particular, no prospective study on isoflavone levels in blood or urine samples has been reported, notwithstanding that, because they are partly determined by individual differences in absorption and metabolism, blood or urine levels might better reflect interperson differences than dietary assessment. The three nested case-control studies which have investigated this association in Western populations have been inconsistent, with one reporting an inverse association with plasma genistein in the Netherlands,[10] the second showing no association with urinary genistein in the Netherlands,[11] and the third finding a positive association with urine and serum phytoestrogens in the United Kingdom.[12] This inconsistency might be in part explained by the apparently small variation in isoflavone levels in Western countries. For example, studies in the Netherlands, which has a high incidence of breast cancer (age-standardized rate per 100,000 world population, 86.7 in 2002),[13] reported a median genistein intake of 0.14 mg/d in women ages 49 to 70 years,[14] and a median plasma genistein level of 4.89 ng/mL in the control group of a nested-case control study.[10] In contrast, a study in Japan, where the incidence of breast cancer is low (age-standardized rate per 100,000 world population, 32.7 in 2002),[13] reported a median genistein intake of 22.3 mg/d and median serum level of 90.2 ng/mL.[15] This substantial variation in isoflavone levels suggests that the Japanese population represents an ideal setting for determining whether an association exists at relatively high levels achievable from dietary intake only.

Herein, to clarify the effect of relatively high-dose isoflavone exposure on breast cancer risk, we conducted a nested case-control study within a large-scale population-based prospective study in Japan.

PATIENTS AND METHODS

Study Population

The Japan Public Health Center–based prospective study, which began in 1990 for cohort I and in 1993 for cohort II, included 140,420 subjects (68,722 men and 71,698 women) living in the municipalities supervised by 11 public health centers (PHC). Details of the study design have been described elsewhere.[16] The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

The study population comprised registered Japanese inhabitants living in each PHC area, ages 40 to 59 years in cohort I and 40 to 69 years in cohort II. In this analysis, one PHC area was excluded since data on cancer incidence were not available. We thus defined a population-based cohort of 67,426 women (27,389 in cohort I and 40,037 in cohort II) after the exclusion of ineligible subjects (n = 95).

Questionnaire Survey

A baseline survey was conducted from 1990 to 1994. A total of 55,891 women (83%) returned the questionnaire, which contained questions concerning demographic characteristics, medical history, menstrual and reproductive history, anthropometric factors, physical activity, smoking and drinking habits, and diet.

Blood Collection

Subjects voluntarily provided 10 mL of blood during health check-ups from 1990 to 1995. Blood samples were divided into plasma and buffy layers and stored at −80°C until analysis. Among respondents to the baseline questionnaire, a total of 24,996 women (45%) donated blood.

Follow-Up

All registered subjects were observed from the start of the study period to December 31, 2002. Data on residential relocation were obtained from residential registries. Among study subjects (n = 24,996), 1,289 subjects (5.2%) moved out of the study area and 5 (0.02%) were lost to follow-up within the study at-risk period.

Selection of Patients and Controls

Incidence data on breast cancer were collected for the Japan Public Health Center cancer registry through two data sources—major local hospitals and population-based cancer registries. Death certificates were used to supplement information on cancer incidence. Site of origin and histologic type were coded by members of our study group (Appendix A1, online only) using the International Classification of Diseases for Oncology, third edition, code C500-509. Up to the end of the study period, 144 new breast cancer cases (97 in cohort I and 47 in cohort II) were identified among the 24,226 women (9,689 in cohort I and 14,537 in cohort II) who had returned the baseline questionnaire, reported no history of breast cancer or ovarian cystoma, and provided blood samples. Diagnosis was microscopically verified in 98% of patients, and based on death certificates only in 0.7%. The mortality/incidence ratio was 0.14.

For each patient, two controls were selected using incidence density sampling from subjects who were not diagnosed with breast cancer during the follow-up period when the patient was diagnosed. Control selection was done without reference to incidence of other cancer sites. Controls were matched with each patient for age (within 3 years), PHC area, area (city or town and village), date of blood collection (within 90 days), time of day of blood collection (within 3 hours), fasting time at blood collection (within 3 hours), and baseline menopausal status.

