Glutathione S-transferases: an overview in cancer research

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Importance of the field: The Glutathione S-transferases (GSTs) have advanced beyond the classic view of their role in metabolism and are encouraging scientists to assess new approaches to cancer risk characterization and chemotherapy resistance and are opening up exciting possibilities in drug discovery.

Areas covered in this review: In this review, the most recent knowledge about the impact of GST genetic polymorphisms in human's cancer susceptibility, ethnic differences in the effects of risk factors and the rise of the GSTs as important targets for drug development are presented. In this context, the ethnic distribution of GST alleles in different populations, which is an important concept that is being incorporated in epidemiologic studies of cancer risk and environmental exposure, was also evaluated. We present up-to-date information about the new generation of GST-activated cytotoxic prodrugs based on GST overexpression in tumor-acquired drug resistance and the newest results of clinical trials.

What the reader will gain: A critical approach of the major advances in research of GST, underlining the new advances of GST genes polymorphisms in cancer susceptibility and target for therapeutic intervention.

Take home message: Although polygenic factors are involved in increased risk of cancer, the interindividual GST variability plays a central role in reduce cells exposure to carcinogens.

Keywords: cancer, ethnicity, genetic variants and pharmacogenetic, glutathione S-transferases

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1. Introduction

The Glutathione S-transferases (GSTs; EC 2.5.1.18) constitute a superfamily of ubiquitous, multifunctional enzymes which play a key role in cellular detoxification, protecting macromolecules from attack by reactive electrophiles, including environmental carcinogens, reactive oxygen species and chemotherapeutic agents [1].

One common feature of all GSTs is their ability to catalyze the nucleophilic addition of the tripeptide glutathione (GSH; γ-Glu-Cys-Gly) to a wide variety of exogenous and endogenous chemicals with electrophilic functional groups (e.g., products of oxidative stress, environmental pollutants and carcinogens), thereby, neutralizing their electrophilic sites and rendering the products more water-soluble, facilitating their elimination from the cell by Phase III enzymes (**Figure 1**) [2]. In addition, GSTs can serve as peroxidases, isomerases and thiol transferases [3]. They also can have non-catalytic functions such as non-substrate ligand binding and modulation of signaling processes [4].

GSTs are widely distributed in nature and are found in both eukaryotes and prokaryotes as the principal Phase II enzymes in metabolic detoxification processes [5]. In humans, GSTs are divided into at least three major families of proteins, namely cytosolic or soluble GSTs, mitochondrial and peroxisomal (κ class) and microsomal GSTs (now termed MAPEG, membrane associated proteins involved in

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Article highlights.

- Displays the Glutathione S-transferase (GST) superfamily; its division into classes, role in cellular detoxification and distribution in human tissues are addressed. Moreover, the review discusses the functional polymorphisms in GST genes and their influence on the development of diseases, such as cancer.
- Describes the most studied genetic variations in α, μ, π, θ and ω GST genes. The genetic variability and its effects on protein and mRNA expression levels in human tissues, enzymatic and functional activity are reviewed.
- Reports the distribution and ethnic variability of GST genetic variants in Caucasians, Asians, Africans, Arabs and native Latin-American populations.
- Reviews the potential impact of GST genetic variants on cancer risk. It shows the most recent findings on this issue in studies of head, neck, oral, hepatocellular, urinary bladder, renal, breast, gastric, lung and prostate carcinomas. Moreover, the influences of ethnic and environmental variability in cancer research are also addressed.
- Describes and discusses the important role of GSTs in the enzymatic activation of anticancer drugs. The potential developments of 'intelligent drugs', such as canfosfamide, and new clinical findings of GSTs' genetic differences in terms of cancer susceptibility and chemotherapeutic treatment effectiveness are also shown.
- Summarizes the functional genetic variants of GSTs and their association with major risk effect for head and neck squamous cell carcinoma, lung, breast and prostate cancer, or protective effect on hepatocellular and urinary bladder cancer.
- Presents the authors' point of view on the GSTs' genetic variability and the risk of developing cancer, in addition to individual clinical responses toward GST-activated prodrugs. The importance of multigene analysis, including other factors such as lifestyle, nutrition and environmental pollutants are also discussed.

This box summarises key points contained in the article.

eicosanoid and glutathione metabolism) [6]. Human cytosolic GST family is the most complex and relevant to disease investigation. Based on amino-acid sequence similarities, physical structure of the genes (i.e., intron number and position) and immunological crossreactivity, cytosolic GSTs are subdivided into seven distinct classes designated as: α (A), μ (M), π (P), σ (S), θ (T), ω (O) and ζ (Z) (see Hayes *et al.* (2005) for more details about classification of GSTs [5]). When only the N-terminal region is considered, the identity increases. The identity may reach 90% of sequence identity when this region comprises part of the active site, with residues that interact with GSH; however, a limit of 50% sequence identity has been set as a criterion for membership of a given class of mammalian GSTs [7]. Almost all soluble GSTs are active as dimmers of subunits of 23 – 30 kDa with subunits of 199 – 244 amino acids in length (identical, homodimers or different, heterodimers) subunits, and each dimmer is encoded by independent genes [8]. The systematic presence of clusters of GST genes in genomes of both plant and animal species is indicative of a common organizational theme within this gene family and reflects their evolutionary history.

Associations between GST genotypes and disease phenotype may reflect a link between specific mutations and cytogenetic damage in target genes. Presumably genotypes, alone or in combination, should identify subjects who are detoxication-deficient and consequently more likely to suffer formation of carcinogen-DNA adducts and/or mutations [9] conferring major susceptibility to 'complex' genetic disorders such as cancer. The genetic determinants of the majority of these disorders are currently poorly understood, but the few examples that exist demonstrate clinically important racial and ethnic differences in gene frequency (genetic and environmental factors). In this review, the current knowledge about the relationship between GST genetic variants and the susceptibility to cancer are summarized. In this sense, the review also discusses the role of ethnic background among different ethnic populations. The best characterized classes, named α (GSTA), μ (GSTM), π (GSTP), θ (GSTT) and ω (GSTO), are considered here due to their relevance in diseases in the general population.

To carry out the review, we have consulted electronic databases such as Medical Literature Analysis and Retrieval System Online (MEDLINE), Scientific Electronic Library Online (SciELO) and Latin American and Caribbean Health Sciences (LILACS). In the stage of research and selection of articles, the following keyword combinations were used: glutathione S-transferases, cancer, ethnicity, genetic polymorphisms and pharmacogenetics. Also, some additional references of selected articles were included; technical reports and official documents of the WHO and American Association of Cancer and the Global Network of the Global Fund for Research on Cancer were analyzed. There were no exclusion criteria concerning the year of publication of the articles.

2. GST family: genetic variants

2.1 GST α class

The human α class GST is encoded by genes clustered within chromosome 6p12. The cluster consists of five genes: *GSTA1*, *GSTA2*, *GSTA3*, *GSTA4* and *GSTA5*, and seven pseudogenes. Variability of expression of the major GSTs of liver, GSTA1 and GSTA2, is thought to affect the efficiency of detoxification of xenobiotics. GSTA class is widely expressed in human tissues, predominantly in the liver, and it has been shown that both *GSTA1* and *GSTA2* genes are polymorphic [5].

