Polymorphic Human Glutathione S-transferase Genes may Predict Susceptibility to Type 2 Diabetes Mellitus: A Minireview

Taiwo Idowu A.¹ and Shittu Mujeeb O.²*

¹Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
²Department of Medical Laboratory Services, Chemical Pathology Unit, Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Nigeria

*Correspondence Info:
Shittu Mujeeb O.
Biomedical Scientist,
Department of Medical Laboratory Services,
Chemical Pathology Unit,
Ladoke Akintola University of Technology Teaching Hospital,
Ogbomoso, Oyo State, Nigeria
E-mail: shifemjay1@gmail.com

Abstract
Among the primary human classes of glutathione S-transferase (GST) genes, GSTM1 and GSTT1 genes exhibit a deletion polymorphism that leads to a lack of active isoforms when in homozygous state (the null genotype). Persons with homozygous deletions of either GSTM1 or GSTT1 locus have no functional enzymatic activity of GST and this in turn exacerbate the damage caused by ROS and RNS to pancreatic β-cells. This causes reduced insulin production and, therefore type 2 diabetes. Therefore, GST polymorphic genes (GSTM1-null and GSTT1-null) could be used as a biological marker to determine the diabetic risk of individual.

Keywords: Glutathione S-transferase genes, Polymorphism, Type 2 diabetes

1. Introduction
Type 2 diabetes mellitus (T2DM) is a common multifactorial genetic syndrome, which is determined by several different genes and environmental factors. Even though little is known about the genetics of type 2 DM, its incidence is increasing rapidly due to secondary factors, such as hypertension, obesity, and lack of physical activity and it now affect an estimated 382 million people worldwide, with type 2 diabetes making up about 90% of the cases[1-3]. Hyperglycemia, an excess of blood sugar resulting from uncontrolled glucose regulation is widely recognized as a causal link between diabetes and diabetic complications, as it causes free radicals hyperproduction in endothelial cells at the mitochondrial level[4].

Substantial data indicate that oxidative stress is one of the several mechanisms that contribute in the pathogenesis of T2DM. Oxidative stress is the result of an imbalance between the amount of reactive oxygen species (ROS) and the capacity of antioxidant defense systems. The hyperglycemia induce overproduction of ROS such as superoxide, hydrogen peroxide, hydroxyl radical along with reactive nitrogen species (RNS) such as nitric oxide causes oxidation of DNA, proteins, and other cellular components leading to their damage[5-7].

Studies have shown that individuals with lowered antioxidant capacity are at risk of type 2 DM[8,9]. Pancreatic β-cells have little expression of antioxidant enzymes and they are also sensitive to cytotoxic stress therefore, they emerge as a putative target of oxidative stress-induced tissue damage. This seems to explain in part the progressive deterioration of β-cell function in T2DM[10].

Different families of antioxidants have been identified in reduction of ROS production. Glutathione S-transferases (GSTs) are the most important detoxifier of a variety of electrophilic compounds, including chemotherapeutic drugs, environmental toxins, carcinogens, and DNA products generated by ROS damage to intracellular molecules. Thus, GSTs play a major role as a cellular...
antimutagen and in antioxidant defense mechanism[11].

Many of the glutathione S-transferase genes (GST genes) undergo polymorphism; therefore, there has been substantial interest in studying the associations between particular allelic variants with altered risk of a variety of diseases. Recent studies have revealed significant interethnic differences in allelic frequencies of polymorphic GST genes and susceptibility to certain diseases[12].

2. Glutathione

Glutathione (γ-glutamylcysteinylglycine, GSH) is the predominant low-molecular-weight thiol in animal cells, it often attain millimolar levels inside cells (0.5-10mmol/L) which makes it one of the most highly concentrated intracellular antioxidants. Glutathione is ubiquitous in animals, plants, and micro-organisms. Being water soluble, it is found mainly in the cell cytosol with the remainder in many organelles (including the mitochondria, nuclear matrix, and peroxisomes)[13-16].

Glutathione exists both in reduced and oxidized forms. The antioxidant “reduced glutathione” tripeptide is conventionally called glutathione and abbreviated GSH; the oxidized form is a disulfide compound, known as glutathione disulfide or GSSG. The GSSG/GSH ratio may be a sensitive indicator of oxidative stress[17]. An increase GSSG-to-GSH ratio is indicative of oxidative stress.

It is noteworthy that a shift in the GSH/GSSG redox toward the oxidizing state activates several signaling pathways (including protein kinase B, apoptosis signal-regulated kinase 1, calcineurin, etc.); thereby reducing cell proliferation and increasing apoptosis[18]. Oxidative stress plays a role in the pathogenesis of many diseases, including cancer, Alzheimer’s disease, Parkinson’s disease, cystic fibrosis, HIV/AIDS, heart attack, stroke, and diabetes[19,20].

Glutathione effectively scavenges free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxy radical, peroxynitrite, and hydrogen peroxide) directly and indirectly through enzymatic reactions. In the process of such reactions, GSH is oxidized to form GSSG which is reduced to GSH by the NADPH-dependent glutathione reductase (Figure 1)[21]. GSH plays a diverse role in biological processes such as protein synthesis, leukotriene synthesis, transmembrane transport, intermediary metabolism, and cell maturation.

![Figure 1: Showing activities of glutathione, GST and GST Genes](image)

3. Glutathione s-transferases

The GSTs are a multigenic superfamily of detoxification enzymes that are essential for cell protection against oxidative damage [22]. They play an important role in the cellular mechanism of detoxification by conjugating reactive electrophilic compounds with soluble glutathione. GSTs also modulate the induction of other enzymes and proteins essential for cellular functions, such as DNA repair. This class of enzymes is therefore important for maintaining cellular genomic integrity and, as a result, may play an important role in cancer susceptibility.

