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Association of Glutathione-S-Transferase P1 (*GSTP1*) and Group-Specific Component (*GC*) Polymorphism with the Risk of Asthma in Pakistani Population

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ABSTRACT

Risk of asthma is associated with complex interplay of genetic, ethnic and environmental factors therefore asthma prevalence in various ethnic groups cannot be predicted from the interethnic genotype and allelic frequencies. Currently there is very limited data available regarding the association of genetic polymorphisms with asthma in Pakistani population. The following study was aimed to assess the association of glutathione-S-transferase P1 (*GSTP1*) and group-specific component (*GC*) with asthma in adult Pakistani population. Genetically unrelated and clinically diagnosed 200 asthmatics along with 200 healthy controls were voluntarily recruited for this study. Genotyping was performed using conventional PCR. *GSTP1* and *GC* polymorphisms were determined by using restriction fragment length polymorphism (RFLP) technique. The homozygous *GSTP1* Ile/Ile genotype was found to be significantly associated (p = 0.003) with the risk of asthma whereas the homozygous *GSTP1* Val/Val genotype (p = 0.19) was found to be more frequent in control population than in the patients (6% and 10%, respectively). The *GC* polymorphism GC2 homozygote was also significantly associated with the risk of asthma (p = <0.001), whereas the GC1S homozygote (p = 0.250) was less frequent in asthmatics and more prevalent in the control group (3.5% and 6.5%, respectively). It is thus concluded that the polymorphisms in both the *GSTP1* and *GC* are significantly associated with the risk of asthma in Pakistani population.

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NAA executed the experimental work and wrote the article. NJ conceived the project and supervised the work.

Key words
***GSTP1, GC,* Asthma, Pakistani Population, Polymorphisms**

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways which affects 300 million people around the World (WHO, 2007.). Genetic predisposition can alter the airways and the innate immune system in protecting itself against inhaled environmental toxic substances (Lazarus *et al*., 2001; Kabesch *et al*., 2004). Chronic exposure of the innate immune system to allergens, pathogens and tobacco smoke components along with the role of certain genetic variants results in inflammation and remodeling of airways leading to COPD and asthma (Zhao and Bracken, 2011). Evidence from studies shows that tobacco smoke and exposure to environmental tobacco smoke (ETS) is responsible for increased prevalence of cough, wheeze and phlegm as well as COPD and exacerbated asthma (Gilliland *et al*., 2002; Bose and Bathri, 2012; Raza *et al*., 2014). Glutathione-S-transferases (*GST*s) in humans represent a diverse and large super family of enzymes associated with the antioxidant defenses and detoxification of xenobiotics and carcinogens such as found in ETS (Hayes and Pulford, 1995; Strange *et al*., 2000). *GST*s (M1, T1, P1) are polymorphic in humans (Bose and Bathri, 2012). *GSTs* confer protection to cells from reactive oxygen species (ROS) by utilizing oxidative stress substrates (Hayes and Pulford, 1995). Glutathione S-Transferase P1 (*GSTP1*) enzyme plays a vital role in the detoxification of reactive oxygen species (ROS) (Kabesch *et al*., 2004; Nickel *et al*., 2005). *GSTP1* is located on chromosome 11q13 (Fryer *et al*., 1986) and its SNP rs1695 (A→G) at codon 105 of exon 5 (Ile105→Val105) has been reported to be associated with the risk of asthma (Spiteri *et al*., 2000; Hemmingsen *et al*., 2001; Mapp *et al*., 2002; Islam *et al*., 2009). Deletion of the *GSTP1* Ile105 polymorphism has been known to eliminate enzyme activity and increase susceptibility to oxidative stress (Hayes and Pulford, 1995; Cotton *et al*., 2000).

Group-Specific Component (*GC*) also known as vitamin D-binding protein (*VDBP*) is a 52-58 kDa plasma protein synthesized by hepatocytes and acts as a major carrier protein for vitamin D (Schmidt-Gayk *et al*., 1977; Safadi *et al*., 1999; Verboven *et al*., 2002). *GC* is located on chromosome 4q13 having 13 exons which encode 474 amino acids. Two common functional polymorphisms in exon 11 of *GC* are rs4588 and rs7041 which are positioned at codons 416 (GAT→GAG, Asp→Glu) and 420 (ACG→AAG, Thr→Lys) respectively (Chishimba *et al*., 2010). Polymorphisms in the co-dominant alleles (rs4588 and rs7041) result in three electrophoretic variants (GC1S, GC1F and GC2) based on the glycosylation differences and amino acid substitutions (Yamamoto and Homma, 1991; Braun *et al*., 1992). The

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GC1F and GC1S isoforms are sialylated (Svasti and Bowman, 1978) whereas GC2 is non-glycosylated (Viau *et al*., 1983). Additionally, *GC* can transform into macrophage-activating factor (GC-MAF) upon contact with T or B lymphocytes (DiMartino and Kew, 1999) and has various inflammatory functions (Daiger *et al*., 1975; Gumireddy *et al*., 2003). Significantly elevated levels of GC-MAF have been detected in asthmatics with sensitization to specific antigens (Jeong *et al*., 2005).

