

## Glutathione S-Transferase Gene Polymorphisms (GSTM1 and GSTT1) in Vitiligo Patients

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**Abstract:** Background: Vitiligo is an acquired pigmentary disorder of the skin characterized by white areas on the skin and pigment-producing cells (melanocytes) are absent from vitiligo lesions. Oxidative stress is a major pathogenesis hypothesis of vitiligo. The glutathione S-transferases (GSTs) are group of polymorphic enzymes that are important in protection against oxidative stress and chemical toxicity. *Objectives:* The aim of this work was to study the relation between glutathione S-transferase gene polymorphisms (GSTM1 and GSTT1) and pathogenesis of vitiligo. *Subjects and Methods:* This study included 40 patients with vitiligo and 10 healthy subjects served as controls, attending the Outpatient Clinic of Dermatology and Venereology Department of Tanta University Hospitals. Blood samples were collected from all patients for detection of GSTM1 and GSTT1 polymorphisms using the multiplex polymerase chain reaction (PCR) and blood samples were collected from control subjects for comparison. *Results:* In this study, there was non significant association with null type of both GSTM1& GSTT1 genotype and vitiligo susceptibility. There was significant association of vitiligo risk with GSTM1 null/ GSTT1 null type as well as GSTM1 present/GSTT1 null and GSTM1 null/ GSTT1 present when compared to the GSTT1 present/ GSTM1 present. There was non significant association with GSTM1 null type of vitiligo in focal, segmental and generalized subtypes. There was significant association with GSTT1 null type of vitiligo in generalized subtypes but GSTT1 null type of vitiligo in focal and segmental types showed non significant association with vitiligo susceptibility. Non significant association was shown in GSTM1 null/GSTT1 null type of vitiligo in focal and generalized types while it was significant in segmental type. Significant association with GSTM1 present/GSTT1 null type of vitiligo in focal and generalized types but non significant in segmental type. The GSTM1 null/GSTT1 present types of vitiligo subtypes showed a significant association with focal and generalized types but non significant association in segmental type. Significant association with GSTM1 present/ GSTT1 present type in segmental and generalized types but non significant in focal type. *Conclusion:* Collectively, the mechanistic study revealed new pieces in the vitiligo "puzzle", such as GST and 4-Hydroxy-2-nonenal (HNE)-protein which, together with the known one, namely hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), may well be included in the hypothetic redox-regulated mechanism of melanocyte loss, and might represent good candidates as therapeutic targets for this skin disease.

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**Key words:** Vitiligo, Glutathione S-transferase, Oxidative stress

### 1. Introduction

Vitiligo is an acquired skin disorder characterized by white depigmented patches due to disappearance of functioning melanocytes and loss of melanin in the epidermis. It is the most common pigmentary disorder affecting 0.1-2% of the world's population, irrespective of race and gender<sup>(1)</sup>. The pathogenesis of vitiligo is proposed to be associated with many factors as genetic, mechanical, biochemical, neurological and immunological factors. The genetic background underlying vitiligo susceptibility had been proposed in various studies<sup>(2)</sup>. Oxidative stress and accumulation of free radicals in the epidermal layer of affected skin have been suspected to be involved in the pathophysiology of vitiligo. There is an impairment of antioxidative system in vitiligo melanocytes with resultant free radical mediated damage in melanocyte<sup>(3)</sup>.

The GST genes represent major group of

detoxification enzymes, and are main defense against oxidative stress<sup>(4)</sup>. They comprise several isoenzymes, including alpha, mu, pi, theta, and zeta gene families and are known to be induced under conditions of oxidative stress<sup>(5)</sup>. The GST contribute in the protection against a broad range of compounds including carcinogens, pesticides, antitumor agents, and environmental pollutants<sup>(6)</sup>. The GST gene polymorphisms (GSTM1 and GSTT1) were thought that they play a role in the susceptibility of several diseases e.g. asthma and rheumatoid arthritis<sup>(7)</sup>. It was suggested to have a role in susceptibility to vitiligo<sup>(8)</sup>.