Polymorphism of the *GSTA1* regulatory sequence determines some of the variation of hepatic GSTA1 expression. The two human *GSTA1* alleles are *GSTA1*A* and *GSTA1*B*, containing three linked basis substituted in the proximal promoter region, at positions -567, -69 and -52. *GSTA*1* have T, C and G at positions -567, -69 and -52, respectively, and individuals with *GSTA*B* have G, T and A. These

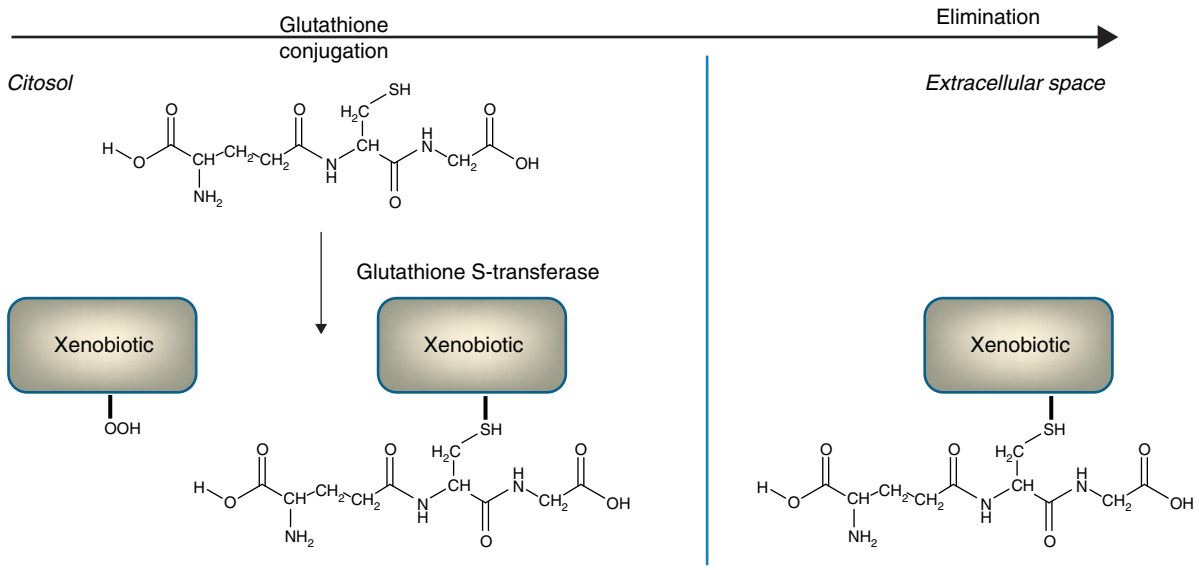

Figure 1. GSH conjugation to a generic xenobiotic via GST results in the formation of a GSH-*S* conjugate. GSTs catalyze reduced GSH, a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine, and conjugation to electrophilic centers via the sulfhydryl group. GST effectiveness depends on the combined actions of, on one hand, glutamate cysteine ligase and GSH synthase to supply GSH and, on the other hand, the actions of transporters to remove GSH conjugates from the cell.
GSH: Glutathione; GST: Glutathione S-transferase.

variants result, under normal conditions, in higher transcriptional activity of the *GSTA1*A* gene (wild type) and lower transcriptional activation with *GSTA1*B* (variant) allele *in vitro* [10]. Liver from individuals who carried the variant *GSTA1*B* showed reduced levels of GSTA1 enzyme [11].

The *GSTA2* locus contains five *GSTA2* allelic variants, *GSTA2*A – E* (**Table 1**), although *GSTA2* polymorphisms (exons 5 and 7) were not thought to affect GSTA2 activity. Ning *et al.* (2004) by analysis of GST protein expression for a set of human liver samples suggested that hepatic expression of the *Ser112* variants (in *GSTA2*A*, **B*, **D* or **E* alleles) was approximately fourfold higher than that of the *Thr112* variant (*GSTA2*C*) [12].

2.2 GST μ class

The human μ class GST is encoded by a 100-kb gene cluster ordered 5′ *GSTM4-GSTM2-GSTM1-GSTM5-GSTM3* 3′, located on chromosome 1p13.3 [13]. *GSTM1* is one of the genes encoding the μ class of enzymes and three polymorphisms have been identified. One polymorphism is a deletion that results in a lack of functional gene product (*GSTM1 null*). The other two, *GSTM1*A* and *GSTM1*B*, differ by a C519G substitution, resulting in asparagine (Asn) to Lys substitution at amino acid 173 [14]. Despite the limited number of substrate types used for comparison tests, no evidence of functional difference between *GSTM1*A* and *GSTM1*B* variants was found; thus, these alleles are typically categorized together as a single functional phenotype [15].

The homozygous deletion (*GSTM1 null*) has been examined extensively in epidemiologic studies. The *GSTM1 null* is caused by a homologous recombination involving the left and right 4.2-kb repeats [13]. Subjects with a homozygous deletion of the *GSTM1* locus have no enzymatic functional activity. Several studies suggest that the *GSTM1 null* genotype can interfere in the drug and carcinogen detoxification. In support of this contention, some studies suggest that *GSTM1 null* genotype is a significant determinant of successful response to chemotherapy in childhood acute lymphoblastic leukemia [16]. Inskip *et al.* (1995) identified two *GSTM3* alleles (*GSTM3*A*, *GSTM3*B*) that differ by the presence of an intronic recognition motif for the YY1 (Yin Yang 1) transcription factor (which is known to have a fundamental role in normal biologic processes such as embryogenesis, differentiation, replication and cellular proliferation). The *GSTM3*B* has been postulated to regulate gene expression. In addition, this study also suggests that *GSTM3*B* and *GSTM1*A* are in linkage disequilibrium [17].

2.3 GST π class

GSTP1 is one of the most extensively studied GSTs genes. Located on chromosome 11q13 and comprising nine exons, this gene encodes the π class of enzymes. *GSTP1* is polymorphic with two common functional variants based on substitutions in amino acids 105, Isoleucine (Ile) to Valine (Val), and 114, Alanine (Ala) to Val, demonstrating different catalytic efficiencies due to changes in the active site [18,19]. Thus, four haplotypes have been identified: the wild-type *GSTP1*A* (*Ile105 + Ala114*) and three variant haplotypes, *GSTP1*B* (*Val105 + Ala114*), *GSTP1*C* (*Val105 + Val114*) and *GSTP1*D* (*Ile105 + Val114*) [20]. In *GSTP1*B*, the most

Table 1. The most relevant polymorphisms of cytosolic GSTs in human diseases.

Class	Chromossome	Gene_accession no.	Alleles	Position of polymorphism	Protein alteration
α	6p12	*GSTA1*_2938	*GSTA1*A*	-567T, -69C, -52G	Reference
			*GSTA1*B*	-567G, -69T, -52A	Low protein levels
		*GSTA2*_2939	*GSTA2*A*	328C, 335G, 588G, 629A	Pro^{110}, Ser^{112}, Lys^{196}, Glu^{210}
			*GSTA2*B*	328C, 335G, 588G, 629C	Pro^{110}, Ser^{112}, Lys^{196}, Ala^{210}
			*GSTA2*C*	328C, 335C, 588G, 629A	Pro^{110}, Thr^{112}, Lys^{196}, Glu^{210}
			*GSTA2*D*	328C, 335G, 588T, 629C	Pro^{110}, Ser^{112}, Asn^{196}, Ala^{210}
			*GSTA2*E*	328T, 335G, 588G, 629A	Ser^{110}, Ser^{112}, Lys^{196}, Glu^{210}
μ	1q13.3	*GSTM1*_2944	*GSTM1*A*	519G	Lys^{173}
			*GSTM1*B*	519C	Asn^{173}
			GSTM1 null	Gene deletion	No protein
		*GSTM3*_2947	*GSTM3*A*	Wild type	Reference
			*GSTM3*B*	3 bp deletion in intron 6	Protein unchanged
π	11q13.3	*GSTP1_2950*	*GSTP1*A*	313A, 341C	Ile^{105}, Ala^{114}
			*GSTP1*B*	313G, 341C	Val^{105}, Ala^{114}
			*GSTP1*C*	313G, 341T	Val^{105}, Val^{114}
			*GSTP1*D*	313A, 341T	Ile^{105}, Val^{114}
θ	22q11.23	*GSTT1*_2952	*GSTT1*A*	310A	Thr104
			GSTT1null	Gene deletion	No protein
			*GSTT1*B*	310C	Pro^{104}
ω	10q24.3	*GSTO1*_9446	*GSTO1*A*	419C	Ala^{140}
			*GSTO1*C*	419A	Asp^{140}
		*GSTO2*_119391	*GSTO2*A*	424A	Asn^{142}
			*GSTO2*B*	424G	Asp^{142}

GST: Glutathione S-transferase.

extensively studied *GSTP1* genetic variant occurs in an A1404G (exon 5) substitution at base pair 313, resulting in an amino-acid difference from Ile to Val at codon 105. Carries of *GSTP1*D* have a nucleotide substitution of C2294T (exon 6) that results in Ala to Val substitution at codon 114. *GSTP1*C* contains both these transitions (*Val105* + *Val114*).