The GSTs possess various activities and participate in several different types of reaction. Most of these enzymes perform their antioxidative activities by catalysing the conjugation of reduced glutathione (GSH) with compounds that contain an electrophilic centre through the formation of a thioether bond between the sulphur atom of GSH and the substrate[23,24]. These enzymes also perform several non-catalytic functions ranging from the sequestering of carcinogens, intracellular transport of a wide spectrum of hydrophobic ligands, and modulation of signal transduction pathway[25-27].

Two distinct superfamilies of GST isoenzymes exist; one family comprises cytosolic, soluble dimeric enzymes[28], and the other superfamily is composed of membrane bound trimeric proteins known as the membrane associated proteins in eicosanoid and glutathione (MAPEG) metabolism[29].

The GST cytosolic enzymes in humans are coded by at least eight distinct loci: Mu (GSTM), Kappa (GSTK), Alpha (GSTA), Pi (GSTP), Omega (GSTO), Theta (GSTT), Zeta (GSTZ), and Sigma (GSTS), each containing one or more homodimeric or heterodimeric isoforms [30] (Table 1).
Table I. Showing Classes of Glutathione S-transferase (GST) Genes

| GST Gene Classes | GST Class Members |
|------------------|-------------------|
| Alpha            | GSTA1,GSTA2,GSTA3,GSTA4,GSTA5 |
| Kappa            | GSTK1             |
| Mu               | GSTM1, GSTM1L, GSTM2, GSTM3, GSTM4, GSTM5 |
| Omega            | GSTO1, GSTO2      |
| Pi               | GSTP1             |
| Theta            | GSTT1,GSTT2,GSTT4 |
| Zeta             | GSTZ1             |

Source: UniProtKB/SwissProt database

4. Glutathione s-transferase genes polymorphism

Among candidate genes related to oxidative stress, three cytosolic GST loci in particular, GSTM1, GSTT1 and GSTP1 were intensively studied in different disease states due to their potential modulating roles in individual susceptibility to environmentally induced diseases[31]. The GSTM1 locus has been mapped on chromosome 1p13.3, while the GSTT1 and GSTP1 locus exist on chromosome 22q11.2 and 11q13 respectively [32].

Among the primary human classes of this system, GSTM1 and GSTT1 genes exhibit a deletion polymorphism that leads to a lack of active isoforms when in homozygosis, known as the null genotype. Persons with homozygous deletions of either the GSTM1 or GSTT1 locus have no functional enzymatic activity of the respective enzyme[22]. In GSTT1-null, which occurs at frequencies of 11-38% in different populations, 50kb of genomic sequence containing the entire gene is deleted. While the GSTM1-null, which occurs at frequencies of 20-70%, has a 15kb sequence deletion [33].

On the other hand, the GSTP1 single nucleotide polymorphism (SNP) present on exon5 is characterized by guanine replacing adenine base at position 313 (A313G) of the gene nucleotides. This eventually results in valine replacing isoleucine amino acid at position 105 in the GSTP1 isoenzyme protein. The result of such replacement is the appearance of a new allele with alteration in specific activity for substrate compared to wild-type allele[34].

5. The association between GST genes polymorphism and diabetes mellitus

The presence of homozygous null genotypes of GSTM1 and GSTT1 affect the functional enzymatic activities of GST and this in turn exacerbate the damage caused by ROS and RNS to pancreatic β-cells. This causes reduced insulin production and, therefore type 2 diabetes (Figure 3).

Figure 3: Showing Pancreatic β-Cell Destruction

A number of epidemiological studies have been carried out to determine the possible associations between polymorphisms of the GST isoforms particularly deletions in the GSTM1 and GSTT1 genes with disease risk or therapy outcomes in different types of pathologies [35,36].

Several population-based studies have reported a GSTM1-null prevalence ranging from 16% to 60% [37]. Asians and Caucasians have the highest frequencies (50-53%) while black populations including Africans have the lowest [38-40]. The GSTT1-null genotype prevalence is highest among Chinese (64%), followed by Koreans (60%), Africa-Americans (22%), Caucasians (29%) and Asian-Indians (16%), and the lowest among Mexican-Americans (10%) [41].

A recent study revealed that the proportion of the GSTT1- and GSTM1-null genotypes was significantly greater in diabetic patients. Patients carrying both null polymorphisms had 3.17-fold increased risk of having type-2 diabetes mellitus compared to those with normal genotypes of these two genes[42]. This finding is in tandem with a previous study[35].

In another study, GSTT1-null genotype resulted in a 3.2-fold increase risk for type-2 DM. Though it was also reported that there was no association of GSTM1 with susceptibility to T2DM[43]. It can be inferred that, the GSTT1 and GSTM1 genes, alone or combined, have an influence on the risk of having T2DM. All together, these results suggest that GSTT1-null type and GSTM1-null type cooperatively play a protective role against the development of T2DM.

6. Conclusions and Future directions

Since significant association was observed in GSTM1-null, GSTT1-null variants alone or combined, these polymorphisms can be screened in a
population to determine the diabetic risk of the individual. The GST polymorphic genes (GSTM1-null and GSTT1-null) could be used as a biological marker to determine the diabetic risk of individual. Though, functional studies are needed to clarify the exact molecular mechanisms by which GST gene variants may exert influence on pancreatic beta cells destruction.

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