Journal of
Carcinogenesis & Mutagenesis

Nissar et al., J Carcinog Mutagen 2017, 8:2
DOI: 10.4172/2157-2518.1000287

Review Article Open Access

Glutathione S Transferases: Biochemistry, Polymorphism and Role in Colorectal Carcinogenesis

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Received date: February 19, 2017; **Accepted date:** March 10, 2017; **Published date:** March 15, 2017

Abstract

Glutathione S-transferases (GSTs) are enzymes detoxifying a wide range of hazardous substances both of endogenous or exogenous origin, such as reactive oxygen species (ROS) or xenobiotics and environmental carcinogens; thereby imparting protection to DNA against oxidative damage. *GST gene* polymorphisms on the other hand, exert an effect on the functioning of enzymes encoded by these genes at both gene expression level and the activity of the protein. In this way it may influence the possibility of detoxification of carcinogens, and consequently, the level of DNA damage; thus it may have an effect on the risk of development of cancer. In this review we aim to understand the function of GSTs in the xenobiotic metabolism and their role in modulation of colorectal cancer (CRC).

Keywords: *GST genes*; Xenobiotic metabolism; Polymorphisms; CRC

Introduction

The Glutathione S-transferases (GSTs) are important Phase II biotransformation enzymes which play a key role in cellular detoxification, protecting macromolecules from attack by reactive electrophiles, environmental carcinogens, reactive oxygen species and chemotherapeutic agents [1]. GSTs are widely distributed in nature and are present in both prokaryotes and eukaryotes as the principal Phase II detoxifying enzymes [2]. They constitute a superfamily of ubiquitous, multifunctional enzymes (GSTs; EC 2.5.1.18) which catalyze the nucleophilic addition of the tripeptide glutathione (GSH; g-Glu-Cys-Gly) to several hazardous xenobiotics, including phase I electrophilic and carcinogenic metabolites [3-5] thereby, neutralizing their electrophilic sites and rendering the products more water-soluble and facilitating their elimination from the cell by Phase III enzymes [6]. In addition, GSTs can serve as peroxidases, isomerases and thiol transferases [7]. They also can play role in non-catalytic functions like modulation of signaling processes and non-substrate ligand binding [8].

Therefore, in this review, we aim to understand the role of GSTs in the metabolism of xenobiotics including carcinogens of both endo- as well as exogenous origin and the impact of GST gene polymorphisms in the human cancer susceptibility especially CRC.

GST Genes and Its Types

Human GSTs are divided into three main families: cytosolic, mitochondrial and membrane-bound microsomal. The cytosolic and mitochondrial GSTs are soluble enzymes with three-dimensional fold structural similarity. Almost all soluble GSTs are active as dimers of subunits of 23–30 kDa with subunits of 199–244 amino acids in length (identical, homodimers or different, heterodimers) subunits, and each dimer is encoded by independent genes [9]. Microsomal GSTs designated as 'membrane associated proteins in eicosanoid and glutathione metabolism' (MAPEGs) are structurally distinct from cytosolic GSTs but are functionally similar in the ability to catalyze the conjugation of GSH to electrophilic compounds [2]. Each class is coded by different genes located on different chromosome (Figure 1 and Table 1).

S.no	Class	enzyme	Chromosomal location
1.	Cytosolic	*ALphA (a1,A2,A3,A4 and a5)* *mu (m1,M2,M3,M4 and m5)* *pi (p1)* *sigma (s1)* *theta (t1 and t2)* *zeta (z1)* *omega (o1 and o2)*	6p12 1p13.3 11q13.3 4q21-22 22q11.23 14q24.3 10q24.3
2.	Mitochondrial	*Kappa k1*	Not Determined
3.	mapeg	*gp I, MGST2* *gp I, FLAP* *gp I, LTC4S* *gp II, MGST3* *gp IV, MGST1*	4q28-31 13q12 5q35 1q23 22p13.1-13.2

		gp IV, PGES1	9q34.3

Table 1: Genetic properties of human *GSTs*.

The cytosolic GSTs are the most complex family with seven sub divisions or classes designated as Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta. Classification is based on amino-acid sequence similarities, physical structure of the genes (i.e., intron number and position) and immunological cross-reactivity [2]. There is greater than 60% identity within a class and relies mainly on the more highly conserved N-terminal domain. 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 [10].

