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Absence of *GSTT1* and polymorphisms in *GSTP1* and *TP53* are associated with the incidence of acne vulgaris

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Abstract

Backgrounds: Acne vulgaris is a chronic inflammatory skin disease of the pilosebaceous unit affecting most teenagers and numerous adults throughout the world. The present study was designed to assess the association of the presence or absence of *GSTM1*, *GSTT1*, and single nucleotide polymorphisms rs1695 in *GSTP1* and rs1042522 in *TP53* gene with acne vulgaris.

Methods: The cross-sectional case–control study was conducted at the Institute of Zoology from May 2020 to March 2021 and included acne vulgaris patients ($N = 100$) and controls ($N = 100$) enrolled in Dera Ghazi Khan district, Pakistan. Multiplex and tetra-primer amplification refractory mutation system-polymerase chain reactions were applied to investigate the genotype in analyzed genes. The association of rs1695 and rs1042522 with acne vulgaris was studied either individually or in various combinations with *GATM1* and *T1*.

Results: A significant association of absence of *GSTT1* and mutant genotype at rs1695 (GG) and at rs1042522 (CC) in *GSTP1* and *TP53*, respectively, was found to be associated with acne vulgaris in enrolled subjects. Subjects aged 10–25 years and smokers were more susceptible to acne vulgaris.

Conclusion: Our results suggest that genotypes of glutathione S-transferases (GSTs) and *TP53* are involved in protection against oxidative stress and may influence disease progression in acne vulgaris.

KEYWORDS
acne vulgaris, *GSTM1*, *GSTT1*, rs1695 in *GSTP1*, rs1042522 in*TP53*

1 | INTRODUCTION

Acne vulgaris is a chronic inflammatory skin disease of the pilosebaceous unit affecting most teenagers and numerous adults across the globe.[1] Globally, acne is ranked eighth in overall disease prevalence, and the incidence of acne vulgaris in teenagers and adults varies among countries and ethnic groups.[2]

It is already known that *Propionobacterium acnes* produces chemotactic factors for neutrophils, leading to the release of hydrolytic enzymes that breakdown the follicle when the neutrophils are

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attracted to these sites for phagocytosis of *P. acnes*.[3] Penetrating the dermis, *P. acnes* stimulates the immune system, causing a foreign body reaction by sebaceous fats, hair, and epithelial cells, which, in turn, leads to inflammation.[4] The clinical features of this disease include seborrhea (excess grease), non-inflammatory lesions (open and closed comedones), inflammatory lesions (papules and pustules), and varying degrees of scarring.[5]

It has also been reported that in addition to environmental factors, genetics also contribute to the development of disease symptoms.[6] Oxidative stress components, such as reactive oxygen species (ROS) and lipid peroxide, are thought to be involved in the pathogenesis and progression of acne vulgaris.[7] Glutathione S-transferases (GSTs) refer to a group of detoxification enzymes that are essential components of cellular defense against oxidative stress.[8] Cytosolic GSTs represent the largest class and consist of seven distinct subclasses. Among them, the glutathione S-transferase mu (*GSTM1*) locus has been mapped to chromosome 1p13.3, and this gene produces an enzyme responsible for detoxifying metabolites of environmental carcinogens, including tobacco smoke.[9] The glutathione S-transferase theta (*GSTT1*) gene (positioned at 1p13.3) plays a major role in phase-II biotransformation of a number of drugs and industrial chemicals.[10] The glutathione S-transferase pi (*GSTP1*) gene (positioned at 11q13) is known to reduce organic peroxides by oxidizing glutathione to glutathione disulfide (GSSG).[11]

The p53 protein is a transcription factor involved in maintaining genomic integrity by monitoring cell cycle progression and cell survival.[12] p53 is known to stimulate the expression of multiple pro-apoptotic signaling molecules, including FOXO1, FOXO3, and TRAIL.[13] The *GST* and TP53 genotype has been linked to a number of diseases in numerous investigations.[14–16] However, there is limited data available from Pakistan that reports the association between various single nucleotide polymorphisms (SNPs) and the incidence of acne vulgaris. Hence, the present investigation was aimed to report the effect of presence or absence of *GSTM1* and/or *GSTM1* and genotypes at SNPs in *GSTP1* (rs1695) and *TP53* (rs1042522) genes on the incidence of acne vulgaris. We are also reporting the risk factors that are associated with this disease among the enrolled subjects.