Candidate genes implicated in the development and prevalence of asthma and atopic diseases vary considerably by ethnicity (Ober *et al*., 2000; Pillay *et al*., 2000) therefore positive association between genetic polymorphisms and asthma cannot be predicted on the basis of intra- and interethnic genotypic or allelic differences (Hanene *et al*., 2007). Currently there is very limited data available regarding the association of genetic polymorphisms with asthma in Pakistani population. This study was aimed to determine the association of *GSTP1* and *GC* polymorphisms with asthma in Pakistani population.

MATERIALS AND METHODS

Study population and ethical approval

This study and all of its protocols were approved by the Advance Studies and Research Board (ASRB) at GC University, Lahore Pakistan. Total 200 clinically diagnosed asthmatics were voluntarily recruited from the outpatient departments of Jinnah Hospital, Lahore and Gulab Devi (Chest) Hospital, Lahore along with 200 healthy individuals randomly recruited from the general population as control subjects. All self-reported cases of asthma were excluded. Patients suffering from pneumonia, tuberculosis, angina and other heart conditions along with pregnant and breast feeding women were excluded from this study. The selected control population was negative for clinical and family history of asthma, rhinitis or eczema/dermatitis.

A standardized questionnaire was used for the collection of data such as the gender, age, BMI, exposure to tobacco and biomass smoke as well as the patient's medical and family history. Written and informed consents were obtained from all participants of this study.

Physical examination and pulmonary function tests (PFTs)

Height and weight of the subjects was recorded for the calculation of the BMI by employing a calibrated weighing machine provided with a stadiometer. Peak expiratory flow (PEF) was measured with the aid of a portable Spirometer (MicroPlus, UK). Spirometry was performed in accordance with the American Thoracic Society (ATS) guidelines (Miller *et al*., 2005). Mean of the best out of three acceptable readings was taken.

Sample collection and DNA extraction

Approximately 3ml blood was collected in EDTA coated tubes from subjects via venipuncture technique. DNA was isolated from whole blood with the aid of Genomic Blood DNA Isolation kit (Invitrogen, USA) in accordance with manufacturer's instructions. Purified DNA was stored at -20°C until further analysis.

Determination of genetic polymorphisms

PCR (Platinum® PCR Super Mix, Invitrogen, USA) was performed for the amplification of the *GSTP1* (rs1695) and *GC* polymorphisms (rs4588 and rs7041). Primer pairs for the targeted SNPs and their respective annealing temperatures are provided in Table I. Genomic DNA (50-80ng) was taken as template in 50µl reaction volume. PCR cycle included initial denaturation at 95°C (5 min) followed by 35 cycles of amplification (30 seconds at 95°C, 30 seconds at 55°C, and 40 second at 72°C) then a final extension for 10 minutes at 72°C.

After PCR, the products were purified with commercially available kit (GF-1 PCR Clean-up Kit, Vivantis, USA) for the removal of excess dNTPs, primers, ethidium bromide and enzymes. Total 25µl of purified PCR product was digested at 37°C with Alw261 (Fermentas, USA) for *GSTP1* polymorphisms. A 176bps band indicated homozygous wild-type, 85bps/91bps fragments represented homozygous mutant genotype whereas the presence of all three bands indicated heterozygous genotype. For the *GC* polymorphisms, Hae III (Fermentas, USA) was used for T/G at 37°C and cleaved the 483bp product into two (297/186bps) fragments and Sty I (Fermentas, USA) was used for C/A at 37°C and cleaved the 483bp product into two (305/178bps) fragments. Three phenotypes occur due to the combination of these alleles GC1F, GC1S and GC2 (Table II) hence there are 6 possible genotypes. The digested products were analyzed on 3% agarose gel and stained with ethidium bromide.

SPSS for Windows was used for the statistical analysis of the accumulated data. Descriptive statistics were expressed as mean ± standard deviation (SD). Clinical data was compared between cases and controls (gender, age, body mass index (BMI) and smoke exposure) by Pearson's chi-square test.

RESULTS

The demographic characteristics of the subjects included in this study are summarized in Table III. Among the total 400 subjects who participated in this

Table I.- List of primers used for PCR.