Assessment of Dietary Intake

Dietary intakes of genistein and daidzein were assessed by a food frequency questionnaire of 44 items for cohort I and 52 for cohort II. Isoflavone intake was defined for this study as the sum of genistein and daidzein intake. We documented the questionnaire assessment of isoflavone intake to be reasonably valid (details in Appendix A1).[15,17]

Laboratory Assay

Plasma levels of isoflavone were analyzed using high-performance liquid chromatography with a coulometric array detector in accordance with the modified methods of Gamache and Acworth.[18] Concentrations of genistein and daidzein were determined by linear regression of the peak height for each standard, and adjusted according to the recovery rate of the internal plasma standard. The regression coefficient of peak height and concentration calculated for isoflavones revealed a linearity range of 0 to 0.75 μg/mL, with correlation coefficient values higher than 0.938. Voltametric response for the standard solution displayed coefficients of variation of 8% for intra- and 11% for interday variation. Recovery rates of isoflavones in plasma samples ranged between approximately 73% and 98%. Detection limits were 2.2 ng/mL for genistein and 2.7 ng/mL for daidzein. Laboratory personnel were blinded to case-control status when performing the analyses.

Statistical Analysis

Comparison of baseline characteristics, as well as plasma levels and dietary intake of isoflavones, between cases and controls was evaluated by the Mantel-Haenszel test using matched-set strata. Spearman's correlation coefficients were calculated among plasma levels and dietary intakes of isoflavone

among control subjects. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% CIs of breast cancer for plasma levels and dietary intake of isoflavone divided into quartiles based on control distribution. The ORs were adjusted for number of births and age at first birth as potential confounders. The adjusted ORs were calculated based on a total of 405 subjects with complete information for covariates. Linear trends for ORs were tested in the conditional logistic regression model using the exposure categories as ordinal variables. All P values reported are two sided, and significance level was set at $P < .05$. All statistical analyses were performed with SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Case subjects and controls had significantly different distribution for number of births (Table 1). Other characteristics, such as age at menarche, age at first birth, body mass index (BMI), alcohol consumption, or dietary intake did not substantially differ between the two groups.

Plasma genistein was significantly lower among cases than controls whereas plasma daidzein values were similar (Table 2). No significant differences between the groups were seen for dietary genistein, daidzein, or isoflavone intake. Median isoflavone intake in the control group was 34.8 mg/d (36.1 in cohort I and 29.9 mg/d in cohort II). Genistein and daidzein were highly correlated for both plasma level ($r = 0.72$) and dietary intake ($r = 0.99$). Correlation coefficients between plasma and dietary levels were relatively low for both genistein ($r = 0.23$) and daidzein ($r = 0.31$).

We found a statistically significant inverse association between plasma genistein and the risk of breast cancer (P for trend, .02), but no statistically significant association for plasma daidzein (P for trend, .54; Table 3). Adjusted ORs for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; $P \leq .01$) and 0.71 for daidzein (95% CI, 0.35 to 1.44; $P = .34$). Moreover, the results did not change substantially after adjustment for dietary intake of isoflavone or other potential confounders such as age at menarche, menopausal status at baseline, age at menopause, height, BMI, and alcohol consumption. Further, exclusion of cases diagnosed before the first 3 years of follow-up did not substantially change the results, nor did the exclusion of subjects who used vitamin supplements or who provided a nonfasting blood sample (ie, within 6 hours after a meal). Regarding dietary intake, we observed inverse associations for both genistein and daidzein but neither was statistically significant (Table 3). In addition, adjusted ORs by isoflavone intake were closely similar to those by genistein intake (data not shown).

A stratified analysis according to baseline menopausal status showed no remarkable difference between two strata for either genistein and daidzein, regardless of whether the values were assessed by plasma or questionnaire, although the inverse association between plasma genistein and risk of breast cancer tended to be more stable in postmenopausal than premenopausal women (Table 4).