### Aim of the Work

The aim of the work was to study the relation between the glutathione S- transferase gene polymorphism (GSTM1 and GSTT1) and pathogenesis of vitiligo.

## 2. Subjects and Methods

This study included 40 patients with vitiligo and 10 healthy subjects served as controls, attending the Outpatient Clinic of Dermatology and Venereology Department of Tanta University Hospitals during the period from February 2010 to April 2011.

### (I) Control group:

They included 10 normal healthy subjects; 6 males and 4 females. Their ages ranged from 24-36 years.

### (II) Vitiligo patients:

They included 40 vitiligo patients; 23 males and 17 females. Their ages ranged from 7-67 years.

#### *Vitiligo patients group was subdivided into:*

Focal type, segmental type and generalized type

#### All the studied persons were subjected to:

1-Full history taking: including personal (age and gender), past, present (onset and duration), family history.

2-Informed consent signing.

3-Full clinical examination : to exclude any other skin or systemic diseases as vitiligo, alopecia areata, systemic lupus erythromatosus and other autoimmune diseases as thyroid disease , rheumatoid arthritis, bronchial asthma and alcoholic liver disease

4-Laboratory investigations:

A-Routine laboratory investigations

B-Specific laboratory investigations which included:

Identification of the distribution of GSTT1 and GSTM1 genotypes

#### Blood samples:

About 2 ml venous blood samples were collected in the morning into an EDTA vacutainer tubes used for genomic DNA extraction.

#### Extraction of genomic DNA from whole blood:

By utilizing silica-based membrane technology in the form of a convenient spin column provided by Gene JET genomic DNA Purification Kit cat. no. K0722.

Blood samples were digested with proteinase K. RNA was removed by treating the samples with RNAase. The lysate was then mixed with ethanol and loaded on the purification column where the DNA binds to the silica membrane. Genomic DNA was then eluted under low ionic strength conditions with the Elution Buffer.

#### Amplification of samples DNA:

DNA amplification by multiplex PCR was used

to detect the presence or absence of the GSTM1, GSTT1 and CYP1A1 genes in the genomic DNA samples, simultaneously in the same tube. The CYP1A1 genes were co-amplified and used as an internal control. The primers of GSTM1 gene were as follow: 5'-GAG GAA CTC CCT GAA AAG CTA AAG-3/ (forward) and 5'- CTC AAA TAT ACG GTG GAG GTC AAG-3/ (reverse). The GSTT1 gene was amplified with the following primers: 5'-TTC CCT ACT GGT CCT CAC ATC TC-3\ (forward) and 5'-TCA CCG GAT CAT GGC CAG CA-3/ (reverse). The primers of CYP1 gene are as follows: 5'-CTC CCT CTG GTT ACA GGA AGC TAT-3/ (forward) and 5'-CAA CCA GAC CAG GTA GAC AGA GTC-3/ (reverse).

The DNA of each sample was introduced in a reagent mixture containing an excess of deoxynucleoside 5'-triphosphates (dNTPs), biotinylated primers, and thermostable DNA polymerase (Taq) supplied by Dream Taq Green PCR Master Mix. Cat. no. K1089. (Fermentas)

The PCR was carried out using a GeneAmp PCR system 2700. The conditions were as follows; 35 cycles, each consisting of denaturation at 94°C for 30 s, annealing at 61°C for 30 s. and extension at 72°C for 30 s. The reaction cycles were preceded by 5 min denaturation at 94°C and were followed by 7 min extension at 72°C.

#### Detection of PCR products:

The PCR products were confirmed by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. DNA from samples positive for GSTM1 and GSTT1 genotypes yielded bands of 216bp and 459bp, respectively while the internal positive control (CYP1A1) PCR product corresponded to 349bp [Fig. 1].

#### Statistical analysis

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, and chi-square test by SPSS V.11. Statistical significance was determined at a level of  $p < 0.05^*$ .  $P < 0.001^*$  was considered highly significant.

## 3. Results

**I- Clinical results** were demonstrated in table-1

#### **II-Laboratory results:-**

In this study, there was non significant association with null type of GSTM1 ( $P=0.790$ ) and null type of GSTT1 genotypes ( $P=0.119$ ) and vitiligo susceptibility ( $P > 0.05$ ) [Table 2].