Although the *Ile105* has a higher catalytic efficiency for 1-chloro-2,4-dinitrobenzene than the *Val105* variant [19], the latter seems to confer higher catalytic efficiency to polycyclic aromatic hydrocarbon (PAH) diol epoxide detoxification [18]. Many studies have shown that *GSTP1*B* is often highly expressed in tumors and has been implicated in both the carcinogenic process and in the development of drug resistance [21]. Thus, elevated *GSTP1*B* expression has been associated with aggressive tumors and a poor prognosis with chemotherapy.

2.4 GST θ class

The θ class of GSTs consists of two genes, *GSTT1* and *GSTT2*, located at 22q11.2 and separated by about 50-kb [22]. θ Is considered the most ancient of the GSTs, and θ-like GSTs are found in almost all organisms investigated [23]. Among the GSTT substrates, there are several environmental carcinogens found in food, air or medications, such as PAHs, found in combustion products, diet and tobacco smoke [24].

Similar to *GSTM1*, the most common genetic variant in *GSTT1* consists of a deletion of the whole gene, resulting in the lack of active enzyme [25]. Complete deletion at the *GSTT1* locus was hypothesized by observing the phenotypic variation in glutathione-related detoxification of halomethanes by human erythrocytes, resulting in 'positive' (*GSTT1*$^+$) and 'negative' (*GSTT1 null*) conjugator phenotypes [26]. Another less common polymorphism (*rs11550605*) that results in a threonine to proline substitution at amino acid 104 was described as also resulting in a nonconjugator phenotype [27].

2.5 GST ω class

The GSTO is a newly identified subfamily of GSTs that has some different characteristics in structure and function from the other members of GST superfamily. They have a cysteine residue in their active site in contrast to serine or tyrosine that is in the active sites of other subfamilies [3]. These GSTs have poor activity with common GST substrates (such as 1-chloro-2,4-dinitrobenzene) but exhibit novel GSH-dependent thioltransferase, dehydroascorbate reductase and monomethylarsonate reductase activities, and modulate Ca^{2+}

release by ryanodine receptors [28]. Expression of *GSTO1* is abundant in a wide range of normal tissues, including the liver, colon, heart, ovary, pancreas, prostate and spleen [29]. The widespread distribution of GSTO1 suggests that it has important biological functions.

In humans, ω class GST contains two expressed gene *GSTO1* and *GSTO2* and a pseudo gene *GSTO3p* [28]. Both *GSTO1* and *GSTO2* genes are composed of six exons and are separated by 7.5-kb on chromosome 10q24.3. Two polymorphisms in human *GSTO* genes have shown to be the most frequent in ethnic groups: *GSTO1*C* (*rs4925*) and *GSTO2*B* (*rs156697*). *GSTO1*C* results in an Ala to acid aspartic (Asp) substitution at amino acid 140, which creates a non-conservative amino-acid change from hydrophobic to hydrophilic residue. Tanaka-Kagawa *et al.* (2003) reported that the thiol transferase activity of *GSTO1*C* was 75% of the wild type, reflecting that it may result in defective protection against cellular oxidation stresses [30]. In *GSTO2* gene, a transition of A424G (*GSTO2*C*) at nucleotide position in exon 4 was reported, which results in an amino-acid difference from Asn to Asp in codon 142. It is reported that the *GSTO2 Asp142* variant allozyme showed 20% reduction in level of expression when compared with the level of the *GSTO2* wild-type (*Asn142*) allozyme [31].

3. Ethnicity and GST polymorphisms

Environmental or genetic factors are of fundamental importance in disease risk and may be influenced by ethnic diversity. Most recent studies have shown the influence of the ethnic component in the distribution of GST genetic polymorphisms [32]. The knowledge of the distribution of these alleles is important to determine whether these variants differ in risk effect among these groups.

Overall, genetic polymorphisms resulting in lack of enzyme activity due to homozygous deletion of the *GSTM1* (*GSTM1 null*) and *GSTT1* (*GSTT1 null*) genes are the most studied. For *GSTM1 null* genotype, the frequencies are higher in Caucasians, Asians and Arabs than in Africans (**Table 2**). The *GSTM1 null* genotype occurs between 34 and 58.3% in caucasian population; 47.6 and 56.2% among Asians; 44 and 56.3% in Arabs; 17 and 46.7% among Blacks; and from 0 to 43% in Native Latin-American populations [20,29,33-59]. Paradoxically, the highest frequencies (64 – 100%) of *GSTM1 null* have been reported in studies carried out among South Pacific individuals who have Negroid origin [60]. This is probably due to the fact that these populations remained isolated during a long period under the effect of endogamy and genetic drift [61].

GSTT1 null genotype is lower in Caucasians and increases significantly in Asian populations. *GSTT1 null* genotype displays similar frequencies among Arabian and African-descendents, with some exceptions, such as in Somalians [43]. While the highest *GSTT1* deletion frequencies are present in Asian populations (64.4%) [41], intriguingly the lowest frequencies appear in South American natives (0 – 38.2%) [39,58], despite their Asian origin.

Few studies addressed *GSTP1* polymorphisms associated with ethnicity. *GSTP1*BB* genotype, responsible for the protein expression with less catalytic activity, may vary between 5 and 11.3% in Caucasians [39,43], 3.1 – 8% in Asians [49] (except the Han ethnicity in China, with 37.3% [48]), 13% in Arabs [53], 8 – 23% in Blacks [43,56] and a frequency of 13.8% among native South-American Ameridians [39].

Xenobiotic-metabolizing enzymes, such as GSTs, constitute an important line of defense against a variety of carcinogens. In this context, we may suppose that some populations could be more susceptible to chemical-induced carcinogenesis. In fact, the double selection of *GSTM1* and *GSTT1 null* genotypes, which may vary from 6.3 to 13.8% in Caucasians [39,41], 24.6 – 29.1% in Asians [33,47], 8.8 – 17.2% in Arabs [42,53], 4.5 – 19% in Blacks [34,37] and also 14.7% in Tupinamba Amerindians [39], confer an increasing risk of development of numerous diseases such as cancer, as discussed later. Besides disease associations, different patterns of allele frequency among ethnic groups are interesting for the studies of the population dynamics [14,33].

4. GST polymorphisms and predisposition to cancer

The Human Genome Project has made easier the identification of inherited genetic variants that increase or decrease the risk of complex diseases. Driven by the common variant disease hypothesis, several genetic variants are being evaluated for their association with physiologic characteristics of humans, common chronic diseases and individual variation in drug response.

A variety of genetic variants are involved in metabolizing carcinogens. The result can be a more or less metabolic process efficiency which may contribute to the individual disease susceptibility, depending on the substrate metabolized. Thus, most population genetic studies have revealed a wide genetic variation in metabolic genes within racial or ethnic subpopulations, but there is no consensus about the impact in disease risk. In addition, the identification of genetic factors underlying these disorders and traits has been problematic because they are influenced by many genetic variants and the disease phenotype is also influenced by environmental factors. Even among patients with identical mutations, other modifying genes, specific environmental influences, other diseases and lifestyle demonstrate powerful effects (**Figure 2**). Although genetic determinants of cancer are currently poorly understood, several studies in recent years have shown an influence of GST polymorphisms in cancer susceptibility due to their important role in the modulation of the biological effects of carcinogens (**Figure 3**). Thus, some association studies that were undertaken in order to evaluate the relationship between GST genetic variants with cancer risk are discussed below.

Table 2. Genotype and allele frequencies of *GSTM1*, *GSTT1* and *GSTP1* in populations worldwide.