Table 1. Characteristics of Patients and Matched Control Subjects at Baseline

Characteristic	Patients (n = 144) No.	Patients (n = 144) %	Controls (n = 288) No.	Controls (n = 288) %	P*
Mean age, years		51.7		51.8	
Standard deviation		7.1		7.1	—
Family history of breast cancer	2	1.4	2	0.7	.48
Premenopausal women	59	42	118	42	—
Postmenopausal women					
Natural menopause	70	50	140	50	—
Surgical menopause	10	7.2	20	7.2	—
Mean age at menopause, years		50.0		49.8	.76
SE†		0.38		0.27	
Mean age at menarche, years		14.6		14.8	.33
SE†		0.15		0.10	
Mean No. of births		2.3		2.8	.01
SE†		0.12		0.09	
Mean age at first birth, years		25.7		25.0	.22
SE†		0.30		0.21	
Use of exogenous female hormones (current use)	4	3.0	2	0.8	.10
Mean height, cm		151.7		151.4	.70
SE†		0.46		0.33	
Mean body mass index, kg/m^2		23.4		23.5	.49
SE†		0.25		0.18	
Smoking (current smoker)	5	3.5	17	5.9	.23
Alcohol drinking (regular drinker)	18	13	26	9.1	.28
Leisure-time physical activity (≥ once per week)	30	21	57	20	.42
Vitamin supplement user	33	24	61	23	.65
Green tea intake (≥ five cups per day)	36	25	71	25	.42
Mean total energy intake, kcal/d		1,269.4		1,271.0	.41
SE‡		26.5		19.2	
Mean fish and shellfish intake, g/d		45.4		45.7	.75
SE‡		2.5		1.8	
Mean meat intake, g/d		30.5		28.5	.15
SE‡		1.7		1.2	
Mean vegetable intake, g/d		121.2		115.9	.20
SE‡		5.7		4.1	
Mean fruit intake, g/d		104.8		99.4	.79
SE‡		5.9		4.3	

*P for Mantel-Haenszel test with matched-set strata.
†Adjusted for age.
‡Adjusted for age and cohort.

DISCUSSION

In this study, we found a statistically significant inverse association between plasma genistein and the risk of breast cancer, but no association for plasma daidzein. This finding suggests that genistein may

Table 2. Plasma Levels and Dietary Intake of Isoflavone in Patients and Matched Controls

Parameter	Patients (n = 144) Median	Patients (n = 144) Interquartile Range	Controls (n = 288) Median	Controls (n = 288) Interquartile Range	P*
Plasma level					
Genistein, ng/mL	131.8	67.9-202.6	144.5	78.8-255.6	.046
Daidzein, ng/mL	16.7	7.0-34.0	17.9	5.5-40.8	.45
Dietary intake					
Genistein, mg/d	19.9	16.6-24.0	21.7	16.8-26.1	.37
Daidzein, mg/d	12.5	10.1-14.8	13.3	10.3-16.3	.36
Isoflavone, mg/d†	32.5	26.8-38.7	34.8	27.0-42.4	.36

*P for Mantel-Haenszel test with matched-set strata.
†Isoflavone intake = sum of genistein and daidzein intake.

Table 3. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone

Parameter	Quartile 1	2	3	4	*P* for trend
Plasma level					
Median genistein, ng/mL	31.9	108.1	190.8	353.9	
No. of patients	41	37	45	21	
No. of controls	72	72	72	72	
OR	1.00	0.84	1.04	0.46	.07
95% CI	Reference	0.47 to 1.51	0.57 to 1.91	0.23 to 0.91	
Adjusted OR*	1.00	0.69	0.87	0.34	.02
95% CI	Reference	0.36 to 1.32	0.45 to 1.67	0.16 to 0.74	
Median daidzein, ng/mL	0	12.0	27.0	53.7	
No. of patients	30	45	44	25	
No. of controls	72	72	72	72	
OR	1.00	1.50	1.44	0.79	.59
95% CI	Reference	0.85 to 2.64	0.80 to 2.61	0.41 to 1.54	
Adjusted OR*	1.00	1.30	1.51	0.71	.54
95% CI	Reference	0.70 to 2.42	0.80 to 2.86	0.35 to 1.44	
Dietary intake					
Median genistein, mg/d	15.7	18.5	22.9	27.3	
No. of patients	42	36	37	29	
No. of controls	69	75	71	73	
OR	1.00	0.78	0.83	0.58	.15
95% CI	Reference	0.46 to 1.35	0.47 to 1.48	0.30 to 1.12	
Adjusted OR*	1.00	0.81	0.92	0.58	.21
95% CI	Reference	0.46 to 1.45	0.50 to 1.70	0.29 to 1.18	
Median daidzein, mg/d	9.4	11.4	14.1	17.1	
No. of patients	40	39	35	30	
No. of controls	70	74	72	72	
OR	1.00	0.91	0.82	0.65	.21
95% CI	Reference	0.52 to 1.58	0.46 to 1.47	0.33 to 1.27	
Adjusted OR*	1.00	0.96	0.94	0.67	.34
95% CI	Reference	0.54 to 1.74	0.50 to 1.74	0.33 to 1.39	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous). Adjusted ORs were calculated based on a total of 405 subjects with complete information of covariates.