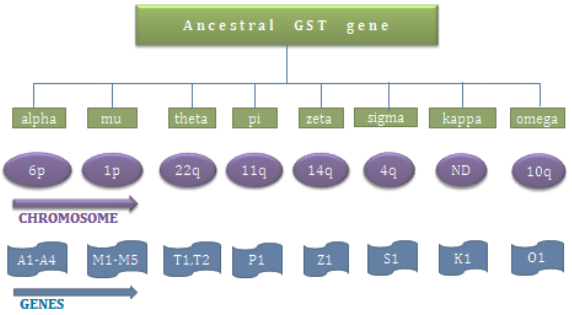

Figure 1: The *glutathione-S-transferase* supergene family.

Metabolism of Xenobiotics by GSTs

GSTs function widely in detoxifying electrophilic xenobiotics, such as chemical carcinogens, environmental pollutants, and antitumor agents but also play an important role in inactivation of secondary metabolites produced during oxidative stress like endogenous α,β-unsaturated aldehydes, quinones, epoxides and hydroperoxides.

Detoxification of exogeneous substrates

GSTs are involved in mercapturic acid synthesis and catalyze the first of four steps of synthesis process. There is subsequent sequential removal of the γ-glutamyl moiety and glycine from the glutathione conjugate, followed by N-acetylation of the resulting cysteine conjugate. GST enzymes form a part of an integrated defense strategy, and their usefulness depends on the combined activities, i.e. supply of GSH by glutamate cysteine ligase and glutathione synthase and alternatively acts on transporters to remove glutathione conjugates from the cell, which are eliminated then by the trans-membrane MRP (multidrug resistance-associated protein) from the cell [2]. There are nine MRP proteins belonging [11], to the C family of ABC transporters. MRP1 and MRP2 can disseminate glutathione conjugates and compounds complexed with GSH [12,13]. The RLIP76 (dinitrophenol-glutathione ATPase), a non-transmembrane protein upholds efflux of glutathione conjugate removal from cells [14].

GST isoenzymes have been shown to detoxify a large number of exogenous substrates including carcinogens, drugs and environmental pollutants. The cancer chemotherapeutic agents are also detoxified by GSTs like adriamycin, 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU), busulfan, carmustine, chlorambucil, cis-platin, crotonyloxymethyl-2-cyclohexenone (COMC-6), melphalan, mitozantrone, and thiotepa, cyclophosphamide, ethacrynic acid [15]. Environmental chemicals and their metabolites detoxified by GST include acrolein, atrazine, DDT, inorganic arsenic, lindane, malathion, methyl parathion, muconaldehyde, and tridiphane [16,17]. A large number of epoxides, such as the antibiotic fosfomycin and those derived from environmental carcinogens, polycyclic aromatic hydrocarbons (PAHs) etc are detoxified by GST. Activated metabolite, N-acetoxy-PhIP of heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP), produced by cooking protein-rich food is also detoxified by cytosolic GST isoenzymes .

Detoxification of endogenous products

As a result of oxidative stress, the reactive oxygen species, the superoxide anion O^{-2}, hydrogen peroxide H_2O_2, and the hydroxyl radical HO• inflict damage on DNA (direct/indirect) on membrane lipid, protein, and carbohydrate. Free radicals arising primarily through oxidative phosphorylation and other oxidase–catalyzed reactions are scavenged by the catalytic activities of superoxide dismutase, catalase and glutathione peroxidase and non-enzymatically by α-tocopherol, ascorbic acid, GSH, and bilirubin. Moreover the by-products of oxidative stress are tackled by number of enzymes like Aldehyde dehydrogenase, alcohol dehydrogenase, aldo-keto reductase, GST, and Selenium-dependent glutathione peroxidase (GPx).

GST isoenzymes exhibit modest role in lipid peroxidation in membranes whereby hydroperoxides that breakdown to yield secondary electrophiles, including epoxyaldehydes, 2-alkenals, 4-hydroxy-2-alkenals, and ketoaldehydes are produced. GSTs exhibit Se-independent glutathione peroxidase activity toward 1-palmitoyl-2-(13-hydroperoxy-cis-9,trans-11-octadecadienoyl)-L-3-phosphatidylcholine and phosphatidylcholine hydroperoxide, reducing lipid hydroperoxides within membranes [18-20]. The transferases can also reduce cholesteryl hydroperoxides [21] and fatty acid hydroperoxides, including (S)-9-hydroperoxy-10,12-octadecadieonic acid and (S)-13-hydroperoxy-9,11-octadecadieonic acid [20]. Among the end-products of lipid peroxidation, GSTs conjugate GSH with the 2-alkenals acrolein and crotonaldehyde, as well as 4-hydroxy-2-alkenals of between 6 and 15 carbon atoms in length [22]. Further, GSTs catalyze the conjugation of cholesterol-5,6-oxide,epoxyeicosatrienoic acid, and 9,10-epoxystearic acid with GSH indicating its role in cellular protection against a range of harmful electrophiles of oxidative stress [2].