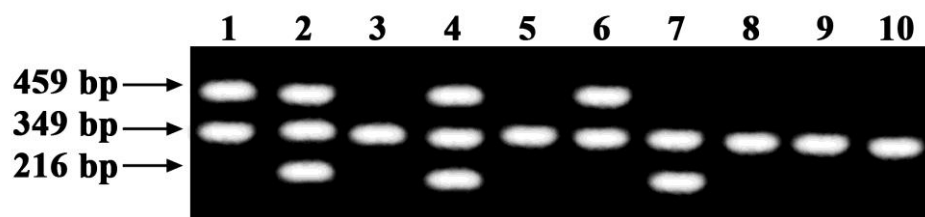
In this study, further analysis of the combined effect of GSTM1 and GSTT1, both present type of GSTM1 and GSTT1 genes were compared to both

null, GSTM1 null, and GSTT1 null types. The results suggested a significant association of vitiligo risk with GSTM1 null/ GSTT1 null type ( $P=0.048^*$ ) as well as GSTM1 present/ GSTT1 null ( $P=0.011^*$ ) and GSTM1 null/GSTT1 present ( $P=0.050^*$ ) when compared to the GSTM1 present/ GSTT1 present [Table 3].

Further analysis of GSTM1 and GSTT1 polymorphism in the vitiligo subtypes, three clinical types of vitiligo, such as focal, segmental, and generalized types were analyzed. As shown in [Table 4], there was non significant association with GSTM1 null type of vitiligo in focal, segmental and generalized subtypes (Focal  $P= 0.342$ ; Segmental  $P=0.793$  and Generalized  $P= 0.752$ ). There was significant association with GSTT1 null type of vitiligo in generalized subtypes (Generalized  $P=0.014^*$ ) but GSTT1 null type of vitiligo in focal and segmental types (Focal  $P=0.813$ ; Segmental  $P=0.583$ ) showed non significant association with vitiligo susceptibility [Table 4].

To analyze combined effect of GSTM1 and

GSTT1 in vitiligo subtypes, both present type of GSTM1 and GSTT1 genes were compared to both null, GSTM1 null, and GSTT1 null types with each subtypes. Non significant association was found in GSTM1 null/GSTT1 null type of vitiligo in focal and generalized types (Focal  $P=0.058$ ; Generalized  $P= 0.099$ ) while it was significant in segmental type (Segmental  $P= 0.028^*$ ). Significant association with GSTM1 present/GSTT1 null type of vitiligo in focal and generalized types (Focal  $P=0.028^*$  and generalized  $P=0.009^*$ ) but non significant in segmental type (Segmental  $P= 0.357$ ). The GSTM1 null/GSTT1 present types of vitiligo subtypes showed a significant association with focal and generalized types (Focal  $P=0.028^*$  and generalized  $P=0.041^*$ ) but non significant association in segmental type (Segmental  $P=0.179$ ). Significant association with GSTM1 present/ GSTT1 present type in segmental and generalized types (Segmental  $P=0.028^*$  and generalized  $P= 0.050^*$ ) but non significant in focal type (Focal  $P= 0.059$ ) [Table 4].



**Fig. (1):** Polymerase chain reaction products were analyzed on 2% ethidium bromide stained agarose gel.

Lanes 1, 6: GSTT1 positive(459, 349 bp).

Lanes 2, 4: GSTT1 and GSTM1 positive (459, 349 and 216 bp).

Lanes 3, 5, 8, 9, 10: GSTT1 and GSTM1 null alleles (349 bp)

Lanes 7: GSTM1 positive (349, 216 bp).