2 | MATERIALS AND METHODS

2.1 | Subjects and data collection

The cross-sectional case–control study was conducted at the Institute of Zoology from May 2020 to March 2021 and included clinically diagnosed acne vulgaris patients and a healthy control group.

Following their informed consent, blood samples from the patients ($N = 100$) were collected in the dermatology ward at District Headquarter Hospital (DHQ) Dera Ghazi Khan (Pakistan). The enrolled subjects haled from different cities of Southern Punjab population and included both men and women of varying ages and different cultural backgrounds. Enrollment of participants in this study was conducted based on inclusion and exclusion criteria, which were determined following a thorough interview by a dermatologist. The study included individuals between the ages of 10 and 30 years who were diagnosed with acne vulgaris and had a regular health status otherwise. Participants who had used anti-acne agents within the last 6 months, had chronic illnesses such as cancer, diabetes mellitus, or endocrine disorders, or were pregnant were excluded from the study. The comprehensive acne severity scale, as described by Masoud et al.,[17] was employed to determine the severity of acne vulgaris in patients. All enrolled subjects had moderate or pustular acne, with multiple papules and pustules on their faces. Controls ($N = 100$) were age-matched and did not have acne vulgaris. We used Solvin's formula to estimate sample size when collecting random samples in this study. Solvin's formula was computed as:

$$n = \frac{N}{1 + N \times e^2}$$

where n is the number of samples, N is the total population, and e is the margin of error.

Following informed consent, all subjects were interviewed using a questionnaire to collect epidemiological data, including age, gender, family history, and smoking habits.[16]

2.2 | Blood collection and DNA extraction

The blood sample (3–5 mL) from each participant was collected and stored at −4°C in vials coated with ethylene diamine tetra acetic acid until further examination.

Deoxyribonucleic acid (DNA) was extracted from whole blood by using inorganic DNA extraction protocol.[16]

2.3 | Amplification and genotyping of *GSTT1* and *GSTM1*

A multiplex polymerase chain reaction (PCR) was carried out to search the presence and absence of *GSTT1* and *GSTM1* in the subjects following Jamil et al.[16] The cytochrome P450, family 1, subfamily A, polypeptide 1 (*CYP1A1*) gene (exon 7) was also amplified as an internal control. The oligonucleotide primers used for the amplification of these genes are presented in Table S1. A 50 μL master mixture was prepared for PCR containing 5 μL template DNA, 5 μL PCR buffer, 3.5 μL $MgCl_2$ (25 mM), 2 μL of each primer (12 Pm), 2 μL of deoxynucleoside triphosphates (dNTPs) (2 mM), and 1 μL of DNA polymerase (Thermo Scientific, USA). The thermal profile for GSTM1, GSTT1, and CYP1A1 amplification was an initial denaturation for 5 min at 94°C followed by 30 cycles of a denaturation for 2 min at 94°C, an annealing step for 1 min at 59°C and an elongation for 1 min at 72°C, and a final extension for 10 min at 72°C.[16]

2.4 | T-ARMS-PCR-based amplification of rs1695 SNP in *GSTP1*

Tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) was conducted to genotype Ileu 105 Val (rs1695 A/G) in exon 5 of *GSTP1* as reported by Hashemi et al.[18]