Gene	SNP	PCR	Primers	T_m	bps
GSTP1	rs1695	PCR-1	5′- GTACCAGTCCAATACCATCC -3′ 5′- CTCCCTCATCTACACCAACT -3′	55°C	514
		Nested-2	5′- ACCCCAGGGCTCTATGGGAA -3′ 5′- TGAGGGCACAAGAAGCCCCT -3′	47°C	176
GC	rs4588 rs7041	PCR-1	5′- AAATAATGAGCAAATGAAAGAAGAC -3′ 5′- CAATAACAGCAAAGAAATGAGTAGA -3′	57°C	483

T_m; Annealing temperatures (°C), bps; Product size in base pairs

Table II.- Nucleotide and corresponding amino acid differences of the studied GC variants.

GC variants	Nucleotides (T/G)	Corresponding amino acid	Nucleotides (C/A)	Corresponding amino acid
GC1F	GA<u>T</u>	Aspartic Acid	A<u>C</u>G	Threonine
GC1S	GA<u>G</u>	Glutamic Acid	A<u>C</u>G	Threonine
GC2	GA<u>T</u>	Aspartic Acid	A<u>A</u>G	Lysine

Table III.- Demographic characteristics of studied population.

Traits	Male	Female
Cases *n*(%)	55 (27.5)	145 (72.5)
Controls *n(%)*	126 (31.50)	86 (21.50)
Mean age (year ± SD)	34.17±14.30	31.21±12.30
BMI (kg/m² ± SD)	22.17±5.51	24.37±4.12
Tobacco smoking *n*(%)	19 (4.75)	5 (1.25)
Exposure to tobacco smoke *n*(%)	93 (23.25)	33 (8.25)

BMI = Body Mass Index

study, 55 (27.5%) were male asthmatics, and 145 (72.5%) were female asthmatics, whereas the male to female ratio in the control population was 126 (31.50%) and 86 (21.50%) respectively. The mean age of males was 34.17±14.30 and the mean age of females was 31.21±12.30. The BMI for the males was 22.17±5.51 whereas that of the females was 24.37±4.12. Total 19 (4.75%) males and 5 (1.25%) females were smokers. About 23.25% (n=93) males and 8.25% (n=33) females were exposed to tobacco smoke either by smoking or from second-hand smoke.

Genotype frequencies were within the Hardy-Weinberg equilibrium. Association of genotype profiles between asthmatics and controls were determined by odds ratio and chi-square test (p value > 0.05). Genotype frequencies of the studied *GSTP1* and *GC* polymorphisms in both asthmatics and controls are provided in Table IV.

A significant association between asthma and the homozygous *GSTP1* Ile/Ile genotype (OR = 2.27; 95% CI, 1.339 - 3.854; p = 0.003) was observed in the studied population whereas the homozygous *GSTP1* Val/Val genotype (OR = 0.57; 95% CI, 0.273 - 1.209; p = 0.19) was more frequently observed in the controls than the cases (6% and 10% respectively). This higher frequency of the Val variable is an indicator of its possible protective role against asthma. As for the *GC* polymorphisms, the GC2 homozygote (GC2/GC2) was found to be significantly associated with asthma (OR = 3.14; 95% CI, 1.786 - 5.535; p = <0.001), whereas the GC1S homozygote (GC1S/GC1S) (OR = 0.52; 95% CI, 0.203 - 1.335; p = 0.250) was observed less frequently in asthmatics and more in the control group (3.5% and 6.5%, respectively). The higher rate of GCIS in control population may be an indicator of the protective role of GC1S variant against the susceptibility to asthma.

DISCUSSION

This case-control study was performed to assess the association of the *GSTP1* and *GC* polymorphisms with asthma susceptibility in Pakistani population. Our results demonstrate the association of both *GSTP1* and GC variants with an increased risk of asthma in Pakistani population as evident from the significant differences in the genotype frequencies of asthmatics and controls.

In present study the asthmatics have exhibited a higher frequency of *GSTP1* Ile (A) variant and a lower frequency of *GSTP1* Val (G) variant as compared to the control population. These results implicate the homozygous Ile/Ile (AA) with susceptibility to the risk of asthma and suggest a protective role of the homozygous

Table IV.- Genotype frequencies for the studied polymorphisms.

Gene	SNP	Genotypes	Asthma *n* = 200 n (%)	Control *n* = 200 n (%)	x^2	OR	95% CI	*p*
GSTP1	rs1695	AA(Ile/Ile)	49(24.5)	25(12.5)	8.77	2.27	1.339 - 3.854	0.003*
		AG(Ile/Val)	51(25.5)	48(24)	0.05	1.084	0.688 - 1.707	0.817
		GG(Val/Val)	12(6)	20(10)	1.66	0.57	0.273 - 1.209	0.19
		A(Ile)	149(74.5)	118(59)	4.45	1.54	1.050 - 2.285	0.035*
		G(Val)	75(37.5)	92(46)				
GC	rs4588	GC1F/GC1F	45(22.5)	34(17)	1.61	1.42	0.865 - 2.359	0.204
		GC1S/GC1S	7(3.5)	13(6.5)	1.32	0.52	0.203 - 1.335	0.250
		GC1F/GC1S	18(9)	12(6)	0.90	0.64	0.301 - 1.377	0.341
	rs7041	GC1F/GC2	33(16.5)	23(11.5)	1.70	1.53	0.859 - 2.724	0.191
		GC1S/GC2	31(15.5)	22(11)	1.41	0.67	0.372 - 1.208	0.235
		GC2/GC2	51(25.5)	20(10)	15.70	3.14	1.786 - 5.535	<0.001*