play a more important role in the etiology of breast cancer than daidzein. Our findings are in general agreement with those of a recent nested case-control study in the Netherlands,[10] albeit that our inverse association occurred at substantially higher plasma concentrations. For example, median plasma genistein values in the control group of the Netherlands study were 3.75 ng/mL for premenopausal and 4.89 ng/mL for postmenopausal women.[10] In contrast, the median value in our control group was 144.5 ng/mL, and only 3.2% of control subjects was under 5 ng/mL. This apparently high level is not surprising considering that the median value of 353.9 ng/mL in our highest plasma genistein quartile group, which had a significantly lower risk of breast cancer than the lowest group, corresponded to a median dietary intake of 28.5 mg/d for genistein and 46.5 mg/d for isoflavone, as estimated by the validation study data. Although some in vivo and in vitro studies have shown risk-enhancing effects of genistein, our study suggests that relatively high-dose isoflavones exposure achievable from dietary intake alone is associated with a decreased rather than increased risk.

We observed an approximately 65% reduction in breast cancer risk in the highest plasma genistein quartile group but no decrease in the other quartiles, indicating that only the highest group benefited from risk reduction. The apparent lack of a dose-response relationship might imply the presence of a threshold level of effect. Interestingly, this idea contradicts findings in Western populations, in whom inverse associations are seen despite materially low levels of isoflavones. Given the differences in hormonal milieu between the two populations, the potential protective effect of isoflavones in breast cancer might act differently between Western and Asian populations: sex hormone levels are higher in Western than Asian women,[19] for example, as is the prevalence of obesity.[20,21] In this regard, a case-control study in Shanghai found that the inverse association between urinary isoflavone level and breast cancer risk was stronger among women in the high BMI, waist-hip ratio, and estradiol level groups and in the low sex hormone-binding globulin level group than in the respectively converse low and high groups.[22] Alternatively, the apparent lack of a dose-response relationship might merely reflect uncontrolled confounding by other dietary characteristics or risk-lowering behaviors.

The reason for a role for genistein but not daidzein in the etiology of breast cancer is unclear, but several possibilities can be speculated. Genistein possesses stronger binding affinity for estrogen receptor than daidzein.[5] Further, a pharmacokinetic study showed higher plasma levels and a 1.5-fold longer half-life for genistein than daidzein

Table 4. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone By Baseline Menopausal Status

