Research article

Investigation of glutathione *S*-transferase zeta and the development of sporadic breast cancer

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Abstract

Background: Certain genes from the glutathione *S*-transferase superfamily have been associated with several cancer types. It was the objective of this study to determine whether alleles of the glutathione *S*-transferase zeta 1 (*GSTZ1*) gene are associated with the development of sporadic breast cancer.
Methods: DNA samples obtained from a Caucasian population affected by breast cancer and a control population, matched for age and ethnicity, were genotyped for a polymorphism of the *GSTZ1* gene. After PCR, alleles were identified by restriction enzyme digestion and results analysed by chi-square and CLUMP analysis.
Results: Chi-squared analysis gave a χ^2 value of 4.77 (three degrees of freedom) with $P = 0.19$, and CLUMP analysis gave a T1 value of 9.02 with $P = 0.45$ for genotype frequencies and a T1 value of 4.77 with $P = 0.19$ for allele frequencies.
Conclusion: Statistical analysis indicates that there is no association of the *GSTZ1* variant and hence the gene does not appear to play a significant role in the development of sporadic breast cancer.

Keywords: glutathione *S*-transferase zeta, *GSTZ1*, sporadic breast cancer

Introduction

Breast cancer is the most common malignancy in women in the Western world, with an incidence approaching one in 10 in the USA in 1980 [1] and one in 11 in Australia in 1991 [2]. Several genetic and environmental risk factors for the development of breast cancer are already known, including mutations within the *BRCA1* and *BRCA2* genes [3]. Other risk factors include a maternal relative with breast cancer, longer reproductive span, obesity, reproductive history and previous breast cancers [2].

Carcinogens in the body are detoxified by specific enzymes that aid in interception and removal from the cell or in modification involving addition of chemical residues to the reactive sites that cause DNA damage, allowing safe storage, prior to removal [4]. Among these enzymes are the glutathione *S*-transferase (GST) superfamily of enzymes, which prevent the action of electrophilic and alkylating carcinogens by binding to glutathione [5]. Several GST class enzymes have been studied to determine their role in breast cancer susceptibility. The *GSTM1* null genotype has been variously reported as showing significant association in some studies [6] and no association with breast cancer [2] in other studies. The *GSTT1* null genotype, however, has not been found to be involved in predisposition to sporadic breast cancer, although there is evidence that it may play a role in other cancers [2]. *GSTP1* has a polymorphism resulting in an amino acid change from isoleucine to valine, which may predispose to certain cancers, including breast cancer, and has variously

bp = base pairs; GST = glutathione *S*-transferase; *GSTZ1* = glutathione *S*-transferase zeta 1; PCR = polymerase chain reaction.

been found to be significant and not significant in breast cancer [2,7]. With the results from other GST classes in mind, it is possible that any new GST class discovered may have an affect on the development of several different kinds of cancer; hence the present investigation of *GSTZ1*.

The *GSTZ1* gene has a polymorphism recently reported by Blackburn *et al*. [8] that is characterised by base changes from A to G at nucleotides 94 and 124 of the coding region of the gene. Hence, *GSTZ1* has four alleles, *GSTZ1*A* (A94A124), *GSTZ1*B* (A94G124), *GSTZ1*C* (G94G124) and *GSTZ1*D* (G94A124), for which individuals may be homozygotic or heterozygotic. Blackburn *et al*. [8] reported the incidences of the alleles of *GSTZ1* as 9% for *GSTZ1*A*, 28% for *GSTZ1*B* and 63% for *GSTZ1*C*; however, they found no incidence of *GSTZ1*D* at all and reported that it was non-existent, most probably due to its rarity. The base changes from A to G at nucleotides 94 and 124 lead to amino acid alterations from lysine to glutamic acid (Lys 32 → Glu) and from arginine to glycine (Arg 42 → Gly), respectively [8]. These amino acid alterations are known to affect the activities of the resultant *GSTZ1* enzyme for different substrates, particularly dichloroacetates and fluroacetates, affecting the efficiency of removal for these substances. To date, the role of this gene in the development of breast cancer has not been investigated [9]. The purpose of the present study is to investigate the role of the *GSTZ1* gene in the development of sporadic breast cancer.

Materials and methods

Subjects

The populations tested comprised 103 female individuals diagnosed with breast cancer and, as a control population, 103 females with no cancer history at all. The affected and control populations were matched for age and ethnicity, and have been previously described [2,10]. The average ages of the affected and control populations were 58 ± 12 and 57.5 ± 11.6 years, respectively. Most of the samples were recruited through collaboration with the Pathology Department of the Gold Coast Hospital. Additional affected samples, as well as the entire control population, were obtained through the Genomics Research Centre of Griffith University.