Further it has been proposed that GST reactivates oxidized 1-cys peroxiredoxin (Prx) VI through glutathionylation followed by reduction of the mixed disulfide thereby combatting oxidative stress indirectly [23]. The Prx VI defends against cellular membrane damage by reducing phospholipid hydroperoxides to their respective alcohols. Also GSTs mediate the conjugation of harmful quinone-containing compounds with GSH preventing redox cycling reaction.

Bioactivation of xenobiotics

Conjugation of *GSTs* is supposed to form less reactive and readily excreted products. However, in some instances the glutathione conjugate is more reactive than the parent compound such as short-

chain alkyl halides that contain two functional groups and 1,2-dihaloethanes, where the glutathione conjugate however, rearranges to form an episulfonium intermediate that is responsible for modifying DNA [24]. Also conjugation of GSH with the solvent dichloromethane results in the formation of the highly unstable S-chloromethylglutathione, containing an electrophilic center capable of modifying DNA [24,25].

Allyl-, benzyl-, phenethyl-isothiocyanates, and sulforaphane, the moderately toxic compounds thatare reversibly conjugated by GST with GSH to yield thiocarbamates which spontaneously degrade to their isothiocyanates releasing GSH, followed by export from cells via MRP1 or MRP2. Thereafter, the isothiocyanate may be taken up again by the cell and re-conjugated with GSH, only to be re-exported as the thiocarbamate and revert to the isothiocyanate. This cyclical process results in depletion of intracellular GSH and assists distribution of isothiocynates throughout the body. Should isothiocyanates be taken up by cells that have a low GSH content, they may not be conjugated with GSH, but rather are more likely to thiocarbamylate proteins, a process that can result in cell death [26].

In the liver conjugation of haloalkenes with GSH, leads to the generation of reactive thioketenes, thionoacylhalides, thiiranes, and thiolactones through the actions of renal cysteine conjugate β-lyase in the kidneys [27]. Further the ability of GST to produce reactive metabolites has been exploited to target tumors that overexpress particular transferases in cancer chemotherapy [28]. For example the latent cytotoxic drug TER286 (now called TLK286) is activated by GST through a β-elimination reaction to yield an active analogue of cyclophosphamide [29]. More recently, the prodrug PABA/NO (O2-[2,4-dinitro-5-(N-methyl-N-4-carboxyphenylamino)phenyl] 1-N,N-dimethylamino)diazen-1-ium-1,2-diolate) has been designed to generate cytolytic nitric oxide upon metabolism by GST [30].

Role of GSTs in biochemical processes

A large number of biochemical processes require the direct involvement of GST enzymes. These enzymes play a pivotal role in the biosynthesis of leukotrienes, prostaglandins, testosterone, and progesterone, as well as the degradation of tyrosine. The detailed role of each of the gene and its enzyme is provided in the Table 2.

Gene	Enzyme	Major Substrates	Functions
GSTA1	GSTA1-1	1-chloro-2,4-dinitrobenzene (CDNB)	PGH2 →PGE2;
		,7-chloro-4nitrobenzo-2-oxa-1,3diazole;	PGH2 →PGF2α
		Δ5-androstene-3,17-dione	Isomerization of androstene
			PGH2 → PGD2
GSTA2	GSTA2-2	CDNB, 7-chloro-4 nitrobenzo-2-oxa-1,3 diazole, cumene hydroperoxide	PGH2 → PGF2α,
			reduction of cumene hydroperoxide
GSTS1	GSTS1	PGD2 synthase	PGH2 → PGD2
			Conjugation of benzo pyrene dioxide with GSH
GSTP1	GSTP1	CDNB, acrolein, adenine propenal, benzyl isothiocyanate	
			GSH dependent Thiol transferase, dehydro ascorbate reductase and monomethyl arsonate reductas
GSTO1	GSTO1	Thiol transferase	
			Conjugation of PGA2 & PGJ2 and other substrates.
GSTM1	GSTM1	CDNB, aflatoxin B1-epoxide	
GSTZ1	GSTZ1	maleylacetoacetate, fluoroacetate,	Maleyl acetoacetate isomerase(MAAI)
		dichloroacetate	
	MGST-I-like-I	PGE2 Synthase	PGH2 → PGE2
			(synthesis of PGE2)
	MGST- II	Leukotriene C4 synthase; 5-HPETE	Reduction of 5-hydroperoxy-8,11,14 cis- 6- trans
MAPEG	MGST-III		eicosatetraenoic acid (5HPETE)
	LTC4	Leukotriene C4 synthase	
	FLAP	5-lipoxygenase- activating protein.	Binding of Arachidonic acid