Parameter	Quartile 1	2	3	4	*P* for trend
Premenopausal women					
Plasma genistein, ng/mL					
No. of patients	24	14	19	2	
No. of controls	41	28	25	24	
Adjusted OR*	1.00	0.76	1.75	0.14	.20
95% CI	Reference	0.31 to 1.86	0.68 to 4.50	0.03 to 0.69	
Plasma daidzein, ng/mL					
No. of patients	17	21	15	6	
No. of controls	27	45	23	23	
Adjusted OR*	1.00	0.80	1.27	0.49	.48
95% CI	Reference	0.34 to 1.88	0.48 to 3.38	0.15 to 1.57	
Dietary genistein intake, mg/d					
No. of patients	21	16	14	8	
No. of controls	35	31	32	20	
Adjusted OR*	1.00	0.92	0.86	0.62	.43
95% CI	Reference	0.41 to 2.05	0.34 to 2.18	0.21 to 1.84	
Dietary daidzein intake, mg/d					
No. of patients	20	17	14	8	
No. of controls	36	30	32	20	
Adjusted OR*	1.00	1.07	0.93	0.67	.53
95% CI	Reference	0.46 to 2.51	0.37 to 2.34	0.22 to 2.03	
Postmenopausal women					
Plasma genistein, ng/mL					
No. of patients	17	23	25	15	
No. of controls	28	41	46	45	
Adjusted OR*	1.00	0.54	0.57	0.36	.10
95% CI	Reference	0.18 to 1.62	0.20 to 1.65	0.12 to 1.12	
Plasma daidzein, ng/mL					
No. of patients	13	23	27	17	
No. of controls	40	27	47	46	
Adjusted OR*	1.00	2.86	2.06	1.16	.95
95% CI	Reference	1.03 to 7.98	0.82 to 5.17	0.43 to 3.15	
Dietary genistein intake, mg/d					
No. of patients	20	20	22	18	
No. of controls	33	42	35	50	
Adjusted OR*	1.00	0.73	0.93	0.52	.31
95% CI	Reference	0.30 to 1.77	0.38 to 2.27	0.19 to 1.42	
Dietary daidzein intake, mg/d					
No. of patients	19	22	20	19	
No. of controls	33	42	36	49	
Adjusted OR*	1.00	0.89	0.93	0.64	.43
95% CI	Reference	0.38 to 2.10	0.38 to 2.29	0.23 to 1.72	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous).

after ingestion of baked soybean powder containing closely similar amounts of the two.[23] Moreover, the absence of an association for plasma daidzein might be attributable to misclassification arising from the metabolization of this compound. Daidzein can be metabolized by intestinal bacteria to equol and O-desmethylangolites; because approximately only 30% to 50% of individuals are capable of equol production, probably due to differences in gut microflora, daidzein-to-equol metabolizers may have lower plasma daidzein levels than nonmetabolizers.[24] Equol has been suggested to have greater biologic activity than daidzein,[24] and an inverse association between equol level and breast cancer risk has been reported.[25] Here, the lowest plasma daidzein quartile group might conversely have had a lower breast cancer risk than the higher groups due to its inclusion of equol metabolizers, and such misclassification, if present, would lead to a null result.

Our study has several methodological advantages over previous studies of isoflavones and the risk of breast cancer. First, the direct measurement of plasma isoflavone levels provides not only an index of intake but also of the absorption and metabolism of isoflavone, an understanding of which is important to elucidating the mechanisms by which isoflavones might influence breast cancer development. Indirect measurement by dietary intake of genistein is likely a major reason for the present smaller and nonsignificant risk reduction of breast cancer than by plasma genistein. Exposure assessment using

blood samples is therefore likely a more sophisticated means of detecting an association. Second, two case-control studies in Australia and China showed an inverse association between urinary isoflavones and breast cancer risk.[25,26] In view of the retrospective design of these studies, however, blood or urine levels of isoflavones in breast cancer cases might have been influenced by metabolic changes after the breast cancer was detected or by altered eating habits among case subjects. In our nested case-control study within a prospective cohort, in contrast, blood samples were collected before cancer diagnosis, obviating any potential bias due to the presence of cancer. Third, cases and controls were selected from the same cohort, thereby avoiding the selection bias inherent to case-control studies.

Several limitations of this study warrant mention. First, we measured plasma isoflavones only once for each individual. The consumption of soy foods is a personal dietary preference, and intake levels of most individuals are assumed to be relatively stable over time in Japan, as suggested by our validation study, which showed high reproducibility of repeated measurements of genistein intake by food frequency questionnaire (correlation coefficient = 0.72 for 1-year interval and 0.61 for 5-year interval).[15,17] By comparison, plasma isoflavone levels may reflect short-term rather than long-term intake: isoflavones have short half-lives in blood (eg, 6 to 8 hours),[23,27] and plasma levels are particularly affected by time elapsed since the last meal. To minimize the attenuation of risk estimates derived from random measurement errors, we matched fasting time between cases and controls. Second, despite a reasonably large cohort population (24,226 women) and long follow-up period (average, 10.6 years), the number of breast cancer cases was relatively small, reflecting the low incidence rate in Japan (age-standardized rate per 100,000 world population, 32.7 in 2002).[13] The interpretability of our results might therefore be limited, particularly in stratified analyses. Third, although our cohort subjects were selected from the general population, subjects were restricted to the 24,226 women respondents (43%) to the baseline questionnaire who provided blood samples. Although health check-up examinees in our previous report had a different socioeconomic status than nonexaminees and a more favorable lifestyle profile,[28] no apparent difference in isoflavone intake and breast cancer risk factors was found between subjects in the subcohort for this study and the original cohort; median isoflavone intake, for example, was 32.5 and 32.1 mg/d, respectively, and the average number of births was 2.8 and 2.7, respectively.[29] Nevertheless, any extrapolation of the results to the general population should be done cautiously, particularly in view of a previous report showing the difficulty of extrapolating relative risk estimates for a subcohort to an entire cohort. This difficulty might in fact be inherent to prospective studies in general.[30]