The primers used in this T-ARMS-PCR are shown in Table S1. A 25 μL reaction mixture was prepared comprising 3 μL of template DNA, 5 μL of PCR buffer, 2 μL of $MgCl_2$ (25 mM), 2 μL of each primer (12 Pm), 2 μL of dNTPs (2 mM), and 1 μL of Taq DNA polymerase. Additionally, rs1695 in GSTP1 was amplified by using thermal conditions, which were an initial denaturation for 5 min at 95°C followed by 35 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 62°C, elongation for 30 s at 72°C, and a final extension for 10 min at 72°C.[18]

2.5 | T-ARMS-PCR-based amplification of rs1042522 SNP in *TP53*

T-ARMS-PCR was conducted to analyze rs1042522 (G/C) SNP in *TP53* gene according to Asadi et al.[19] The primers used in this T-ARMS-PCR are presented in Table S1. A reaction mixture of 25 μL was prepared comprising 3 μL of template DNA, 5 μL of PCR buffer, 1.5 μL of $MgCl_2$ (25 mM), 2 μL of each primer (12 Pm), 2 μL of dNTPs (2 mM), and 1 μL of Taq DNA polymerase.[19] For the amplification of rs1042522, the thermal profile was as follows: initial denaturation for 10 min at 95°C followed by 25 cycles of denaturation at 95°C for 45 s, annealing at 56°C for 30 s, elongation at 72°C for 45 s, and a final extension for 10 min at 72°C.[19]

2.6 | Statistical analysis

Data were analyzed using Minitab version 18 (Mini Tab, USA). Genotypic frequencies were determined by direct counting, while an online calculator of Hardy Weinberg equilibrium was used for allelic frequency counting. The chi-square test was applied to compare the genotype and allelic frequencies between cases and controls and also to report the correlation between acne vulgaris and the studied risk factors.

3 | RESULTS

3.1 | Presence or absence of *GSTM1* and *GSTT1* and their association with acne vulgaris

Multiplex PCR amplified 480, 215, and 312 base pair (bp) products for *GSTT1*, *GSTM1*, and *CYP1A1*, respectively. The chi-square test results indicated that the presence or absence of *GSTM1* had no association with the incidence of acne vulgaris ($p = 0.9$) (Table 1). A significant correlation ($p < 0.001$) was observed when the presence or absence of the *GSTT1* gene was analyzed among the enrolled cases and controls. It was observed that the majority of cases were *GSTT1* null (Table 1).

TABLE 1 Distribution of presence or absence of *GSTT1* and *M1* genes among acne cases and controls enrolled during present study.

Genotype	Control	Case	Chi-square	*p*-Value
GSTT1				
Present	8	27	12.5	<0.0001***
Null	92	73		
GSTM1				
Present	43	44	0.02	0.9
Null	57	56		

Note: *p*-Value represents the outcome of chi-square test calculated for each combination. $p > 0.05$: not significant.
***$p < 0.001$: highly significant.

3.2 | Genotypic and allelic frequency at rs1695 in *GSTP1* and their association with acne vulgaris

When the T-ARMS-PCR was applied to genotype rs1695 in *GSTP1* gene, outer primers generated an amplicon of 467 bp that was visible for all enrolled subjects. The primers specific for homozygous wild (AA) genotype and homozygous mutant (GG) genotype amplified products of 233 and 290 bp, respectively. Subjects with a heterozygous genotype (AG) at rs1695 amplified both 233 and 290 bp products.

When the genotype at rs1695 in *GSTP1* gene was compared among controls, it was observed that (AA) wild type was most common genotype (91%), followed by heterozygous (AG, 8%) and homozygous mutant (GG, 1%). A similar trend was observed in acne vulgaris patients enrolled during this study (AA [55%] > AG [32%] > GG [13%]) (Table 2). Chi-square test analysis revealed that the genotypic frequency varied significantly ($p < 0.001$) when compared between healthy controls and acne vulgaris patients, with the AA genotype more common in controls, while the AG and GG genotypes were more common in cases (Table 2).