Table 2: Biochemical properties of human GSTs.

Role of GSTs in Regulation of Cellular Signaling *Via* Kinases

Decreased detoxification of possible carcinogens due to the absence or decreased expression of GSTP may result in the malignant transformation and disease progression. In addition, its increased expression is highly correlated with multidrug resistance due to weak affinity of GST-mediated conjugation of GSH for the majority of anticancer drugs. Therefore, the regulation of kinase-dependent proliferation pathways by GSTs is more significant than their catalytic properties alone [31].

GSTs undergo protein:protein interactions with critical kinases during regulation of cellular signaling involved in controlling stress response, apoptosis and proliferation. It negatively regulates signaling pathways through sequestration of signaling kinases.

GSTP has been characterized as a Jun kinase (JNK) inhibitor and GSTM1 binds to and inhibits the activity of ASK1 [32,33]. JNK has been implicated in pro-apoptotic signaling and ASK1 is an MAP kinase kinase. The mechanism of action involves activation of JNK, initiated by the phosphorylation of c-Jun which in turn results in subsequent activation of downstream effectors. During non-stressed condition, there is low JNK1 catalytic activity due to its sequestration within the protein complex including at least GSTP and JNK [34]. However, under conditions of oxidative or chemical stress, a dissociation of the GSTP:JNK complex occurs releasing *GSTP* for oligomerization and JNK, allowing it for the subsequent commencement of apoptosis (Figure 2) [32,35]. The high levels of

GSTP in many tumors may be a consequence of an acquired dependence on the protein. Many kinase pathways are dysregulated during proliferation, and subsequently tumor cells try to enhance GSTP expression in compensation to control kinase activity.

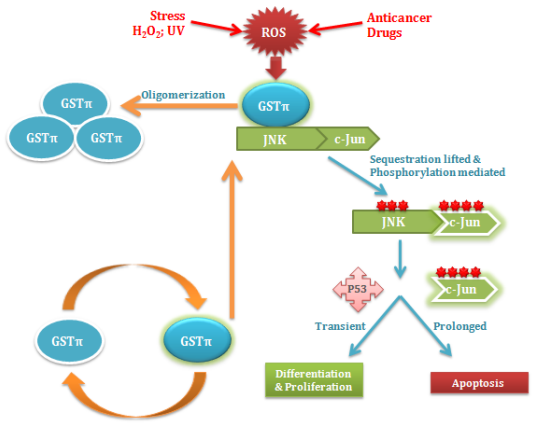

Figure 2: Role of GSTS in regulation of cellular signaling *via* kinases GSTP mediated activation of JNK.

Likewise, mechanism of *GSTM1:ASK* is similar to the one proposed for *GSTp:JNK*. *ASK1* activates the *JNK* and p38 pathways leading to cytokine and stress-induced apoptosis [36]. Under normal conditions, *ASK1* exhibits low activity as it is sequestered by *GSTM1* forming *GSTM1:ASK1* complex, which is dissociated under stressful conditions leading to the release and activation of *ASK1* [37,38]. In oxidative stress or heat shock, *GSTM1* oligomerizes and releases *ASK1* which subsequently induces apoptosis [37]. Thus an altered expression of *GSTM1* is found to be associated with impaired clinical response to therapy in a variety of tumor types.

Moreover GSTP has also been shown to play a necessary role in the glutathionylation of 1-cys peroxiredoxin (1-cysPrx). Oxidation of the catalytic cysteine of 1-cysPrx has been associated with its loss of peroxidase activity. The heterodimerization of 1-cysPrx with GSTP mediates the glutathionylation of the previously oxidized cysteine thus restoring its peroxidase activity [39]. Also the findings by Townsend et al. [40] suggest that GSTP may play a direct role in control of post-translational glutathionylation reactions.

