Report

**GSTM1 and GSTT1 polymorphisms and postmenopausal breast cancer risk**

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**Summary**

Glutathione S-transferases (GSTs) are a family of important enzymes involved in the detoxification of a wide variety of known and suspected carcinogens, including potential mammary carcinogens identified in charred meats and tobacco smoke. A substantial proportion of the Caucasian population has a homozygous deletion (null) of the *GSTM1* or *GSTT1* gene, which results in lack of production of these isoenzymes. We conducted a case-control study in a cohort of postmenopausal Iowa women who in 1986 completed a mailed questionnaire on lifestyle factors including information on cigarette smoking and breast cancer risk factors. DNA samples and information related to charred meat intake were obtained, in the case-control study, from breast cancer cases diagnosed during 1992–1994, and a random sample of cancer-free cohort members. Included in this study were 202 cases and 481 controls who were genotyped for *GSTM1* or *GSTT1* gene polymorphisms. Compared to women who had both *GSTM1* and *GSTT1* genes, a 60% elevated risk (95% CI = 1.0–2.5) was observed among those whose *GSTM1* or *GSTT1* gene was deleted. When stratified by meat eating habits, the risk of breast cancer associated with null *GSTM1* or *GSTT1* genotype was observed primarily among women who ate meats consistently well- or very well-done. Women who carried either one of the null genotypes and consumed meat consistently well- or very well-done had a 3.4-fold elevated risk of developing breast cancer (95% CI = 1.6–7.1). Cigarette smoking was not a risk factor for breast cancer among women who had either the *GSTM1* or *GSTT1* genes. Among those with the null *GSTT1* genotype, however, a significantly elevated risk of breast cancer was associated with cigarette smoking (OR = 2.5, 95% CI = 1.1–5.4) and the association was stronger among former (OR = 4.4, 95% CI = 1.5–12.8) than current smokers (OR = 1.3, 95% CI = 0.4–4.1). This study suggests that certain null GST genotypes may be associated with an elevated risk of breast cancer and the association may be modified by charred meat intake and cigarette smoking.

**Introduction**

Glutathione S-transferases (GSTs) are a family of multifunctional enzymes involved in the metabolism of a variety of xenobiotic compounds, including mammary carcinogens, such as polycyclic aromatic hydrocarbons (PAHs) [1–3]. These enzymes catalyze the conjugation of diverse electrophilic compounds with glutathione, giving rise in most cases to less reactive, water-soluble metabolites that are more readily excreted in urine [1–3]. In humans, four classes of cytosolic GST isoenzymes (α, μ, π, and θ) have thus far been identified, and several of them are polymorphic [1, 2]. A substantial proportion of the Caucasian population has a homozygous deletion of the *GSTM1* gene (chromosome 1p13.3, encoding...
GSTµ or the GSTT1 gene (chromosome 22q11.2, encoding GST0), resulting in a complete loss of these isoenzymes [1–3]. Because GSTµ and GST0 are important in the detoxification of carcinogens implicated in breast cancer [1–5], absence of these enzymes may increase the risk of this common malignancy.

A number of epidemiological studies have investigated the association of GST genetic polymorphisms with breast cancer risk [6–14]. The results from these studies, however, have been very inconsistent. Part of the inconsistency may be due to the fact that few previous studies included relevant exposure data in the evaluation of any gene effect on breast cancer risk. Charred meat intake and cigarette smoking are the major sources of PAH exposure in humans. Some epidemiological studies have suggested these two lifestyle factors may be associated with the risk of breast cancer [15–17]. In this study, we report the role of GSTM1 and GSTT1 gene polymorphisms and their potential interactions with charred meat intake and cigarette smoking on the risk of female breast cancer.

**Subjects and methods**

Women included in this study were participants in the Iowa Women’s Health Study, a prospective cohort study of 41,836 women, aged 55–69 at baseline, who completed a self-administered questionnaire in January, 1986. This cohort of women has been followed for mortality and cancer incidence through computer linkage of study participants with Iowa death certificate files, the National Death Index, and cancer diagnosis data collected by the Iowa State Health Registry, which is part of the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute. The baseline questionnaire focused on dietary and other major risk factors for cancer, including family history of cancer, prior medical conditions, cigarette smoking, reproductive factors, and hormone use. Details on the methodology of the cohort study have been published elsewhere [18, 19].

From 1995 to 1996, a case-control study was conducted in the Iowa Women’s Health Study to collect DNA samples and additional information on meat consumption habits [15, 20–23]. Eligible cases for this case-control study were cohort members who were diagnosed with breast cancer between January 1, 1992 and December 31, 1994 (n = 456). Controls were randomly selected from women who were cancer free as of January 1, 1992 (n = 900). Of the 900 controls, 24 were excluded from the control group because they were later found either to have a breast cancer diagnosis (n = 3) or to have been selected to participate in other Iowa Women’s Health Study ancillary projects (n = 21). All eligible women were asked to complete a self-administered questionnaire on meat consumption habits during the ‘reference’ year (1991, 1992, 1993). The reference year for cases was the year immediately prior to breast cancer diagnosis. Controls were divided randomly into three groups corresponding to the three reference years for cases and answered the same questionnaire. A series of color photographs were used to represent the various doneness (charring) levels of hamburger, beefsteak, and bacon and these photographs were included in the questionnaire to facilitate the assessment of usual doneness levels of meat consumed by the study participants [15]. These three meats account for over 60% of the red meats consumed in this population. Of all women selected for the study, 273 cases and 657 controls responded, representing approximately 60 and 75% response rates, respectively. The major reasons for non-participation were refusal (29.1% of cases and 18.7% of controls), inability to locate (4.9% of cases and 3.8% of controls), and death before contacting (5.7% of cases and 2.5% of controls).

Among those who completed the supplemental questionnaire, 96.6% of them (267 cases and 631 controls) provided a sample of buccal cells using a cytobrush [24]. Genomic DNA from buccal cells was extracted using the method described by Richard et al. [24] and stored temporarily at 4°C until genotyping assays. Four hundred eighty-eight women (156 cases and 332 controls) donated a blood sample to the study, for an overall response rate of 53% (57% for cases, 50% for controls). Blood collection was also accomplished through the mail. Specifically, a blood collection kit, including vacutainer tubes, biological specimen packaging containers and envelopes, and instructions, was mailed to all women who agreed to donate a blood sample. Study participants were instructed to contact their physicians to have their blood drawn, and return samples via overnight express mail using preaddressed, prepaid envelopes provided by the study. Genomic DNA from peripheral blood leukocytes was extracted using a standard protocol and stored at a low temperature for subsequent assays.

Polymerase chain reaction- (PCR) based assays were used to determine the GSTM1 and GSTT1 genotypes of study participants. Although these assays did not distinguish between heterozygote and homo-