*Significant
x^2 = Chi-Square, OR = Odds ratio, CI = Confidence interval, *p* = Probability

Val/Val (GG) allele against asthma. Several studies concur with our results and have reported similar protective role of the *GSTP1* Val/Val genotype and its association with lower risk of asthma than the *GSTP1* Ile/Ile (AA) genotype (Spiteri *et al*., 2000; Hemmingsen *et al*., 2001; Aynacioglu *et al*., 2004; Miller *et al*., 2004). Fryer *et al*. (2000) studied a population of Northern European white and concluded a 4-10 fold lower risk of asthma for *GSTP1* Val/Val homozygotes which corresponds to our own findings. Lee *et al*. (2004) demonstrated a significantly increased risk of asthma in Ile homozygotes (OR = 5.52; 95% CI, 1.64–21.25) as compared to Val carriers in a Taiwanese cohort. Asthmatics from South-East Anatolia, Turkey have also exhibited significantly lower *GSTP1* Val allele frequency than in control group (3.8% and 12.1% respectively, $p = 0.01$) (Aynacioglu *et al*., 2004). Similar observations were concluded by Spiteri *et al*. (2000) where the frequency of *GSTP1* Val/Val genotype was found to be higher in non-atopic asthmatics rather than in atopic asthmatics, furthermore a significant increase in *GSTP1* Ile/Ile genotype frequency was observed with increased asthma severity and a parallel decreased in frequency of *GSTP1* Val/Val genotype. *GSTP1* Ile/Ile frequency was found to increase in patients with established asthma whereas *GSTP1* (Val105→Ala114) and *GSTP1* (Val105→Val114) confer protective effects in Northern Europeans (Kabesch *et al*., 2004). Occurrence of the *GSTP1* Val/Val homozygote was found to be more frequent in the control group than the asthmatics in a study carried out on Tunisian Children, moreover there was a higher prevalence of *GSTP1* Ile allele in the asthmatic children than the control group (43.8% and 33.5%, respectively; $p = 0.002$) (Hanene *et al*., 2007).

However, contrary to our findings, one study has reported a higher prevalence of *GSTP1* Val/Val among asthmatic subjects rather than the control group (22.8% and 7.8%, respectively) and showed that the subjects with homozygous *GSTP1* Val/Val genotype had a 3.55 fold increased risk of developing atopic asthma as compared to non-atopic asthma (OR = 3.55; 95% CI = 1.10–12.56) (Tamer *et al*., 2004). Nonetheless, the catalytic efficiency of the Val variant is higher when compared to the Ile variant (Watson *et al*., 1998) which indicates the possibility of the role of *GSTP1* polymorphisms in the risk of asthma by ROS product modulation.

Our study has shown significant association of GC2 homozygote with asthma, similar results were demonstrated by a study in Chinese Han population (Li *et al*., 2011) and GC2 allele was found to be associated with increased susceptibility to asthma (OR = 1.35; 95% CI = 1.01–1.78, $p = 0.006$). GC2-2 allele was found to be more significantly associated with asthma than the GC1-1 allele (OR = 13.13; 95% CI = 2.42–7.13, $p = 0.001$).

In the following study, the GC1S homozygote was more prevalent in the control group which concurs with the findings of Jung *et al*., (2014) as demonstrated in Korean population (GC1S variant 29.0% vs. 21.4%; $p = 0.020$). Similar results were reported in the Hispanic population of inner-city New Haven, Connecticut where GC1S/GC1S genotype was found to be under transmitted in asthmatics as compared to the wild-type GC1F/GC1F conferring protection against development of asthma (Navas-Nazario *et al*., 2014).

These results confirm the association of polymorphisms of *GSTP1* and *GC* with asthma in Pakistani population and establish *GSTP1* and *GC* as susceptible genes for the risk of asthma as previously demonstrated by similar studies in populations belonging to various ethnic groups.

CONCLUSION

Our results lead to the conclusion that there is a significant association of *GSTP1* Ile/Ile and GC2 homozygotes with susceptibility to asthma in Pakistani population. The *GSTP1* Val/Val and GC1S homozygotes were more frequently observed in the controls and may be protective against the development of asthma.

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Statement of conflict of interest

Authors declare no conflict of interest.

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