Intonation of Signaling Pathways by GSTs

GSTs antagonize expression of genes trans-activated by the peroxisome proliferator-activated receptor γ (PPARγ) and nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) in consequence to GSH conjugation of the signaling molecules 15-deoxy-_12,14-prostaglandin J2 (15d-PGJ2) and 4-hydroxynonenal substrates and GST may enhance gene expression driven by nuclear factor-κB (NF-κB) through metabolism of *15d-PGJ2*. *15d-PGJ2*, a downstream metabolite of *PGD2*, synthesis and breakdown is regulated by GSTs mainly as compared to other transferases. *15d-PGJ2* serves as an activating ligand for the peroxisome proliferator-activated receptor γ (PPARγ) and is a critical regulator of adipocyte differentiation and also represents the molecular target of the thiazolidinedione class of insulin sensitizing drugs. GST over-expression reduces the transactivation of gene expression by *15d-PGJ2* mediated by PPARγ through conjugation of the prostanoid with GSH [41].

15d-PJ2 can stimulate Nrf2-mediated induction of gene expression through the antioxidant response element (ARE) via mechanism whereby GSH conjugation of 15d-PGJ2 eradicates its ability to modify cytoskeleton-associated protein Keap1 (Kelchlike ECH-associated protein 1) [42,43]. *15d-PGJ2* modifies cysteine residues in the Keap1 rendering itunable to target Nrf2 for proteasomal degradation [44,45]. Similarly 15d-PGJ2 tends to inactivate the β subunit of the inhibitor of κB kinase (IKKβ) and inhibit NF-κB-dependent gene expression [46]. Thus the scope to which GST-catalyzed synthesis and/or metabolism of *15d-PGJ2* intrudes on these signaling pathways demands more research in this area (Figure 3).

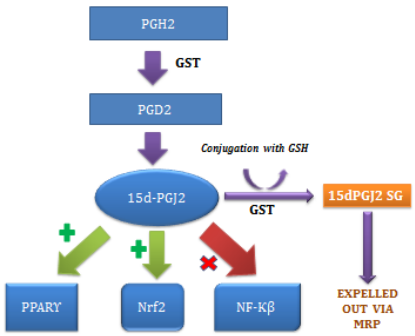

Figure 3: Attenuation of *15d-PJ2* signaling by *GST*.

Further the endogenous lipid peroxidation product 4-hydroxynonenal (4-HNE) is believed to act as an intracellular signaling molecule that stimulates several components in signal transduction pathways, such as JNK, p38, and protein kinase C, as well as increases p53 protein and promotes apoptosis [47-49]. Its conjugation with GSH will influence a number of signal transduction pathways and modulates the activity of transcription factors, including NF-κB, c-Jun, and Nrf2. Like 15d-PGJ2, it can stimulate gene expression through the ARE [50] and also prevents activation of NF-κB by inhibiting IκB phosphorylation. Collectively with 15d-PGJ2, it is plausible that Nrf2 mediates induction of ARE-driven genes by 4-HNE [2]. Reportedly, it modulates several cell-surface receptors; activate epithelial growth factor receptor and platelet-derived growth factor-β receptor, and upregulate transforming growth factor receptor β1.

Polymorphism of *GSTs*

A number of polymorphisms have been identified within each class of GSTs (Table 3). The M and T class of *GST* have a null phenotype (*GSTM*0* and *GSTT*0*) whereby individuals do not express catalytically active protein.

class	gene	allele	gene/nucleotide change	amino acid/ protein affect
alpha	*gsta2*	*gsta2*a*	*c335,a629*	Thr112,Glu210
		*gsta2*B*	*g335,c629*	Ser112,Ala210