Ethnic groups/country	n	*GSTM1 null*	*GSTT1 null*	*GSTP1*B*			*GSTM1 null + GSTT1 null*	Ref.
				Ile/Ile	*Ile/Val*	*Val/Val*		
Caucasians								
Portuguese	501	58.3	-	-	-	-	-	[33]
British	1122	57.8	20.5	-	-	-	-	[33]
Swedes	544	55.9	13	-	-	-	-	[33]
White Brazilians: Southeast	1214	48 – 55.4	20 – 26	51.4	34.2	14.4	9.9 – 13	[34-38]
White Brazilians: Northeast	32	37.9	27.6	30	65	5	13.8	[39]
White Brazilians: Centerwest	91	34	22	-	-	-	11	[40]
North Americans	2303	51 – 54.3	15 – 27.6	42	51	7	6.3	[33,41-43]
Danes	537	53.6	12.9	-	-	-	-	[33]
French	1184	53.4	16.8	-	-	-	-	[33]
Denmark	100	52	14	37	53	9	-	[44]
Germans	1618	44.9 – 51.6	13.4 – 19.5	47.9 – 55.1	36.2 – 40.8	8.7 – 11.3	-	[33,45,46]
Canadians	591	51.3	17.2	42	51	7	-	[33,43]
Norwegians	423	50.6	-	-	-	-	-	[33]
Dutch	419	50.4	22.9	-	-	-	-	[33]
Spanish	312	49.7	27.6	-	-	-	-	[33]
Italians	810	49.4	16.3	-	-	-	-	[33]
Greenland	100	47	46*	42	52	6	-	[44]
Finns	482	46.9	13	-	-	-	-	[33]
Total n/range	**12,383**	**34 – 58.3**	**12.9 – 27.6**	**30 – 55.1**	**34.2 – 65**	**5 – 14.4**	**6.3 – 13.8**	
Asians								
Singaporeans	244	56.2	51.9	-	-	-	-	[33]
Koreans	2017	52.1 – 53.8	51.5 – 60.2	61 – 68.4	29.1 – 37	2.5 – 3.9	29.1	[29,33,41, 47,48]
Asians	1511	52.9	47	-	-	-	24.6	[33]
Chinese: Han	102	54.9	52	62.7	37.3*	-	[49]	
Chinese	119	50.4	45.4 – 64.4	70.6	28.6	8	-	[41,50]
Japanese	896	47.6	35.3	71.6	25.3	3.1	-	[33,51]
Indians: North	370	33*	18.4	44.3	50.3	5.4	-	[52]
Indians: South	225	22.4*	17.6	58.4	38.4	3.1	-	[53]
Total n/range	**5484**	**47.6 – 56.2**	**17.6 – 64.4**	**44.3 – 71.6**	**25.3 – 50.3**	**2.5 – 8**	**24.6 – 29.1**	
Arabs								
Saudis	1405	55 – 56.3	25	33.5	53.5	13	17.2	[33,54]
Egyptians	255	44 – 55.5	14.7 – 29.5	-	-	-	8.8	[42,55]
Total n/range	**1660**	**44 – 56.3**	**14.7 – 29.5**	**33.5**	**53.5**	**13**	**8.8 – 17.2**	
Blacks								
African-Americans	1603	28 – 46.7	17 – 26.7	6.7 – 22	55 – 80	13.3 – 23	-	[41,56,57]
Africans and African-Americans	479	-	26.7	-	-	-	-	[33]
Somalians	100	40	44	53	39	8	-	[44]
Black Brazilians: Southeast	469	28 – 35	26 – 36	46.2	45.5	8.3	4.5 – 19	[34,35, 37,38]
Black Brazilians: Centerwest	106	34	22	-	-	-	8	[36]
Black Brazilians: Northeast	140	33.8	28.9	36.4	49.5	14.1	11.3	[39]
Quilombolas Brazilians: Northeast	206	17 – 35	25 – 44	-	-	-	6 – 9	[40]

*Values statistically different from the rest of the group (values not computed in the range).

Table 2. Genotype and allele frequencies of *GSTM1* (continued).

Ethnic groups/country	n	*GSTM1 null*	*GSTT1 null*	*GSTP1*B*			*GSTM1 null* + *GSTT1 null*	Ref.
				Ile/Ile	*Ile/Val*	*Val/Val*		
Quilombolas K. Brazilians: Centerwest	68	26	33	-	-	-	13	[40]
Zimbabweans	148	24	26	-	-	-	-	[58]
South Africans	96	23	20	-	-	-	-	[58]
Total n/range	**3415**	**17 – 46.7**	**17 – 44**	**6.7 – 53**	**39 – 80**	**8 – 23**	**4.5 – 19**	
Native South americans								
Mexican-Americans	-	-	9.7	-	-	-	-	[41]
Native Brazilians: North	157	0 – 43	0 – 27	-	-	-	-	[59,60]
Native Brazilians: Centerwest	153	3.9 – 27	11.8 – 30.3	-	-	-	-	[59,60]
Native Tupinamba Brazilians: Northeast	31	26.5	38.2	62.1	24.1	13.8	14.7	[39]
Native Aché Paraguayans	67	35.8	17.9	-	-	-	-	[59]
Total n/range	**408**	**0 – 43**	**0 – 38.2**	**62.1**	**24.1**	**13.8**	**14.7**	

*Values statistically different from the rest of the group (values not computed in the range).
-: Not available; GST: Glutathione S-transfer

4.1 Head and neck carcinoma

Among the most studied cancer with GST polymorphisms is the head and neck squamous cell carcinoma (HNSCC). This cancer is one of the most prevalent in the world. Besides its correlation with other environmental pollutants, which are preferentially deactivated by the GSTs, it has a strong association with the carcinogens and pro-carcinogens present in tobacco, such as PAHs. Tobacco habit is the most important risk factor for HNSCC. For example, death from laryngeal squamous cell carcinoma is 13.56 times more likely for heavier smokers than for non-smokers [62]. Thus, variations in the expression of GSTs due to heritable genetic polymorphisms probably modulate the process of carcinogenesis by altering the exposure levels of tobacco-derived carcinogenesis. However, many studies that evaluate the association between GST polymorphisms and HNSCC are quite divergent, reporting weak-to-moderate associations in the risk for the main effect of the gene [63]. A meta-analysis in 31 studies with 4635 cases and 5770 controls and one pooled analysis of nine studies with 2334 cases and 2766 controls in HNSCC patients found an OR (odds ratio) of 1.23, 95% CI = 1.06 – 1.42 and 1.32 (95% CI = 1.07 – 1.62) for *GSTM1 null*, 1.17 (95% CI = 0.98 – 1.40) and 1.25 (95% CI = 1 – 1.57) for *GSTT1 null*, and 1.10 (95% CI = 0.92 – 1.31) and 1.15 (95% CI = 0.86 – 1.53) for *GSTP1*B* allele, respectively. When the analysis was combined among the three genes, an increase in OR to 2.06 (95% CI = 1.11 – 3.81) was found [64]. Thus, there is a high probability that *GSTM1* and *GSTT1 null* genotypes and *GSTP1*B* may result in a synergetic risk for carcinogenesis in HNSCC [14].

In the same manner, for *GSTM1 null*, a study that included 63 African-Americans and 101 caucasian patients with histologically confirmed primary oral cancer, as well 133 African-Americans and 213 Caucasians matched control subjects showed OR = 3.1 (95% CI = 1.1 – 8.5) in African-Americans, and oral cancer risk was higher in heavy smokers OR = 5.4 (95% CI = 1.2 – 24) [65]. In a study that included 294 HNSCC cases and 333 controls, a positive association was found between homozigote individuals for *GSTP1*B* and *NQO1* (quinone oxidoreductase 1) *Arg139* allele. These authors reported that tobacco in a dose-dependent manner increased the HNSCC risk [66].

On the other hand, some studies did not find a positive association between GST polymorphisms and HNSCC. In a review of 11 recent works that related *GSTM1 null* with HNSCC, five of the evaluated studies did not show a positive association. Likewise, out of other six studies, four of the analyzed ones did not obtain this association [67]. In a similar manner, another study suggests that *GSTT1 null* also does not have a significant association [68].