Allowing for these methodological issues, we found an inverse association between plasma genistein and the risk of breast cancer in a nested case-control study in Japan. This finding suggests a risk-reducing rather than a risk-enhancing effect of isoflavones on breast cancer, even at relatively high concentrations within the range achievable from dietary intake alone.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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REFERENCES

1. Parkin DM, Whelan SL, Ferlay J, et al: Cancer incidence in five continents vol. VIII. IARC Scientific Publications no. 155. Lyon, France, IARC, 2002
2. Adlercreutz H: Epidemiology of phytoestrogens. Baillieres Clin Endocrinol Metab 12:605-623, 1998
3. Magee PJ, Rowland IR: Phyto-oestrogens, their mechanism of action: Current evidence for a role in breast and prostate cancer. Br J Nutr 91:513-531, 2004
4. Limer JL, Speirs V: Phyto-oestrogens and breast cancer chemoprevention. Breast Cancer Res 6:119-127, 2004
5. Kuiper GG, Lemmen JG, Carlsson B, et al: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139:4252-4263, 1998
6. Day JK, Besch Williford C, McMann TR, et al: Dietary genistein increased DMBA-induced mammary adenocarcinoma in wild-type, but not ER alpha KO, mice. Nutr Cancer 39:226-232, 2001
7. Ju YH, Allred KF, Allred CD, et al: Genistein stimulates growth of human breast cancer cells in a novel, postmenopausal animal model, with low plasma estradiol concentrations. Carcinogenesis 27: 1292-1299, 2006
8. Trock BJ, Hilakivi Clarke L, Clarke R: Meta-analysis of soy intake and breast cancer risk. J Natl Cancer Inst 98:459-471, 2006
9. Yamamoto S, Sobue T, Kobayashi M, et al: Soy, isoflavones, and breast cancer risk in Japan. J Natl Cancer Inst 95:906-913, 2003
10. Verheus M, van Gils CH, Keinan-Boker L, et al: Plasma phytoestrogens and subsequent breast cancer risk. J Clin Oncol 25:648-655, 2007
11. den Tonkelaar I, Keinan Boker L, Veer PV, et al: Urinary phytoestrogens and postmenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev 10:223-228, 2001
12. Grace PB, Taylor JI, Low YL, et al: Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European prospective investigation of cancer and nutrition-norfolk. Cancer Epidemiol Biomarkers Prev 13:698-708, 2004
13. Ferlay J, Bray F, Pisani P, et al: GLOBOCAN 2002 Cancer Incidence, Mortality and Prevalence Worldwide, IARC Cancer Base No. 5, version 2.0. Lyon, France, IARC Press, 2004
14. Keinan Boker L, van Der Schouw YT, Grobbee DE, et al: Dietary phytoestrogens and breast cancer risk. Am J Clin Nutr 79:282-288, 2004
15. Yamamoto S, Sobue T, Sasaki S, et al: Validity and reproducibility of a self-administered food-frequency questionnaire to assess isoflavone intake in a japanese population in comparison with dietary records and blood and urine isoflavones. J Nutr 131:2741-2747, 2001
16. Watanabe S, Tsugane S, Sobue T, et al: Study design and organization of the JPHC study. J Epidemiol 11:S3-S7, 2001 (suppl)
17. Tsubono Y, Kobayashi M, Sasaki S, et al: Validity and reproducibility of a self-administered food frequency questionnaire used in the baseline survey of the JPHC study cohort I. J Epidemiol 13:S125-S133, 2003
18. Gamache PH, Acworth IN: Analysis of phytoestrogens and polyphenols in plasma, tissue, and urine using HPLC with coulometric array detection. Proc Soc Exp Biol Med 217:274-280, 1998