	gstm1	*gstm1*a*	*g519*	Lys 173
		*gstm1*b*	*c519*	Asn173
		*gstm1*0*	*gene deletion*	No Protein
		*gstm1*1x2*	*Gene duplication*	Overexpression
mu				
	gstm3	*gstm3*a*	*Wild type*	Wild type
		*gstm3*b*	*3bp deletion in intron 6*	Primary structure intact
				Wild type
		*gstm4*a*	*Wild type*	Unchanged
	gstm4	*gstm4*b*	*intron change*	
		*gstp1*a*	*A313,C341,C555*	Ile105,Ala114,Ser185
	gstp1	*gstp1*b*	*G313,C341,T555*	Val105,Ala114,Ser185
pi		*gstp1*c*	*G313,T341,T555*	Val105,Val114,Ser185
		*gstp1*d*	*A313,T341*	Ile105,Val114
	gstt1	*gstt1*a*	*Unique Gene*	Unique Protein
theta		*gstt1*o*	*Gene Deletion*	No Protein
	gstt2	*gstt2*a*	*A415*	Met139
		*gstt2*b*	*G415*	Ile139
		*gstz1*a*	*A94;A124;C245*	Lys32;Arg42;Thr82
zeta	*gstz1*	*gstz1*b*	*A94;G124;C245*	Lys32;Gly42;Thr82
		*GSTz1*c*	*G94;G124;C245*	Glu32;Gly42;Thr82
		*gstz1*d*	*G94;G124;T245*	Glu32;Gly42;Met82
	MGST1	*MGST1*A*	*T598(noncoding3')*	wild type
		*mgst1*b*	*g598(noncoding3')*	unchanged
	ltc4s	*ltc4s*a*	*a-444(promoter)*	Wild type
MAPEG		*ltc4s*b*	*c-444(promoter)*	
	flap	*flap*a*	*No Hindiii site*	wild type
		*flap*b*	*t-c forming Hindiii site*	Unchanged

Table 3: Polymorphism of different *GST* genes.

The *GSTM1*0* allele is observed in approx. 40 to 60% of the Caucasian population [51] and is associated with an increased risk of lung, prostate, gastric and bladder cancer and is a risk factor for head and neck cancer [52,53]. The GSTT*0 phenotype varies between ethnic groups and is found to be highest in Chinese (65%) and lowest in Mexican American (9%) populations [54]. The GSTT*0 phenotype is associated with an increased risk of tumors of the head and neck, oral cavity, pharynx, and larynx [55,56].

The *GSTM1 gene* contains four alleles and has been the most widely studied. GSTM1 polymorphism M1*A 0.2 is associated with decreased risk of bladder and breast cancer in Caucasians. M1*B 0.2 with decreased risk of pituitary adenomas; M1*0 0.59 has been shown to increase the risk of lung, colon, bladder, and post-menopausal breast cancer. *GSTM1*A* has been associated with a decreased risk of bladder cancer and has an allele frequency of 20% [57].

It is estimated that 10–20% of the Caucasian population are carriers of the GSTP1 null genotype. *GSTP1* gene polymorphism is most often a point mutation SNP (single nucleotide polymorphism) within exon 5 Ile105 Val. Thus, the results of mutation are *GSTP1* genotypes Ile/Ile, Ile/Val and Val/Val. The exchange of isoleucine and valine in the amino acid chain results in decreased enzymatic activity of protein [58,59].

Also polymorphisms at the *GSTP1* locus result in four alleles, *GSTP1*A–D*, that differ structurally and functionally. The promoter region contains a TATA box, two SP1 sites, an insulin response element and an anti-oxidant response element within an AP1 site [60]. *GSTP1*A* plays a role in the acquisition of resistance to cisplatin (CDDP) by enhancing the capacity of the cell to form platinum–GSH conjugates/CDDP-GSH adducts [61]. *GSTP1*B* is an allele in which a single nucleotide (A→G) substitution at position 313 substantially diminishes catalytic activity [62]. Homozygosity for *GSTP1*B* is favorable in the treatment of cancer patients because they have a diminished capacity to detoxify platinum based anticancer agents [63]. *GSTP1*C* is an allelic variant that is more predominant in malignant glioma cells and differs from other *GSTP1* variants by two transitions resulting in Ile104Val and Ala113Val [60]. No major functional property has yet been assigned to this polymorphism.

Four (*GSTZ1*A–D*) polymorphisms have been identified. As *GSTZ1*A* (Lys32;Arg42;Thr82); *GSTZ1*B* (Lys32;Gly42;Thr82); *GSTZ1*C* (Glu32;Gly42;Thr82); *GSTZ1*D* (Glu32;Gly42;Met82). The isozyme *GSTZ1*A* has the highest catalytic activity in contrast to GSTZ1*D which has been shown to be associated with inborn errors in tyrosine metabolism, along with mutations in other enzymes. Rodent models deficient for *GSTZ1* provide insight into its role in metabolic deficiencies [31].