Additionally, many studies directed for the anatomical placing of the cancer may suggest GSTs roles in various tissues. The main difficulties for the elucidation of these variations are in assessing the level of tissue exposure to the carcinogen and the response by the integration with local detoxification machinery. For oral cavity and pharyngeal cancers, a study conducted in France found OR = 1.6 (95% CI = 1 – 2.8) for *GSTP1*BB* or *AB* genotype and OR = 2 (95% CI = 1 – 4) for *GSTT1 null* genotype. In subjects with a history of > 30 years of smoking, the respective ORs were 2 (95% CI = 1 – 3.9) and 3.3 (95% CI = 1.3 – 8.1) [69]. In Germany, the frequencies of the *GSTM1*AB* heterozygotes and *GSTM3*BB* homozygotes were significantly lower in cases than controls [70]. For oral squamous cell carcinoma (OSCC), two works have found a positive association [71] with *GSTT1 null*, while for *GSTM1*

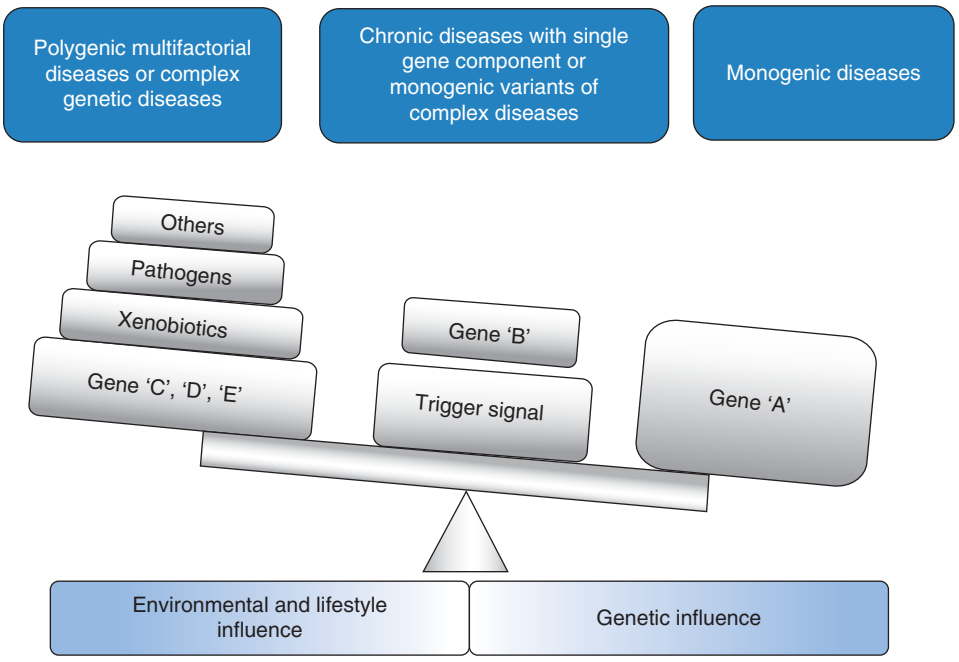

Figure 2. The balance of risk. Genetic profile and environmental factors determine the risk of a disease. For each disorder, different weights can be assigned as a risk factor.

null some authors did not observe any association [69]. A work conducted in Brazil showed OR = 1.94 (95% CI = 1.04 – 3.66) for OSCC in *GSTM3*B* combined with *NAT2* heterozygotes [72].

Analizing nasopharyngeal squamous cell carcinoma, the demonstrated risk was of 1.9-fold for *GSTM1 null* [73]. Despite some studies that also analyzed the association between *GSTM1 null*, *GSTT1 null* and HNSCC, Cheng *et al.* (1999) showed that 53.1% of 162 cases and 42.9% of 315 healthy controls were carriers of *GSTM1 null*, whereas 32.7% of cases and 17.5% of controls were *GSTT1 null* carriers ($p < 0.05$ and < 0.001), respectively. Furthermore, 19.8% of cases and 7.9% of controls were carriers of deletion for both genes ($p < 0.001$) [74]. When the authors made multivariate analysis (age, sex, ethnicity, smoking status, alcohol status and GST genotypes) using logistic regression models, it was found that both of these genotypes remained independent risk factors for disease, OR = 1.50 and 2.27 (95% CI = 1.01 – 2.23 and 1.43 – 3.60), respectively [74].

In another study with 149 esophageal cancer patients and 200 nonmalignant controls, the authors found that patients who were heterozygous carriers of *GSTM3*AB* genotype had an enhanced risk for developing esophageal cancer, OR = 2.1 (95% CI = 1.1 – 3.7; $p = 0.01$). In males, the risk due to *GSTM3*AB* genotype was higher, OR = 3.4 (95% CI = 1.7 – 6.8; $p < 0.001$). In addition, the interaction of *GSTM3*AB + BB* and *GSTM1 null* genotypes obtained the OR = 2.3 (95% CI = 1.1 – 3.7; $p = 0.01$) [75].

There is evidence that ethnicity and the presence of GST polymorphisms may confer different risks of HNSCC. Park *et al.* (2000) reported an increased risk for oral cancer in African-Americans with the following genotypic combinations: *GSTM1*$^+$ and *GSTM3*AA* or *AB*, OR = 2.2 (95% CI = 0.82 – 6); *GSTM1 null* and *GSTM3*BB*, OR = 4.3 (95% CI = 1.1 – 16); and *GSTM1 null* and *GSTM3*AA* or *AB*, OR = 6.6 (95% CI = 1.2 – 38) [65]. Among the Japaneses, the frequency of the *GSTM1 null* genotype was significantly higher in cancers patients (58.7%) compared with controls (46.3%) [71]. In another study, patients with *CYP1A1*C* and *GSTM1 null* genotypes contracted oral carcinoma after fewer cigarettes than those with other genotypes [76].

Because HNSCC is considered as an ideal model for the study of gene–environment interaction, the divergences may be attributed to the difficulty that the researchers have in controlling the individuals environmental exposure, especially among those culturally influenced, which may contribute to the differences observed among population groups.

4.2 Breast cancer

Breast cancer (BC) is also the target of many studies that attempt to elucidate some triggering genetic factors. Many authors have associated GSTs polymorphisms with a greater risk for developing this disease that affects thousands of women over the world. While the well-studied mutations in genes *BRCA1*, *BRCA2* and *p53* presented high risks for the development of BC, the frequencies of these risk alleles are quite low. Conversely, the mutations in xenobiotic metabolism genes (including genes that code for GSTs) have high allelic frequencies in the general population.

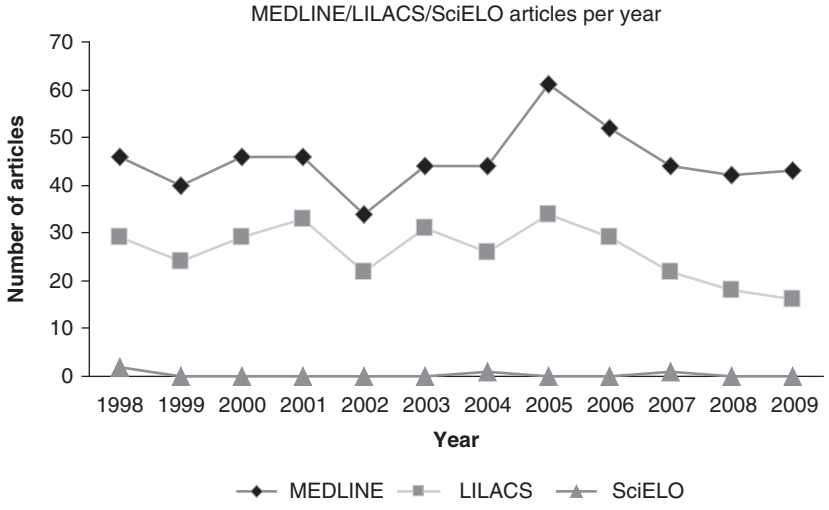

Figure 3. Publications involving Glutathione S-transferase and cancer susceptibility. The number of articles published in the last 11 years in different databases using the keywords glutathione S-transferase and cancer.

A study examined 278 cases and 271 African-Americans controls and 410 cases and 392 white controls in the Carolina Breast Cancer Study. The authors showed some positive genetic associations, and women with *GSTM1 null* obtained OR = 2.1 (95% CI = 1 – 4.2), while among carriers of *GSTT1 null* the OR was 1.9 (95% CI = 0.8 – 4.6) [56]. Mitrunen *et al.* (2001) associated *GSTM1*, *GSTM3*, *GSTP1* and *GSTT1* polymorphisms with the risk of BC development in 483 patients and observed a positive association with *GSTM1 null*, OR = 1.49 (95% CI = 1.03 – 2.15) and *GSTM3*B* allele. For *GSTP1 Ile105/Ile105* genotype, the observed OR was of 2.07 (95% CI = 1.02 – 4.18), being significantly higher in women with *GSTT1 null*, where the risk was strongly increased, OR = 9.93 (95% CI = 1.10 – 90). In this sense, with the intention of evaluating new combinations, these authors found that in the combination *GSTM1 null-GSTT1 null-GSTP1 Ile105/Ile105*, the OR was of 3.96 (95% CI = 0.99 – 15.8) [77]. Gudmundsdottir *et al.* (2001) investigated 388 BC patients and 395 controls, observing a positive association in patients carrying *GSTT1*A* combined with *p53* mutations between cases and controls (24.6 versus 12.4%; p = 0.019) [78].