**Table (1): Clinical characteristics of 40 Egyptian vitiligo patients and 10 healthy controls**

	Vitiligo patient (NO=40)	Healthy controls (NO=10)	p. value	
<i>Average age</i> (year, mean $\pm$ SD)	27.90 $\pm$ 19.8	29.60 $\pm$ 3.89	0.790	>0.05
<b>Male/Female</b>	23/17	6/4	0.886	>0.05
<i>Duration</i> (year, mean $\pm$ SD)	5.12 $\pm$ 5.84	-	-	-
<i>Onset age</i> (year, mean $\pm$ SD)	22.77 $\pm$ 17.91	-	-	-
<i>Clinical types</i> (Focal/ segmental/ generalized)	8/2/30	-	-	-
<i>With/without family history</i> +ve/ -ve	1/39	-	-	-

**P.value <0.05 significant\***

**Table (2): Genotype and allele frequencies of the GSTs polymorphism among the patients and controls and the associations with risk of vitiligo**

Genotypes	Patients (NO=40)		Controls (NO=10) <sup>1</sup>		X <sup>2</sup>	p. value	
	NO	%	NO	%			
<b>GSTM1</b>	—	—	—	—	0.504	0.790	>0.05
Present (+)	19	47.5	6	60			
Null (-)	21	52.5	4	40			
<b>GSTT1</b>	—	—	—	—	2.42	0.119	>0.05
Present (+)	17	42.5	7	70			
Null (-)	23	57.5	3	30			

**Table (3): Frequencies of the combined genotypes of GSTM1 and GSTT1 among the patients and controls and the associations with risk of vitiligo**

Combined genotypes	Patients (NO=40)		Controls (NO=10)		X <sup>2</sup>	p. value	
	n	%	n	%			
<i>GSTM1/GSTT1</i>	—	—	—	—			
<i>Null/null(-/-)</i>	12	30	2	20	3.256	0.048	<0.05*
<i>Present /Null(+/-)</i>	11	27.5	1	10	4.523	0.011	<0.05*
<i>Null/present(-/+)</i>	9	22.5	2	20	2.996	0.050	<0.05*
<i>Present/present(+/+)</i>	8	20	5	50	1.582	0.052	>0.05

P. value &lt;0.05 significant\*

**Table (4): Comparison of GSTM1/GSTT1 frequencies between controls and clinical subtypes of vitiligo patients**

Genotypes	Control (n=10)	Focal (n=8)			Segmental (n=2)			Generalized (n=30)		
	n (%)	n(%)	X <sup>2</sup>	P	n (%)	X <sup>2</sup>	P	n (%)	X <sup>2</sup>	P
<b>GSTM1</b>										
Null (-)	4 (40)	5 (12.5)	0.900	0.342	1 (2.5)	0.075	0.793	15(37.5)	0.63	0.752
Present(+)	6 (60)	3 (7.5)			1 (2.5)			15(37.5)		
<b>GSTT1</b>										
Null (-)	3 (30)	2 (5)	0.063	0.813	1 (2.5)	0.305	0.583	20(50)	2.636	0.014
Present(+)	7 (70)	6 (15)			1 (2.5)			10(25)		
<b>GSTM1/GSTT1</b>										
-/-	2 (20)	2 (5)	1.325	0.058	1 (2.5)	1.865	0.028*	9(22.5)	2.72	0.099
+/-	1 (10)	-	2.63	0.028*	-	0.85	0.357	11(27.5)	3.25	0.009*
-/+	2(20)	3 (7.5)	1.963	0.028*	-	1.80	0.179	6(15)	2.93	0.041*
+/+	5 (50)	3 (7.5)	0.28	0.059	1 (2.5)	1.865	0.028*	4(10)	2.14	0.050*

### 3. Discussion

The results of the present study showed that, the genotype distribution of the GSTM1 and GSTT1 polymorphism between the patients and controls were detected as; there was a non-significant association with null type of GSTM1 and null type of GSTT1 genotypes and vitiligo susceptibility. These results agreed with *Guarneri et al.*<sup>(9)</sup> as their study showed a non-significant associations of GSTM1-null genotype and also GSTT1-null genotype with vitiligo patients. Also the current study agreed with *Uhm et al.*,<sup>(10)</sup> as they showed a non-significant associations between the disease and GSTT1-null genotype. But our results were different from *Uhm et al.*,<sup>(10)</sup> as they showed significant associations between the disease and GSTM1-null genotype. On other hand *Liu et al.*,<sup>(11)</sup> showed significant associations between the disease and GSTT1-null genotype, whereas the GSTM1-null genotype showed a trend toward association with vitiligo patients, in contrast to the current study, which showed neither associations.