*GST*s and colorectal cancer

A vast literature is currently available regarding the single nucleotide polymorphisms (SNPs) of *GST* genes as risk modulating factors in different cancers including gastrointestinal cancer. These SNPs affect the functioning of *GST* enzymes at the gene level as well as protein level, thereby influencing the detoxification process of carcinogens, and consequently, the level of DNA damage; thus it may have an indirect effect on the risk of development of cancer [64]. Multiple studies have been carried out from time to time to assess the impact of GST gene polymorphisms in colorectal cancer development and progression (Table 4). The results of these studies are inconsistent: some suggesting no association, some a low risk whereas others show increased risk and are summarized as follows:

STUDY	SAMPLE POPULATION	RISK	CONCOMITANCE

Loktionov et al. [65]	206 cases; 355 controls	*GSTM1* and *GSTM3* polymorphism is associated with high CRC risk	
Hlavata et al. [68]	Czech population(495)	Moderate increase in risk by *GSTM1* deletion No risk by *GSTP1* gene polymorphism	Simultaneous deletion of the *GSTM1* and *GSTT1* genes causes a significantly higher risk.
Economopoulos et al. [70]	Caucasian population	*GSTM1* as well as *GSTT1* null carriers exhibit increased CRC risk.	
Aghajany-Nasab et al. [69]	Iran (140 cases; 90 controls)	*GSTM1* null predisposes to the development of CRC in individuals aged over 60	
Wang et al. [66]	Indian Hindus (300)	*GSTM1* null increased risk of rectal cancer *GSTT1* null increased risk of colon cancer	Predisposing to the development of CRC
Darazy et al. [67]	Lebanese population	*GSTM1* null genotype increased risk of CRC	
Hezova et al. [59]	Czech population(197 cases; 218 controls)	No significant risk of development of CRC by *GSTM1* and *GSTT1*.	
Khabaz MN et al. [71]	Jordan	*GSTP1* Ile105Val polymorphism does not exert any risk of CRC	
Zhao et al. [72]	China	*GSTP1*,*GSTT1* and *GSTM1* gene polymorphisms are not Colorectal Adenoma risk factors	No risk
Kassab et al. [51]	Tunisian population (150 cases; 128 controls)	No significant risk with *GSTM1* and *GSTT1* null genotypes but significant risk for CRC with *GSTP1*.	
Nissar et al. [73]	Kashmiri population (160 case; 200 controls)	No significant risk of CRC with *GSTM1* and *GSTT1* null genotypes	No risk

Table 4: *GST* polymorphisms and risk of colorectal cancer.

A study by Loktionov et al. [65] was conducted on 561 subjects (206 cases; 355 controls) and showed association of GSTM1 and GSTM3 polymorphisms with high risk of CRC development. Wang et al. [66] studied more than 300 patients belonging to the Indian Hindu population and proved that the *GSTM1* null genotype is considerably related to an increased risk of rectal cancer and the *GSTT1* null genotype to an elevated risk of colon cancer. Their study also recommended that the concomitance of polymorphism in three genes i.e, *GSTM1, GSTT1* and *GSTP1*, may have an influencing role in the development of CRC.

Darazy et al. [67], suggested a significantly increased risk of colorectal cancer in individuals with the *GSTM1* null genotype in the Lebanese population indicating similarity of results with the studies done on the Caucasian population. Hlavata et al. [68] also suggested an association of *GSTM1* null genotype with a moderately increased risk of colorectal cancer development in the Czech population, whereas the simultaneous deletion of the both *GSTM1* and *GSTT1* genes causes a significantly higher risk of the development of CRC, in relation to the presence of both genes. Another study by Aghajany-Nasab et al. [69] conducted in Iran on 230 subjects (140 cases; 90 controls) indicated the *GSTM1* null genotype predisposes to the development of CRC in individuals greater than 60 years of age. Economopoulos et al. [70] showed *GSTM1* as well as *GSTT1* null carriers' exhibit increased CRC risk in Caucasians.

However there are number of studies which show opposite results, like a study by Hezova et al. [59] on Czech population shows no significant risk of development of CRC by *GSTM1* and *GSTT1* null genotypes. Another study from Jordan by Khabaz MN [71] revealed no statistically significant differences between *GSTP1* genotypes and CRC risk.