When the allelic frequency at rs1695 in *GSTP1* gene was compared among controls, it was found that "A" (wild type) allele (95%) was the most common, followed by the "G" (mutant) allele (5%) (Table 2). A similar trend was observed in acne vulgaris patients tested in the present study (A [71%] > G [29%]) (Table 2).

The chi-square test revealed that the allelic frequency varied significantly ($p < 0.001$) when compared between healthy controls and acne vulgaris patients, with controls having more wild-type allele and cases having more mutant alleles, indicating that the mutant allele is associated with the incidence of acne vulgaris in analyzed subjects (Table 2).

TABLE 2 Genotype and allelic frequency distribution at single nucleotide polymorphism rs1695 in *GSTP1* and rs1042522 in *TP53* genes among acne cases and controls enrolled during present study, and their possible association with acne vulgaris.

Gene and SNP	Parameters	Genotypic frequency			*p*-Value	Allelic frequency		*p*-Value
TP53 rs1042522	Nucleotide	GG	GC	CC	<0.0001***	G	C	<0.0001***
	Controls	45 (45%)	50 (50%)	5 (5%)		140 (70%)	60 (30%)	
	Case	17 (17%)	51 (51%)	32 (32%)		85 (43%)	115 (57%)	
GSTP1 rs1695	Nucleotide	AA	AG	GG	<0.0001***	A	G	<0.0001***
	Controls	91 (91%)	8 (8%)	1 (1%)		190 (95%)	10 (5%)	
	Case	55 (55%)	32 (32%)	13 (13%)		142 (71%)	58 (29%)	

Note: Chi-square test was applied to compare the genotype and allelic frequency between case and controls.
Abbreviation: SNP, single nucleotide polymorphism.
***$p < 0.001$: highly significant.

3.3 | Genotypic and allelic frequency at rs1042522 in *TP53* and their association with acne vulgaris

When T-ARMS-PCR was applied to amplify rs1042522 in *TP53* gene, outer primers generated a 493 bp amplicon that was visible for all subjects. The primers specific for homozygous "GG" wild genotype and homozygous "CC" mutant genotype amplified products of 200 and 247 bp, respectively. Subjects with heterozygous genotype "AG" at rs1042522 amplified both 200 and 247 bp products.

When the genotype at rs1042522 in *TP53* gene was compared among controls, it was observed that GC heterozygous was the most common genotype (55%), followed by GG wild homozygous (45%) and CC homozygous mutant (5%). A similar trend was observed in acne vulgaris cases tested during this study (GC [51%] > CC [32%] > GG [17%]) (Table 2). The chi-square test analysis revealed that the genotypic frequency varied significantly ($p < 0.001$) when compared between healthy controls and acne vulgaris patients with a more frequent GG (wild) genotype in controls and CC (mutant) were more common in cases (Table 2).

When the allelic frequency at rs1042522 in *TP53* gene was compared among controls, it was observed that "G" wild-type allele (70%) was the most common, followed by "C" mutant allele (30%) (Table 2). While an opposite trend was observed in cases with a higher incidence of mutant allele (C, 57%) than wild-type allele (G, 43%) (Table 1), and the chi-square test results revealed that this allele frequency distribution was statistically significant ($p < 0.001$) (Table 2).

3.4 | GST interactions and their association with acne vulgaris

Analysis of genotype frequencies indicated that certain genotypes combination with two or more GSTs significantly increased the risk of developing acne vulgaris. All genotype combinations having a significantly higher frequency among the controls were protected combinations, while those having a higher frequency among cases had a higher risk of developing acne vulgaris (Tables 3 and 4). Our results indicated that subjects having both *GSTMI* and *T1* present ($p = 0.026$), subjects having either *GSTM1* ($p < 0.001$) or *GSTT1* ($p < 0.001$) absent with a homozygous mutant (GG) genotype at *GSTP1* ($p < 0.001$) and subjects having both *GSTM1* and *GSTT1* absent with a homozygous mutant (GG) at *GSTP1* ($p < 0.001$) were more susceptible to acne vulgaris (Table 3 and 4).