Vogl *et al.* (2004) revised seven studies that associate *GSTM1*, *GSTT1* and *GSTP1*B* polymorphisms at the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens, with 2048 BC patients and 1969 controls. The authors obtained OR = 0.98 (95% CI = 0.86 – 1.12) for *GSTM1 null*, OR = 1.11 (95% CI = 0.87 – 1.41) for *GSTT1 null* and OR = 1.01 and 0.93 (95% CI = 0.79 – 1.28 and 0.62 – 1.38) for *GSTP1*B* heterozygous and homozygous mutants, respectively [79].

Like other types of cancer, the majority of these studies concentrated on genes *GSTM1*, *GSTT1* and *GSTP1*. For the gene *GSTA1*, most of the evidence for *GSTA1*B* polymorphism has a negative association with BC as well as *GSTO2*B* [80]. On the other hand, *GSTO1*BB* genotype had higher risks of BC when compared with carriers of the *GSTO1*AA* genotype, with incidence rate ratio = 1.62 (95% CI = 1.01 – 2.61). This association was strongest with regard to estrogen receptor positive BC, incidence rate ratio = 2.16 (95% CI = 1.21 – 3.84) [81].

4.3 Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a primary malignancy and accounts for 80 – 90% of all liver cancers [82]. Interestingly, the liver and kidney are two organs that express the highest level of GSTT in the human body. Elevated levels of GSTT1 protein, obviously in *GSTT1*A* carriers, show an elevated risk to the development of HCC for halogenated solvents exposure [83]. Thus, *GSTT1* genotype seems to modulate the hepatic cancer risk, considering the source of the xenobiotic exposure.

A meta-analysis in 15 studies associated the HCC risk with *GSTA1*, *GSTA4, GSTM1*, *GSTM2*, *GSTM3, GSTT1*, *GSTP1*, *GSTO1* and *GSTO2* polymorphisms in Asian, African and European populations. The authors observed that only *GSTT1* and *GSTM1 null* carriers showed a positive association, however, with weak risk, OR = 1.19 and 1.16 (95% CI = 0.99 – 1.44 and 0.89 – 1.53), respectively [84].

A study carried out in India found that *GSTT1 null* individuals showed a risk increased by 2.23-fold ($p < 0.05$) for HCC when compared to the control group, while *GSTM1 null* genotype presented a protective effect. In gene–gene interaction analysis, *GSTM1-GSTT1* and microsomal epoxide hydrolase polymorphisms demonstrated a synergistic association for HCC development [85].

Although most studies estimate a weak association between HCC and GST polymorphisms in the absence of environmental risk factors, additional studies are needed with larger samples, especially in different populations. This is reinforced due to the fact that HCC cases are not homogeneously distributed in the world (> 80% of HCC cases occur in either Sub-Saharan Africa or in Eastern Asia).

4.4 Gastric cancer

Despite many studies showing that infection by *Helicobacter pylori* is the cause of most gastric cancer (GC), various genetic factors are associated with increasing risk levels. In relation to genes *GSTM1* and *GSTT1*, the majority of publications do not show association between the respective deletions and the increase in the risk of GC [86]. However, a study with 304 GC patients and 427 control subjects showed a 1.48-fold increased risk (95% CI = 0.97 – 2.25) in patients with *GSTT1 null* genotype, but not with *GSTM1*, *GSTM3* or *GSTP1* genotypes. Furthermore, when the authors stratify the patients with the *GSTT1 null* genotype by age, it was observed that younger patients (< 50 years old) obtained an increasing GC risk (OR = 3.85) [87]. In addition, a case-control study performed in 108 GC patients and 195 healthy controls demonstrated that carriers of *GSTM1 null* genotypes obtained an increasing GC risk, OR = 1.98 (95% CI = 1.22 – 3.21; p = 0.006). Interestingly, smokers and high salted tea consumers were at higher risk with OR = 8.98 (95% CI = 5.16 – 15.62; p = 0.0001) [88]. However, some authors show that *GSTM1* and *GSTT1 null* genotypes (in addition to the presence of *GSTP1*B* and *GSTO2*B* alleles) confer a reduction in the susceptibility to GC [89].

In an attempt to evaluate the genetic predisposition to GC, Al-Moundhri *et al.* (2009), despite not having found a positive association with *GSTM1 null* separately, demonstrated a positive interaction and an increase in GC risk in *GSTM1 null* carriers combined with carriers of *IL-1RN*2* (IL-1 receptor antagonist) polymorphism. It was suggested that the individual variation in both the cellular inflammatory modulator (*IL-1RN*) and the antioxidative property of *GSTM1* may predispose individuals to an increased GC risk [86].

On the other hand, a few works show that *GSTM1* and *GSTT1* may present a GC risk, even when combined [90]. In this direction, a study shows a greater risk for *GSTT1 null* carriers, OR = 2.58 (95% CI = 1.53 – 4.36), and for simultaneous carriers of both *GSTM1* and *GSTT1 null* genotypes, the OR = 3.32 (95% CI = 1.62 – 6.77) for GC risk. Carriers of the *GSTP1*BB* genotype showed a protective effect, OR = 0.20 (95% CI = 0.02 – 0.86) [90]. Boccia *et al.* (2007) carried out a meta-analysis that associated *GSTT1* polymorphisms in 2508 GC cases and 4634 controls. These authors suggested that the *CYP2E1 PstI/RsaI* polymorphism may be a risk factor for GC in Asians and that a synergic relation among *GSTM1* and *CYP2E1* may account for a proportion of GC cases [91].

4.5 Lung cancer

Lung cancer (LC) is the leading cause of death from cancer worldwide, being responsible for 1.3 million deaths annually [92]. Among the GSTs, GSTP1 is the most commonly expressed in the pulmonary tissue, having among its main metabolic agents the PAHs present in cigarette smoke [76]. Cote *et al.* (2009) published a meta- and a pooled analysis of 27 (8322 cases and 8844 controls) and 15 studies (4282 cases and 5032 controls), respectively, in which they examined the association between *GSTP1*B* and LC risk. The meta-analysis did not show a significant association with *GSTP1*B*, even when stratified by ethnicity. In the pooled analysis, the authors found OR = 1.11 (95% CI = 1.03 – 1.21) between LC and members who carried the *Val105* compared with *Ile105*. After stratification by ethnicity and adjustment for age, sex and smoking status, an increased risk was associated with *GSTP1*B* presence in Asians, but not in White population [93]. In another work with 1921 LC cases and 1343 healthy Caucasians, *GSTP1*B* homozigotes did not show a positive association for increased risk for LC, OR = 1.02 (95% CI = 0.78 – 1.34). However, when stratified by age to evaluate whether *GSTP1*B* is associated with early-onset LC, the individuals with age under 50 years old showed greater susceptibility to LC with OR = 2.67 (95% CI = 1.36 – 5.22) than older individuals, who presented OR = 0.87 (95% CI = 0.65 – 1.2) [94].