In the present study, further analysis of the combined effect of GSTM1 and GSTT1, both present type of GSTM1 and GSTT1 genes were compared to both null, GSTM1 and GSTT1 types. The present results found a significant association of vitiligo risk with GSTM1 null/ GSTT1 null types as well as GSTM1 present/ GSTT1 null and GSTM1 null/GSTT1 present when compared to the GSTM1 present/ GSTT1 present and these results agreed with the study by *Liu et al.*,<sup>(11)</sup> as they showed the same significant association. Also the present results agreed with the study by *Uhm et al.*,<sup>(10)</sup> that showed a significant association of the disease with GSTM1 null/GSTT1 null type as well as GSTM1 null/GSTT1 present type but not go with them as they found a non-significant association of the disease with GSTM1 present/ GSTT1 null type. This study go with that done by *Guarneri et al.*,<sup>(9)</sup> as it showed a significant association of GSTM1 null/GSTT1 null genotype and different from the present study as it showed a non-significant association of the disease with GSTM1 present/ GSTT1 null genotype and GSTM1 null/GSTT1 present genotype when compared to the GSTM1 present/ GSTT1 present and *Guarneri et al.*,<sup>(9)</sup> who explained it on the basis that; increased of vitiligo in GSTM1/GSTT1 double null subject can be expected because of the significant reduction in the basal antioxidant potential of melanocytes and inability to upregulate GSTM1/GSTT1 expression in response to oxidative stress caused by simultaneous lack GSTM1 and GSTT1.

Further analysis of GSTM1 and GSTT1 polymorphism on the vitiligo subtypes, such as,

focal, segmental, and generalized were analyzed. In the present study there was non-significant association of GSTM1 null type of vitiligo patient in focal, segmental and generalized subtypes and these results were agreed with the studies by *Uhm et al.*,<sup>(10)</sup> and *Liu et al.*,<sup>(11)</sup> as they showed that GSTM1 null type of vitiligo in segmental subtype showed non-significant association with the disease but these studies showed a significant association of the GSTM1 null type of vitiligo patients in focal and generalized subtypes and this explained by *Uhm et al.*,<sup>(10)</sup> as different pathogenic mechanisms have been proposed according to the clinical subtypes of vitiligo, it is accepted that the pathogenic mechanism of segmental type vitiligo differs from focal and generalized types. Segmental type vitiligo is distinct in that distributed in dermatomal pattern and neurohumoral mechanism is considered to be involved.

In the present study, there was significant association with GSTT1 null type of vitiligo in generalized subtypes but GSTT1 null type of vitiligo in focal and segmental showed non-significant association with vitiligo susceptibility and these results agreed with *Liu et al.*,<sup>(11)</sup> as regard generalized and segmental subtypes and disagreed the present study; as it showed a significant associations of the GSTT1 null type with vitiligo susceptibility in focal vitiligo. *Uhm et al.*,<sup>(10)</sup> agreed with the current study about the association between GSTT1 null type and focal, segmental vitiligo subtypes and disagreed the current study as they showed a non-significant associations of the GSTT1-null type of vitiligo in generalized subtypes.

In the current study, further analysis of the combined effect of GSTM1 and GSTT1 in vitiligo subtypes, both present type of GSTM1 and GSTT1 genes were compared to both null, GSTM1, and GSTT1 types in each vitiligo subtypes. The present study showed a non-significant association of the GSTM1 null/GSTT1 null types of vitiligo in focal and generalized, but both were significant association with segmental type and these results disagreed with *Uhm et al.*,<sup>(10)</sup> and *Liu et al.*,<sup>(11)</sup> as they showed a significant association in GSTM1 null/GSTT1 null types of vitiligo in focal and generalized subtypes, but non-significant in segmental subtype.