Further it was observed that *GSTP1, GSTT1* and *GSTM1* gene polymorphisms are not colorectal adenoma risk factors in a Chinese meta- analysis study conducted by Zhao et al. [72] and Kassab et al. [51] also showed that there is no significant risk with *GSTM1* and *GSTT1* null genotypes but a significant risk for CRC with *GSTP1* in the Tunisian population.

In our own study in Kashmiri population, we found a non-significant ($p>0.05$) association of *GSTM1*null and *GSTT1*null polymorphism with the CRC. However the individuals with doublenull genotype *(GSTM1−/GSTT1−)* were found to have 3.5fold increased risk for development of CRC [73].

The dissatisfaction of such studies in establishing some positive associations between *GST* polymorphisms and colorectal cancer does not inevitably eliminate the possibility of other variants or combinations of alleles on multiple positions in the same genes as relevant to the cancer. Thus such inconsistent results reflect the complexity in the role of *GST*s and refer to the fact that the metabolic pathways involved in the carcinogen metabolism are complex and facilitated by the actions of multiple genes. Despite all these observations, there is still no consensus regarding the significance of *GST* gene polymorphism in the development of colorectal cancer risk.

GSTs, colorectal cancer and diet

The variations in the metabolism of genotoxic compound by xenobiotic metabolising enzyme (XME) genes result in the genetic predisposition to cancer. Although the risk is fairly modest, but the impact of environmental exposure and/or diet may be dangerous [74,75]. Both nutrients and xenobiotics tend to modulate the inducible

sequences in promoter regions, called responsive elements thereby affectingthe gene expression. Conversely, a genetic polymorphism in XME governs the effects of specific nutrients by alterations in their biotransformation [76,77] henceforth; the link between diet and genes seems to be bidirectional. These 'antioxidant responsive element' (ARE) found in the promoter region of numerous XME are prompted by both mono-functional inducers like transcription factor NF-E2-related factor-2 and Maf proteins, as well as bi-functional inducers like phytochemicals. These ARE's then activate the gene expression and hence are crucial in cancer protection functionality [78,79]. The individual genotypes of common polymorphisms modify the bioavailability, metabolism, affinity and activity of several dietary constituents with potential carcinogenic activity (e.g. heterocyclic amine (HCA), polycyclic aromatic hydrocarbon (PAH), aflatoxin).The process of activation by phase I enzymes and detoxification by phase II enzymes includes environmental, dietary xenobiotics as well as protective components of the diet [80], which can influence the modulation of biotransformation enzymes [81]. Evidently the diet and genetic polymorphism of detoxifying enzymes is associated with PAH–DNA adduct formation and cancer risk. Several studies on gene–nutrient interactions show an association between nutrient level and DNA adduct formation and *GST* genetic polymorphismin CRC.

Further an efficient review including 2,500 studies proposed a relationship between both *GSTT1* and *GSTM1* gene polymorphism and dietary factors in the risk of development of CRC; however, it may require validation by some other independent potential studies [82]. Hence there is no consensus vis-à-vis *GST* gene polymorphism and the development of CRC. The results in various populations do not overlap, and sometimes are even contradictory. This may possibly be due to the fact that such studies are specific to individual genes of the *GST* family in the risk of developing CRC and do not consider the effect of other environmental factors.

Conclusion

In conclusion, we can suggest that the field of xenobiotic metabolism and the genes associated has drawn a lot of audience to research upon the effect of various gene SNPs on the assembly and functioning of the glutathione dependent enzymes vis-a-vis the risk of CRC. However, due to huge genetic variance among various populations and the different set of environmental exposures, the results of various studies vary substantially. Nevertheless, all studies have shed a light upon the association of *GST* gene polymorphisms and the development of CRC. But, future epidemiological studies should focus on continuing to clarify the role of gene–nutrient interactions in the etiology of CRC.

Moreover, independent population-based studies assessing the influence of *GST* family gene variations and environmental factors, such as diet and lifestyle, on the risk of modulating the CRC carcinogenesis is the need of an hour. So keeping this in mind, epidemiological studies focusing on the interactions between nutrients and genes have great potential not only for understanding the relevant underlining mechanisms of carcinogenesis but also in identifying the susceptible populations/individuals as well. This will further allow the translation of these findings to clinical practice for better management of the disease. Therefore, further large scale studies on this gene family are suggested so as to help in the development of effective diagnostic and prognostic tools for the management and treatment of CRC.

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