Some studies have shown a strong association between the tobacco habit and the development of LC in *GSTM1* and *GSTT1 null* genotypes carriers. Hosgood *et al.* (2007) have also carried out a meta-analysis of six studies with 912 cases and 1063 controls in Asian populations. The authors observed a slight increase in LC risk for *GSTM1* and *GSTT1 null* genotypes, OR = 1.31 and 1.49 (95% CI = 0.95 – 1.79; p = 0.100 and 1.17 – 1.89; p = 0.001), respectively. However, these authors did not observe any association with *GSTP1*B* and also suggest that the LC risk is higher in populations with coal exposure [95]. In fact, gene–environment interactions have been extensively studied in LC. Lee *et al.* (2006) studied the association among *CYP2E1*, *GSTM1* and *GSTT1* genetic variants with tobacco habits and LC. Smokers have shown a significant increase in LC risk (p < 0.001); however, among the genetic variants studied, only carriers of *GSTM1 null* showed a significant risk for LC, OR = 1.9 (95% CI = 1.04 – 3.60), even among smokers [96]. Raimondi *et al.* (2006) carried out a meta-analysis that included 34 studies, with 7629 cases LC and 10,087 controls and one pooled analysis including 34 studies with 7044 cases and 10,000 controls. The meta-analysis results suggest a positive association for *GSTT1 null* in Asians, OR = 1.28 (95% CI = 1.10 – 1.49), but not in caucasian subjects, OR = 0.99 (95% CI = 0.87 – 1.12). In the pooled analysis, the ORs were not significant for either Asians, OR = 0.97 (95% CI = 0.83 – 1.13) or Caucasians, OR = 1.09 (95% CI = 0.99 – 1.21), as well as no significant interaction was observed between *GSTT1* and smoking on LC [97].

There is a significant relationship between high adduct levels and the NSCLC. Many authors findings imply that the *GSTM1 null* and *CYP1A1* exon 7 polymorphisms may influence PAH-DNA adduct levels in target tissue from NSCLC patients, especially in the squamous cell carcinoma group. Moreover, individuals carrying the *GSTM1 null* and *CYP1A1*Ile/Val* genotype might exhibit a greater predisposition to a peripheral type of LC [98].

Some studies suggest that *GSTM1* and *GSTT1* polymorphism effects in non-smokers are similar to that found in LC smokers [97,99]. Despite some authors having indicated a relation among these genetic deletions with the increase levels of DNA adducts and deficiency in the detoxification of the tobacco carcinogens, there were works that did not find a positive association between these genotypes and LC [61]. Especially in LC, a work demonstrated that the two main risk factors (DNA adducts and genotypes) seem to be independent predictors of LC risk [100]. Other studies show a significant participation of *GSTM1* deletion with the disease course. Sweeney *et al.* (2003) evaluated the survival of patients diagnosed with LC and found that *GSTM1 null* carriers had shorter survival, with relative death risk of 1.36 (95% CI = 1.04 – 1.80) [101].

There is a tendency in current studies to try to evaluate the interaction of more than one gene with a determined disease. Despite the existence of many divergences, most of studies observed that LC risk and protection is higher when interactions with more GST polymorphisms and other risk factors (e.g., cigarette smoking) are evaluated.

4.6 Renal and urinary bladder cell carcinoma

Renal cell carcinoma (RCC) is an aggressive malignancy that is associated with a high rate of metastasis and the most common type of kidney cancer, responsible for ~ 80% of cases [102]. Some studies show that *GSTM1*A* or *GSTM1*B* individuals present high risk of RCC compared to *GSTM1 null* individuals exposed to metals or pesticides [103]. However, other works demonstrated that *GSTM1 null* genotype frequency does not differ between RCC patients and healthy individuals [101]. Like with *GSTM1*, *GSTT1* studies demonstrated a positive association for both *GSTT1 null* and *GSTT1*A* homozigote genotype.

Some studies show that individuals with positive *GSTT1* genotype exposed to metals and pesticides present higher RCC risk [103]. The role of *GSTP1* polymorphims in RCC was little investigated. Despite some authors demonstrating an association between *GSTP1* polymorphisms and RCC risk, others did not achieve this result [101].

Transitional cell carcinoma (TCC) is a common malignancy affecting the genitourinary tract and is the most common type of urinary bladder cancer (TCCUB). Some authors suggest that *GSTM1 null* carriers obtained predisposition for TCC. The roles of *GSTT1* and *GSTP1* polymorphisms in TCC are still controversial [83].

Golka *et al.* (2008), including 293 urothelial and bladder transitional TCC patients, showed that *GSTM1 null* carriers presented a substantial increase in the risk of disease, among smokers or non-smokers [104]. The same way, another study has not showed an increased risk associated with tobacco or certain toxicants, such as asbestos, rubber and chlorinated solvents [105]. Engel *et al.* (2002), carried out meta- and pooled analysis of 17 studies, with 2149 urinary bladder TCC cases and 3646 controls, obtaining OR = 1.44 (95% CI = 1.23 – 1.68) for *GSTM1 null* genotype, but did not show a significant increased risk in smokers (additive interaction = 0.45) [106]. For the gene *GSTT1*, some studies suggest that *GSTT1 null* individuals have an increased urinary bladder TCC risk [107]. By contrast, other findings have shown that the *GSTT1 null* genotype is a protective factor against bladder cancer [107]. The *GSTA1* polymorphisms have demonstrated to be relevant in TCC risk associated with tobacco. One of the suggestions for this fact is that *GSTA1*BB* individuals have four times less liver expression for this enzyme when compared to the ones that present the *GSTA1*A* allele [83].

Concerning the association between *GSTP1* polymorphisms and urinary bladder TCC, an increased risk was already shown in carriers of both *GSTP1* wild-type (*GSTP1*AA)* [108] and *GSTP1*BB* genotypes [109]. Harries *et al.* (1997) demonstrated a threefold of increase in bladder cancer risk in *GSTP1*BB* compared to *GSTP1*AA* individuals [110]. Kellen *et al.* (2007) examined the association between *GSTP1*B* and urinary bladder TCC through a meta-analysis in 16 studies, with 4273 cases and 5081 controls. The ORs found for *GSTP1*AB* and *BB* compared with *AA* were 1.54 (95% CI = 1.21 – 1.99; $p < 0.001$) and 2.17 (95% CI = 1.27 – 3.71; $p = 0.005$), respectively. This result suggests a carcinogenic tendency for *GSTP1*B* carriers. Yet, the risk was stronger among Asians than European descendents [111].

4.7 Prostate cancer

Prostate cancer (PC) is the most common type of cancer in Western countries and the second cause of death from cancer, after LC. As in the other types of cancer, it was much speculated that *GSTM1*, *GSTT1* and *GSTP1* polymorphisms could be associated with the carcinogenic development in the prostate; however, the results are inconclusive. Recently, through a meta-analysis with 29 studies (4564 cases and 5464 controls), Mo *et al.* (2009) reported that *GSTM1 null* increased PC risk, OR = 1.3 (95% CI = 1.15 – 1.55), obtaining also similar results when the analyses were stratified by ethnicity (Asians and Caucasians). Interestingly, among Africans and African-Americans who shared risk similarities (OR = 0.66 and 0.47, respectively), the values obtained were far from the general population (OR = 1.3). In this study, the authors did not observe any type of association with *GSTT1 null* (22 studies with 3837 cases and 4552 controls) as well as *GSTP1*B* (24 studies with 5301 cases and 5621 controls) polymophisms with PC risk, even after ethnic stratification [112].

Similarly, another meta-analysis including 11 studies that associated *GSTM1 null* (2063 cases and 2625 controls),

10 studies with *GSTT1 null* (1965 cases and 2554 controls) and also 12 studies with *GSTP1*B* polymorphisms (2528 cases and 3076 controls) did not obtain significant frequency differences between PC patients and healthy controls [113]. Lima *et al.* (2008) evaluated the polymorphisms of the *GSTM1*, *GSTO1*, *GSTP1* and *GSTT1* genes in PC and benign prostatic hyperplasia patients paired for ethnic and lifestyle characteristics (lifetime occupational history, dietary patterns, cigarette-smoking and others) in a Brazilian population. Using a combination with uni- or multivariate regression logistic analysis, the authors also did not obtain any association between the studied polymorphisms and the many clinical factors evaluated [114].

The role of genetic variation at the *GSTM3* in the PC risk, though little studied, suggests evidence of an association. Althought no increasing PC risk was observed in *GSTM1* or *GSTT1* deletion genotypes carriers, OR = 1.20 (95% CI = 0.75 – 1.90; p = 0.420) and OR = 0.87 (95% CI = 0.50 – 1.51; p = 0.550), respectively, men carrying *GSTM3*BB* have an increased PC risk with OR = 5.50 (95% CI = 1.2 – 25.8; p = 0.016) [115]. In another study that included 135 PC patients and 169 controls, a 2.5-fold increased risk was found in *GSTM3*AB* and *BB* carriers (p = 0.028) when compared to wild-type genotype. The authors still showed that patients who were either smokers and/or had alcohol habits demonstrated an association with *GSTM3*B* genetic variant, OR = 4.11 (p = 0.046) and OR = 4.38 (p = 0.027), respectively [116].