In the present study, there was a significant association of the GSTM1 present/GSTT1 null type of vitiligo in focal and generalized types but non-significant in segmental type and these results agreed with study by *Liu et al.*,<sup>(11)</sup> as it showed that the GSTM1-present/GSTT1-null type were significantly associated with focal and generalized vitiligo but

non-significant association in segmental type. The present study results agreed with *Uhm et al.*,<sup>(10)</sup> as it showed a non-significant association with GSTM1 present/ GSTT1 null type in segmental subtypes of vitiligo and disagreed with *Uhm et al.*,<sup>(10)</sup> as it showed a non-significant association with GSTM1 present/ GSTT1 null type in focal and generalized subtypes of vitiligo.

In the current study, the GSTM1 null/GSTT1 present types of vitiligo subtypes show a significant associations with focal and generalized types but non-significantly associated in segmental type of vitiligo and these results agreed with a study by *Liu et al.*,<sup>(11)</sup>. Also the present study agreed with *Uhm et al.*,<sup>(10)</sup> who showed that GSTM1 null/GSTT1 present types of vitiligo subtypes showed a significant association with focal and generalized types and the present study different from *Uhm et al.*,<sup>(10)</sup> as they showed a significant association of the GSTM1 null/ GSTT1 present type with segmental subtype of vitiligo.

Discrepancies between the studies done in these fields suggest that the role and the relative importance of each GST isoform in the pathogenesis of vitiligo are variable and probably correlated with several factors. Indeed, GSTs are only a part of the system of enzymatic and non-enzymatic component that maintain the redox homeostasis of the organism. The functional equilibrium among these components is the results of evolutionary selection and adaptation to environmental conditions; consequently, multiple configurations of the system exist in different populations, reflecting the wide variability of genetic, geographical, environmental, and lifestyle factors. This view is also supported by literature data on the frequency of GSTM1 null and GSTT1 null in healthy subjects, 36-54.67% and 8-52.6, respectively.<sup>(9)</sup>

As for GST activity, its inhibition could also be due to polymorphic changes in two GST isoforms (GSTM1 and GSTT1), that are characteristic for vitiligo patients,<sup>(10)</sup> and cause partial loss of the total enzyme activity and impaired HNE detoxification.<sup>(12)</sup> The GSTs, and in particular the isoform GSTM1, exert several nonenzymatic functions relevant to programmed cell death to the control of intracellular nitric oxide (NO) levels, acting as a NO carrier, to the direct regulation of kinase pathways, to inducible NO synthase upregulation through nuclear transcription factor kappa  $\beta$  translocation and other cellular functions.<sup>(13)</sup>

As oxidative stress is due to increased epidermal levels of H<sub>2</sub>O<sub>2</sub> is a leading cytotoxic mechanism of melanocyte loss in vitiligo. Therefore, abnormal levels of H<sub>2</sub>O<sub>2</sub> due to HNE-induced formation and to decreased neutralization by

defective catalase(CAT)<sup>(3)</sup> may shift its regulatory action towards the expression of various cytokines and growth factors controlled through redox signaling, among which are vascular endothelial growth factor, platelet-derived growth factor, monocyte chemotactic protein-1<sup>(14)</sup>, IL-6, IL-8, and TNF- $\alpha$ <sup>(15)</sup>.

The HNE- protein is a stable product of lipid peroxidation, easily reacting with proteins and DNA, thus possessing important regulatory properties towards cell growth and differentiation,<sup>(16)</sup> as well as towards cellular death and survival<sup>(17)</sup>. HNE homeostasis in the organism is controlled by GSTs gene which is a family of conjugating enzymes playing a key role in the phase II biotransformation of organic xenobiotics, in the metabolism of endogenous electrophils, including HNE, and in the deactivation of reactive oxygen species, that are involved in cellular processes of inflammation and degenerative diseases.<sup>(13)</sup> Of note, HNE can be a substrate for GST, either inhibiting enzyme activity, or regulating its expression, depending on concentration<sup>(12)</sup>. Impaired HNE metabolism consequently increases the probability of HNE protein adduct formation, followed by the inactivation of HNE-sensitive enzymes such as CAT and GST<sup>(18)</sup>. Of interest, it has been shown that HNE-CAT complexes possess neoauto-antigen properties, inducing the consequent autoimmune reaction frequently observed in the patients affected by vitiligo.<sup>(19)</sup>