3.5 | Interactions of GSTs with *TP53* gene and their association with acne vulgaris

Our results indicated that subjects having either *GSTMI* present or absent ($p < 0.001$) with a homozygous mutant (CC) genotype at *TP53* were more susceptible to develop acne vulgaris (Table 4). Similarly, subjects having either *GSTTI* present or absent ($p < 0.001$) with a homozygous mutant (CC) genotype at *TP53* were more susceptible to develop acne vulgaris (Table 4). When the genotype combinations at rs1695 in GSTP1 were analyzed with genotypes at rs1042522 in *TP53*, it was observed that subjects homozygous mutant for both rs1695 (GG) and rs1042522 (CC) were more prone to develop acne vulgaris ($p < 0.001$) (Table S2).

3.6 | Association of risk factors and acne vulgaris

When different risk factors were compared between cases and controls tested during this study, chi-square test results revealed that smoking habit ($p = 0.03$) and age ($p < 0.001$) were associated with the incidence of acne vulgaris in the present study. It was also observed that subjects in the age range of 10–25 years and smokers were more susceptible to acne vulgaris (Table S3).

4 | DISCUSSION

Over the past decades, various studies have indicated that patients with acne are under increased cutaneous and systemic oxidative stress and the imbalance between skin antioxidation and oxidative stress may be a pathogenic factor of skin diseases.[20] A deficiency in the antioxidant system may originate from genetic polymorphisms or as a result of intense ROS activity exceeding the capacity of the

TABLE 3 Genotype and allelic frequency distribution among cases and controls for combinations of glutathione S-transferases (GSTs) and their possible association with acne vulgaris.

Genotype	Control	Case	Chi-square	*p*-Value
GSTM1 and *GSTT1*				
Both present	3	13	7.314	0.026*
Either null	45	45		
Both null	52	42		
GSTM1 and *GSTP1*				
M1(+/+) and P1 (AA)	39	27	31.625	<0.0001***
M1(+/+) and P1 (AG)	4	13		
M1(+/+) and P1 (GG)	1	4		
M1(−/−) and P1 (AA)	51	28		
M1(−/−) and P1 (AG)	4	19		
M1(−/−) and P1 (GG)	1	9		
GSTT1 and *GSTP1*				
T1(+/+) and P1 (AA)	8	14	36.655	<0.0001***
T1(+/+) and P1 (AG)	0	9		
T1(+/+) and P1 (GG)	0	4		
T1(−/−) and P1 (AA)	83	41		
T1(−/−) and P1 (AG)	8	24		
T1(−/−) and P1 (GG)	1	8		
GSTT1, *GSTM1*, and *GSTP1*				
T1 and M1(+/+) and P1 (AA)	3	7	39.306	<0.0001***
T1 and M1(+/+) and P1 (AG)	0	2		
T1 and M1(+/+) and P1 (GG)	0	4		
T1(+/+), M1(−/−) and P1 (AA)	5	7		
T1(+/+), M1(−/−) and P1 (AG)	0	6		
T1(+/+), M1(−/−) and P1 (GG)	0	1		
T1(−/−), M1(+/+) and P1 (AA)	35	19		
T1(−/−), M1(+/+) and P1 (AG)	4	11		
T1(−/−), M1(+/+) and P1 (GG)	1	1		
T1(−/−), M1(−/−) and P1 (AA)	48	21		
T1(−/−), M1(−/−) and P1 (AG)	4	13		
T1(−/−), M1(−/−) and P1 (GG)	0	8		

Note: +/+ represents that two copies specific gene present. −/− represents that two copies specific gene present. $p > 0.05$: not significant.
Abbreviations: *GSTM1*, glutathione S-transferase mu; *GSTP1*, glutathione S-transferase pi; *GSTT1*, glutathione S-transferase theta.
*$p < 0.05$: least significant.
***$p < 0.001$: highly significant.