19. Shimizu H, Ross RK, Bernstein L, et al: Serum oestrogen levels in postmenopausal women: Comparison of American whites and Japanese in Japan. Br J Cancer 62:451-453, 1990

20. Yoshiike N, Seino F, Tajima S, et al: Twenty-year changes in the prevalence of overweight in Japanese adults: The National Nutrition Survey 1976-95. Obes Rev 3:183-190, 2002

21. Flegal KM, Carroll MD, Ogden CL, et al: Prevalence and trends in obesity among US adults, 1999-2000. JAMA 288:1723-1727, 2002

22. Dai Q, Franke AA, Yu H, et al: Urinary phytoestrogen excretion and breast cancer risk: Evaluating potential effect modifiers endogenous estrogens and anthropometrics. Cancer Epidemiol Biomarkers Prev 12:497-502, 2003

23. Watanabe S, Yamaguchi M, Sobue T, et al: Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). J Nutr 128:1710-1715, 1998

24. Atkinson C, Frankenfeld CL, Lampe JW: Gut bacterial metabolism of the soy isoflavone daidzein: Exploring the relevance to human health. Exp Biol Med (Maywood) 230:155-170, 2005

25. Ingram D, Sanders K, Kolybaba M, et al: Case-control study of phyto-oestrogens and breast cancer. Lancet 350:990-994, 1997

26. Zheng W, Dai Q, Custer LJ, et al: Urinary excretion of isoflavonoids and the risk of breast cancer. Cancer Epidemiol Biomarkers Prev 8:35-40, 1999

27. Lampe JW: Isoflavonoid and lignan phytoestrogens as dietary biomarkers. J Nutr 133: 956S–964S, 2003 (suppl)

28. Iwasaki M, Otani T, Yamamoto S, et al: Background characteristics of basic health examination participants: The JPHC Study Baseline Survey. J Epidemiol 13:216-225, 2003

29. Iwasaki M, Otani T, Inoue M, et al: Role and impact of menstrual and reproductive factors on breast cancer risk in Japan. Eur J Cancer Prev 16:116-123, 2007

30. Iwasaki M, Yamamoto S, Otani T, et al: Generalizability of relative risk estimates from a well-defined population to a general population. Eur J Epidemiol 21:253-262, 2006

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Appendix

Assessment of Dietary Intake. The cohort I questionnaire asked about average consumption during the previous month of 44 food items, including three items contributing to isoflavone intake, namely, (1) miso soup, (2) soybeans, tofu, deep-fried tofu, and natto, and (3) vegetables other than yellow and green vegetables, such as Chinese cabbage, radish, tomato, cucumber, and so on—whose items includes soybean sprouts. The cohort II questionnaire asked about average consumption during the previous month of 52 food items, including three items contributing to isoflavone intake: miso soup, tofu, and natto. The cohort I questionnaire had four frequency categories: rarely, 1 to 2 days/week, 3 to 4 days/week, and almost everyday, while the cohort II questionnaire had five frequency categories of never, less than 1 day/week, 1 to 2 days/week, 3 to 4 days/week, and almost everyday. Portion size and isoflavone content were estimated from a validation study (Yamamoto S, Sobue T, Sasaki S, et al: J Nutr 131:2741-2747, 2001; Tsubono Y, Kobayashi M, Sasaki S, et al: J Epidemiol 13:S125-133, 2003), in which 14- to 28-day dietary records and blood samples were collected from subsamples among the Japan Public Health Center cohort members, comprising 107 women in cohort I and 178 women in cohort II. Spearman's correlation coefficients between genistein and daidzein intake estimated from the questionnaire and from dietary records were 0.54 for genistein and 0.55 for daidzein in cohort I, respectively (Tsubono Y, Kobayashi M, Sasaki S, et al: J Epidemiol 13:S125-133, 2003), and 0.53 for genistein and 0.50 for daidzein in cohort II, respectively (unpublished data). To include intakes calculated from the two different questionnaires in the analysis, we estimated genistein and daidzein intake for each subject by cohort from the questionnaires based on a regression function derived from the validation study data.

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