Genotyping

The *GSTZ1* allele for each individual was amplified by simple PCR, which produced a DNA fragment 311 bp long. Details of the primers are displayed in Table 1. Since the *GSTZ1* polymorphisms are characterised by two base changes from A to G at positions 94 and 124 [8], identification of each allele was made using restriction enzyme digestion using two restriction enzymes, *Fok*I and *BsmA*I. Once digested, samples were subjected to electrophoresis on an agarose gel and the fragment patterns analysed. With the base changes for each sample identified, the polymorphisms possessed by that individual could be determined, except where the individual possessed both base changes at both locations. In this case, an allele-specific PCR was used, which amplified only the copy of the gene with a G nucleotide at position 124 on the gene. This PCR product was then digested by *BsmA*1 to determine which nucleotide was at position 94. The composition of the second gene could then be deduced without additional PCR. Genotypes were determined as AA (308 bp), GG (115, 93 bp) or AG (308, 115, 93 bp) for the *Fok*I polymorphism, and AA (186, 125 bp), GG (159, 125, 26 bp) or AG (186, 159, 125, 26 bp) for the *BsmA*I polymorphism.

Table 1

Primer compositions for polymerase chain reactions

Primer name	Sequence (5′–3′)
GSTZ1-1F	TGACCACCCAGAAGTGTTAG
GSTZ-1R	AGTCCACAAGACACAGGTTC
GSTZ1-124G	TTCTTACCTGTTGGCCCGC

Statistical analysis

To determine whether any significant differences in polymorphism frequencies occurred between the case and control populations, allele and genotype frequencies were compared using the chi-square method and the Monte Carlo style CLUMP analysis program [11]. This program is best suited for analysis of multiallelic polymorphic markers and has been designed to overcome problems relating to sparse contingency tables. Power calculations indicated that the study was expected to have a >85% power to detect a twofold increase in allele or genotype frequency in the tested populations.

Results

Genotypes and alleles or *GSTZ1* were determined in 103 breast cancer affected individuals and in 103 control individuals, with the frequencies summarised in Tables 2 and 3. The resulting frequencies resemble those noted by Blackburn *et al*. [8], except for the presence of the newly detected *GSTZ1*D* allele (the rarest) occurring in 5.6% of individuals in the sample populations. The frequencies among the genotypes displayed several small differences that were not significant and, due to the presence of low numbers in the frequencies, the genotypes could not be tested using the chi-square method (CLUMP T1 = 9.02, $P = 0.45$). With the frequencies for each allele alone considered, both chi-square and CLUMP methods were usable. Again, however, no significant differences between the allele frequencies were encountered, although the P value obtained from the tests was much lower ($\chi^2 = 4.77$, $P = 0.19$; CLUMP T1 = 4.77, $P = 0.19$).

Table 2

***GSTZ1* genotype frequencies in affected and control populations**

		Genotype frequencies									
Group	*N* (alleles)	AA	BB	CC	DD	AB	AC	AD	BC	BD	CD
Affected	206	1	5	45	2	3	7	6	31	0	3
	%	0.97	4.85	43.69	1.94	2.91	6.8	5.83	30.1	0	2.91
Control	206	5	10	39	1	5	8	5	27	2	1
	%	4.85	9.71	37.86	0.97	4.85	7.77	4.85	26.21	1.94	0.97

CLUMP T1 = 9.02; $P = 0.45$.

Table 3

***GSTZ1* allele frequencies in affected and control populations**

		Allele frequencies			
Group	*N* (alleles)	A	B	C	D
Affected	206	18	44	131	13
	%	8.74	21.36	63.59	6.31
Control	206	28	54	114	10
	%	13.59	26.21	55.34	4.85

Chi-square = 4.77, $P = 0.19$; CLUMP T1 = 4.77, $P = 0.19$.

Discussion

GSTZ1 is a member of the GST superfamily of enzymes. A polymorphic variant of the gene was discovered by Blackburn *et al*. in 1999 [8], and the present study investigated the role of this gene variant in sporadic breast cancer. The results indicate that there is no evidence of a significant relationship between any *GSTZ1* allele or allele combination and the development of sporadic breast cancer within the tested population. It is possible, however, that other factors such as exposure to certain carcinogens or lifestyle may influence this result. *GSTZ1* may also interact with other detoxifying enzymes and, to accurately assess its role, analysis of these additional enzymes should be taken into account in further studies. It is also a possibility that the *GSTZ1* gene is not expressed, or is only poorly expressed, in breast tissue because GST enzymes are differentially expressed in various tissues [12]. Additional studies that take other risk factors into account, as well as matching tumour types, may shed additional light on the role of *GSTZ1* in sporadic breast cancer.

Conclusion

The results of this preliminary study indicate that the *GSTZ1* gene does not appear to play a role in the development of sporadic breast cancer. This extends to both allele and genotype frequencies. The lack of association with breast cancer indicated in the present study does not, however, preclude a role in other cancers. Neither do the results invalidate further studies on *GSTZ1* taking different factors such as gene expression or clinical factors (such as menopausal status) into account.

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