antioxidant system.[21] Since GSTs are known to reduce the oxidative stress, GST polymorphisms have been evaluated by various research groups in relation to the molecular pathogenesis of T-cell-associated autoimmune diseases affecting the skin,[15,22,23] but the association of GST and *TP53* polymorphisms has never been explored in the Pakistani population with acne vulgaris. Hence, the presented data are first of its kind based on the subjects enrolled from Dera Ghazi Khan district, Punjab, Pakistan.

Hereditary deficiencies in enzyme activity caused by homozygous gene deletion that lead to an absence of enzymatic activity have been elucidated for *GSTT1* gene and subjects null for this gene are particularly susceptible to oxidative or chemical stress and therefore they should develop more intense inflammatory reactions.[24] During this study, *GSTT1* null genotype was found to be associated with the incidence of acne vulgaris (Table 1). The literature review indicated that previously *GSTT1* association with acne vulgaris has never been investigated. This is therefore the first report of association of GSTT1 null genotype with acne vulgaris in the Pakistani population. However, similar findings were reported by Yazici et al.[22] as they had reported that absence of *GSTT1* genotype led to the development of acne rosacea

TABLE 4 Genotype and allelic frequency distribution among cases and controls for combinations of GSTT1 and M1 with rs1042522 in *TP53* and their possible association with acne vulgaris.

Genotype	Control	Case	Chi-square	*p*-Value
GSTM1 and *TP53*				
M1(+/+) and *TP53* (GG)	19	5	33.867	<0.0001***
M1(+/+) and *TP53* (GC)	24	29		
M1(+/+) and *TP53* (CC)	0	10		
M1(−/−) and *TP53* (GG)	27	12		
M1(−/−) and *TP53* (GC)	26	22		
M1(−/−) and *TP53* (CC)	6	22		
GSTT1 and *TP53*				
T1(+/+) and *TP53* (GG)	3	4	36.696	<0.0001***
T1(+/+) and *TP53* (GC)	5	10		
T1(+/+) and *TP53* (CC)	0	7		
T1(−/−) and *TP53* (GG)	43	13		
T1(−/−) and *TP53* (GC)	45	41		
T1(−/−) and *TP53* (CC)	6	25		

Note: +/+ represents that two copies specific gene present. −/− represents that two copies specific gene present. $p > 0.05$: not significant.
Abbreviations: *GSTM1*, glutathione S-transferase mu; *GSTT1*, glutathione S-transferase theta.
***$p < 0.001$: highly significant.

in the subjects enrolled from Turkey. Contrary to these observations, Solak et al.[23] reported that the incidence of psoriasis skin disease had no association with *GSTT1* null genotype and disease susceptibility in subjects enrolled from Turkey. In the present study, we found no association of *GSTM1* genotypes with the incidence of acne vulgaris ($p = 0.9$) (Table 1). As mentioned above, GSTs have not been explored in relation to acne vulgaris before, but Tugba et al.[25] recently reported that subjects with a *GSTM1* null genotype have a higher risk of developing acne rosacea. Similarly, Tsai et al.[26] reported that GSTM1 null subjects were more prone to skin damage induced by polychlorinated biphenyls and dibenzofurans in Taiwan.

During the present study, rs1695 in *GSTP1* gene was found to be associated with the incidence of acne vulgaris and mutant allele "G" was more frequent in cases than in controls (Table 2). A review of the literature indicated that previously the association of rs1695 in *GSTP1* with acne vulgaris has not been investigated. However, Ibarrola-Villava et al.[27] reported that rs1695 in *GSTP1* gene was strongly associated with malignant melanoma in subjects tested in Spain. Cilingir et al.[15] also reported a higher frequency of mutant G allele at rs1695 in Turkish patients with alopecia areata.