Notably, HNE has been recognized as an endogenous ligand for epidermal growth factor receptor,<sup>(11)</sup> which may result in NADPH oxidase induction and increased H<sub>2</sub>O<sub>2</sub> formation.<sup>(15)</sup> The defects of GST expression and activity towards HNE in vitiligo keratinocytes result in dysregulated levels of this electrophile. Normally, HNE at low concentrations regulates stress gene response and apoptosis via the activator protein-1 dependent pathways inhibiting the pro-survival extracellular regulation kinase (ERK1/2) and protein kinase B-regulated pathways<sup>(20)</sup>

Accordingly, *Townsend et al.*,<sup>(21)</sup> found an early time-dependent inhibition of ERK1/2 and protein kinase B- phosphorylation in normal human keratinocytes exposed to HNE. They also observed inhibited UV-induced protein kinase B, and enhanced spontaneous +UV-induced p53 phosphorylation in vitiligo keratinocytes, probably caused by endogenous HNE. Impaired GSTM1 expression/induction may also negatively affect cellular functions, by acting in a nonenzymatic mode, for example, inducing apoptosis signal regulating kinase-1 controlled apoptosis normally inhibited by GSTM1,<sup>(17)</sup> or dysregulating the protein

kinase cascade<sup>(21)</sup>.

In cultures of keratinocytes from patients with vitiligo, *Kostyuk et al.*,<sup>(22)</sup> observed: (i) reduced expression of GSTM1 mRNA and protein, (ii) increase level of HNE- protein, and (iii) dysregulated production of major cytokines, chemokines and growth factors (and alteration of the corresponding plasma level). Addition of exogenous HNE to normal keratinocytes also induces a vitiligo like cytokine pattern and H<sub>2</sub>O<sub>2</sub> overproduction but, in this case, adaptive upregulation of CAT and GSTM1 genes occurs, compensating the excess of oxidative stress. The experiment did not consider GSTT1 expression, but a GSTM1-like behavior can be reasonably hypothesized, based on the close functional similarity of the two enzymes.<sup>(22)</sup> Depending on how much of the individual redox homeostasis relies on the activity of a given GST isoform, possession of the corresponding null allele may or may not significantly increase such a risk because of the severe decrease in the deficiency of the antioxidant system caused by the simultaneous lack of GSTM1 and GSTT1 can not be easily compensated by other components<sup>(9)</sup>.

Cruciferous vegetables, such as broccoli, and members of the allium family, such as garlic and onion, have been shown to be potent inducers of these enzymes, which would be expected to increase clearance of potential toxins from the body.<sup>(23)</sup>

Collectively, the mechanistic study revealed new pieces in the vitiligo "puzzle",<sup>(24)</sup> such as GST and HNE which, together with the known ones, namely H<sub>2</sub>O<sub>2</sub> and CAT, may well be included in the hypothetic redox-regulated mechanism of melanocyte loss, and might represent good candidates as therapeutic targets for this skin disease<sup>(22)</sup>.

### Conclusion

The GSTM1 and GSTT1 polymorphisms play an important role in the pathogenesis of vitiligo. Null genotype of both genes either significant or not significant; increase a risk of the disease depending on how much of the individual redox homeostasis relies on the activity of a given GST isoform, because of the severe deficiency of the antioxidant system caused by the simultaneous lack of GSTM1 and GSTT1 can not be easily compensated.

### Recommendation

The current study advise the patients and susceptible people to avoid the oxidant agents and to increase the antioxidant agents; as the oxidative stresses may affect GST gene polymorphism (GSTM1 and GSTT1) which have a role in pathogenesis of vitiligo. Further research (using a

larger number of patients) is advisable to achieve a better understanding of the pathophysiological role of GST polymorphism. Depending on the genes involved, their normal functions, and the genetic changes as it might be possible to design new treatments based on understanding those gene

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