



## ABSTRACT

Asthma and allergy are common and complex diseases often co-occurring within the same individual. Genetic as well as environmental factors such as consanguinity and early sensitization to allergens significantly influence the onset and progress of the disease. Aim of the following study was to investigate the association of environmental factors and selected single nucleotide polymorphisms (SNPs) from candidate genes with asthma in Pakistani population. The studied population consisted of 368 genetically unrelated volunteers. Three SNPs from 2 genes Group Specific Component gene *GC* (rs4588 and rs7041) and Glutathione S-Transferase P1 gene *GSTP1* (rs1695) were selected for genotype analysis by Restriction fragment length polymorphism (RFLP), whereas 15 SNPs from Interleukin 33 gene *IL33* (rs1412426, rs1342326, rs992969, rs928413, rs2066362, rs996029, rs10815388, rs10975501, rs17498196, rs10975516, rs10975519, rs7047921, rs1332290, rs16924241 and rs8172) were selected for ABI's Taqman 7900 genotyping. Mean age was  $34.04 \pm 16.30$  for males and  $33.89 \pm 13.15$  for females whereas the mean BMI was  $22.01 \pm 4.04$  for males and  $23.23 \pm 5.21$  for females. About 68.20% subjects lived in urban areas and 31.79% lived in rural areas. Parents of 87.5% of the studied population had married within their own caste system, 60.05% of which were first cousins. Family history of Asthma was prevalent in 48.91% of the Asthmatic population. Total 60.86% asthma cases were classified as adult and 39.13% cases were classified as pediatric onset asthma, based on the age at which the diagnosis of asthma was concluded. Asthma severity was based on the percent predicted FEV1 and categorized as intermittent (2.71%), mild persistent (0.54%), moderate persistent (40.21%) and severe persistent (56.52%). Total serum IgE levels were  $121 \pm 2.91$  IU/ml for the control population and  $584.2 \pm 4.61$  IU/ml in asthmatics. Males had higher IgE levels (181IU/ml) than the females (99IU/ml). Amongst the atopic manifestations cough was the most prevalent symptom (82.06%) closely followed by wheeze (80.97%) whereas skin allergies were the least prevalent (22.82%). Seasonal variations were reported to be the most significant asthma trigger (95.65%) followed by dust (72.82%) and food allergies (60.32%). Asthma attacks were more frequent in winter season (57.06%) and in transitional period between changing seasons (48.36%). Only 3.80% reported no seasonal correlations with asthma. Smoking asthmatics constituted 11.41% of the studied asthmatic population whereas 62.5%



were exposed to tobacco smoke for long periods of time, 51.08% asthmatics were exposed to tobacco smoke but never smoked themselves. Homeopathic medicine was used by 14.67% and 33.69% turned to Hakeems however; the use of complimentary alternate medication remained the most popular (39.13%) mostly due to the lack of side effects associated with such treatments. Total 18 SNPs from 3 candidate genes were genotyped using RFLP and ABI's Taqman. RFLP was carried out in 2 genes (GC/VDBP, GSTP1). Significant association of GC/VDBP GC2 homozygote asthma was established (OR = 3.14, 95% CI = 1.786 - 5.535,  $p = <0.001$ ) through 90% reproducibility. GSTP1 Ile/Ile homozygote showed significant association with asthma (OR = 2.33, 95% CI = 1.347 - 4.043,  $p = 0.003$ ) in Pakistani population. 15 SNPs from Interleukin-33 were genotyped on the ABI platform. rs17498196 (OR = 1.78, 95% CI = 1.18- 2.68  $p = 0.005$ ) and rs992969 (OR = 1.42, 95% CI = 0.99- 2.04  $p = 0.05$ ) were significantly associated with the risk of asthma, however the risk becomes non-significant with cousin parents removed rs17498196 (OR = 2.42, 95% CI = 1.23- 4.74  $p = 0.01$ ) and rs992969 (OR = 1.41, 95% CI = 0.80- 2.48  $p = 0.23$ ) respectively. These results highlight the importance of consanguineous unions in inheritance of asthma as a genetic disorder. LD analysis between the SNPs revealed strong linkage disequilibrium between rs10975516, rs10975519, rs7047921 and rs1332290 even with cousin parents removed remaining good predictors of each other. rs1412426, rs1342326, rs992969 and rs928413 showed moderate LD which further decreased with cousin parents removed, implicating consanguinity in the inheritance of asthma risk allele.