There is substantial evidence that the tumor suppressor gene, better known as p53, inhibits cell growth after acute stress by regulating gene transcription.[28] We hypothesized that ROS-mediated stress might trigger the p53-mediated response in skin cells; hence, polymorphisms in this gene may play a role in the progression of acne vulgaris. The SNP, rs1042522, in *TP53* has never been correlated with acne vulgaris and our results indicated a significant correlation of the mutant allele "C" with disease incidence (Table 2). These results provide evidence that rs1042522 should be explored in patients suffering from skin disorders, especially in subjects suffering from acne. This suggestion is based on the fact that subjects carrying allele C of rs1042522 have previously been reported to be associated with an increased risk of occupational skin neoplasms in the Russian population.[14]

Disease progression can be due to the interaction of several affected proteins. Therefore, the results of studies dealing with individual SNP and correlating them to a particular disease are inconsistent, as each individual SNP alters the function of a single gene among the many genes implicated in disease progression.[16] Hence, biological events that are modestly affected by a single SNP may be more greatly affected by the combination of SNPs in different genes. Keeping this observation in view, we performed SNP–SNP interaction analysis and tried to examine various possible SNP combinations from GSTs and TP53 (rs1042522), which were screened in the present study in order to identify the SNP combinations most likely to be associated with the risk of developing acne vulgaris (Tables 3 and 4 and Supporting Information S1). Interestingly, the presence or absence of *GSTM1* had no association with the incidence of acne vulgaris when considered alone (Table 1), but when the presence or absence of *GSTM1* was analyzed in various combinations of *GSTT1*, *GSTP1*, and *TP53*, it was observed that several SNP combinations enhanced the susceptibility to develop acne vulgaris (Tables 3 and 4 and Supporting Information S1). The homozygous (GG) mutant at rs1695 in GSTP1, when present in subjects who also have *GSTM1* or *GSTT1* or both null genotypes, was found to increase the risks of acne vulgaris. The same was found to be true when *GSTM1* and *GSTT1* null genotypes were analyzed in various combinations of rs1042522 in *TP53* gene (Table S2). These observations support our hypothesis that the combination of genotypes with more than one SNP plays an important role in health and disease.

Analysis of risk actors in this study indicated that subjects with a smoking habit were more susceptible to acne vulgaris (Table S3).

Our results are in line with Capitanio et al.[29] which reported a correlation between smoking and adolescent acne in Italian population and defined it as "Smoker's acne." Contrary to our observations, Klaz et al.[30] found a lower prevalence of severe acne in active smoking male subjects than in non-smokers. Our results also showed that subjects aged 10–25 years were more susceptible to acne vulgaris (Table S3). Our results are in agreement with those of Smith et al.[31] who reported a higher incidence in men aged between 18 and 25 years.

5 | CONCLUSION

Despite the fact that the number of samples in this study is not elevated, this is the first time that a significant association of absence of *GSTT1* and presence of mutant allele "G" at rs1695 in *GSTP1* and mutant allele "C" at rs1042522 in *TP53* with acne vulgaris was reported in tested subjects from Pakistan. Subjects aged 10–25 years and smokers were more susceptible to acne vulgaris. In conclusion, our results suggest that the studied GSTs and rs1042522 in *TP53* are involved in protection against oxidative stress and may influence disease progression in acne vulgaris. Our results indicated that the SNPs studied in the present investigation should be considered when determining the risk of acne vulgaris.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest with anyone.

FUNDING INFORMATION

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ETHICS STATEMENT

Ethical Research Committee of the Department of Zoology at Ghazi University Dera Ghazi Khan (Pakistan) approved all the experimental procedures and protocols applied in this study.

CONSENT TO PARTICIPATE

Informed consent was obtained from all the subjects before including them in this study.

CONSENT FOR PUBLICATION

Not applicable.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

DATA AVAILABILITY STATEMENT

All the data generated during this study are presented in the manuscript.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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