In a study carried out in Japan, the authors found OR = 1.72 for PC patients *GSTA1*B* carriers. However, the genetic polymorphism distribution was not statistically different observed for genotypes of *GSTM1* and *GSTP1* when compared to healthy control. Interestingly, this work also demonstrated an increase of PC risk (OR = 2.08) in carriers of the following combined genotypes: *GSTA1*AB* or *BB* and *GSTT1* nondeletion [117]. However, Ning *et al.* (2004) evaluating various *GSTA1* and *GSTA2* genetic polymorphisms, did not find any type of association with PC risk [12]. Thus, there is no sufficient evidence to evaluate the impact of the GST α family genes in the PC risk.

5. GST and drug discovery in cancer

Clinical correlation studies show that genetic differences within the human GSTs may play a role in cancer susceptibility and treatment, as extensively discussed. In cancer chemotherapy, pharmacogenetic studies have traditionally focused on single gene candidates that interfere on pharmacokinetic characteristics of a specific drug, which reduces their detoxification, conferring high toxicity. Their typical function is to detoxify reactive metabolites, but their role in the formation of cytotoxic metabolites has also been documented [5]. From GST family, GSTP1 has been associated with an increased cancer incidence, therapy-related cancers and toxicity following chemotherapy [5,118-120]. On the other hand, some *GST* genetic polymorphisms have also been associated with chemotherapeutic efficacy, although this seems to be disease and polymorphism-specific [121].

GST demonstrates unexpected contributions and furthers the classical view, especially with drug resistance and new approaches as target of new drugs such as tumors proteins. In different studies, it has become clear that high levels of GSTP1 were a rather consistent feature of some tumors; in this way the prognosis in several tumor types was inversely correlated with GST expression in the tumor tissue [122,123]. Such observations lead to an initial conflict as not all drugs that were selected for cancer resistance were substrates for catalysis by GSTs. However, GSTs non-enzymatic functions were shown to interact, for example, with activated protein kinases (such MAPKs and PKC), TNF receptor-associated factor and transglutaminase 2, and some authors suggested an alternative way to understand heterologous gene-induced cancer drug resistence [124,125].

Considering the overexpression GSTP1 in tumor cells, 'intelligent drugs' were developmented, such as the prodrug canfosfamide (TER286), a GSH analogue that is activated by GSTP1 [126]. Preclinical studies demonstrated the increased sensitivity of tumors expressing high levels of GSTP1-1 to the cytotoxic effects of canfosfamide [127]. Following activation, the apoptotic activity of canfosfamide is mediated through the pathway of stress response, resulting in the induction of MKK4 (mitogen-activated protein kinase), p38 kinase, JNK and caspase 3 [128]. As expected, the cytotoxic activity of canfosfamide was more observed in human cancer cell lines that are resistant to conventional agents [129].

Phase II studies with combination regimens with platinums, taxanes and anthracyclines demonstrated that canfosfamide works better in combination than as a single agent [129,130]. In spite of canfosfamide not meeting the primary end point in a recent Phase III study, it increased overall survival with third-line therapy in patients with ovarian cancer [131]. However, to date, unequivocally clinical results have not been demonstrated, and new randomized clinical trials should provide further evidence for canfosfamide in cancer treatment. It is worthwhile to mention that a few proteins such as GST encourage scientists from different areas of research to develop and investigate how this specific protein contributes as risk factor of diseases, adverse drug reactions, cancer cell resistances and even as a 'targeted protein approach'.

6. Conclusion

Inherited genetic traits co-determine the susceptibility of an individual to a drug response and toxic chemical. Allelic variants of relevant xenobiotic metabolizing result in a differential risk of cancer susceptibility. Special emphasis has been put on GSTs, which are involved in Phase II detoxification, protecting cells from attack by reactive electrophiles or reducing the cell's ability to metabolize toxins. Although

polymorphisms have been described in several of GST gene families, most attention has focused on allelism in μ, θ and π GST families. In *GSTM1* or *GSTT1 null* individuals, a positive association was observed in LC for smokers (*GSTP1*B, GSTM1 null e GSTT1 null*, OR = 1.9 – 2.67), HNSCC (*GSTM1 null*, OR = 1.10 – 3.9) and especially in BC (*GSTM1 null*, OR = 1.49 – 7 and *GSTT1 null*, OR = 1.9 – 9.93). On the other hand, a protective role was observed in hepatocelular cancer risk (*GSTM1 null* and *GSTT1 null*), TCC (*GSTT1* and *GSTP1*) and TCCUB (*GSTT1 null*). However, some studies have reported conflicting and inconsistent results. In this context, further studies with larger sample sizes, prospective cohorts and more accurate analysis of interactions with other relevant factors, such as gene–environmental and gene–gene interactions, should be considered.

7. Expert opinion

While rare alterations of tumor suppressor originated by single determinants dramatically raise cancer risk, far more common and less dramatic differences in genes encoding for metabolism enzymes may be responsible for a relatively small, but rather frequent increase of cancer risk among individuals. In fact, recent studies have also suggested that although the risk associated with each variant may be small, the effects of the polymorphisms may be increased in combination with other genetic and/or environmental factors (such as cigarette smoking and nutrition). 'Low penetrating' polymorphisms in metabolism genes tend to be much more common in the population than allelic variants of 'high penetrating' cancer genes, and are of considerable importance for public health authorities (**Figure 2**).

The failure of some studies to demonstrate some positive associations between GST polymorphisms and cancer do not necessarily exclude the possibility of other variants (or combinations of alleles on multiple positions) in the same genes as relevant to the cancer. The inconsistencies in some results in different studies reflect the complexity in the role of GSTs. Furthermore, the underlying genetic predisposition of each patient will reflect combinations of poor- and extensive-metabolizer phenotypes for each enzyme involved in a particular metabolic pathway. The final result should be a wide interindividual difference in the risk of toxicity or cancer in the future.

The pathways of carcinogen metabolism are complex and mediated by the activities of multiple genes. For practical purposes, a screening of metabolizing genes seems to be possible in situations of carcinogenic exposures well defined, and when it is performed by the analysis of coordinated enzyme activities concurring to the metabolism of the considered carcinogen(s). For example, the individuals with deletion of the *GSTM1* gene and the *CYP1A1*2B* allele and naturally exposed to PAHs have an increased risk factor to develop cancer than those with only one of these polymorphisms [72], indicating a connection between these alleles and the metabolism of xenobiotics. It is also important to inform that the genotyping methods based on PCR technique used in most studies did not distinguish *GSTM1* and *GSTT1* homozygous wild-type +/+ from heterozygous +/- individuals. Thus, it still remains undetermined whether this is clinically significant.

In addition, nutritional factors may be directly related to generation of reactive species in the body, causing oxidative stress, resulting in DNA damage and thereby increasing the risk of cancer. The recommendations in the report's overview of the *World Cancer Research Fund* and *American Institute for Cancer Research, Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective* is clear in this regard. For these reasons, the study of enzymes related to maintaining the oxidative balance, such as GSTs, has become increasingly necessary in an attempt to stratify ethnic groups, determine susceptibility to the development of cancer and determine therapeutic regimens diets or antioxidant nutritional programs that are adequate to the genetic profile of each population, contributing to individualized therapy. Individual susceptibility to cancer should be monitored as a function of the nature, mechanism of action, carcinogen(s) to which the individual is known to be exposed and with the main target organ of the considered type of exposure. This approach may have future implications for preventive and earlier intervention strategies, and more practical results as well.

Besides the well-defined role of GST in catalyzing the inactivation of various electrophile-producing anticancer drugs, the overexpression of some GSTs (in particular GSTP1) seems to be envolved in acquired resistance of several tumors. The apparent complexity of this problem has challenged researchers to investigate and develop GST inhibitors and GST-activated cytotoxic prodrugs, such as canfosfamide. In fact, even though an old fashioned metabolic enzyme for some researchers, the GST family continues to be an amazing target for cancer understanding and drug discovery.

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Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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