

**MCP3 POLYMORPHISM, ENVIRONMENTAL RISK FACTORS AND ASTHMA- A HOSPITAL BASED STUDY IN VELLORE****MANJIMA CHATTERJEE¹, UPASANA MUKHERJEE¹, NARMADA ASHOK², RAJA DAS³,
SUVRAJIT DAS¹ AND RADHA SARASWATHY^{1*}**¹*School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu.**TT-120, Biomedical Genetics Research Laboratory (BMGRL), VIT University, Vellore, Tamil Nadu, India.*²*Nalam Medical Centre and Hospital, Vellore, Tamil Nadu.*³*School of Advanced Sciences, VIT University, Vellore, Tamil Nadu.***ABSTRACT**

Asthma being a chronic disease imposes a substantial burden on the community with greater morbidity and frequent exacerbations. Environment and genetic factors strongly contribute towards asthma. This study aims to understand the demographic characteristics of asthma patients and to investigate the presence of genetic polymorphism in the MCP3 gene in Vellore, India. A total of 119 confirmed asthma patients (clinically and pulmonary function tests) were analysed for the demographic profile with the presence of genetic polymorphism in MCP3 promoter (-1381T/C) and intronic regions (+563C/T) using PCR-SSCP (Single Strand Conformation Polymorphism) and Sanger sequencing. Two to three of the risk factors and symptoms were significantly associated with asthma. Only one patient with a novel variation (+563C/T) in MCP3 second intronic region was observed. The study being the first of its kind infers a modification of triggering factors and environmental constraints could lead to a better control of the disease.

KEYWORDS: Asthma, Demographic, Morbidity and Polymorphism**RADHA SARASWATHY**

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INTRODUCTION

Asthma affects an estimated 300 million individuals worldwide. It is a serious global health problem affecting all age groups, with increasing prevalence in many developing countries, rising treatment costs and a rising burden for patients and the community. Asthma causes symptoms such as wheezing, shortness of breath, chest tightness and cough that vary over time in their occurrence, frequency and intensity.¹ There are two types of this multifactorial condition – intrinsic and extrinsic asthma. Genetic variance along with environmental factors like urbanization, trigger higher incidence of extrinsic asthma.² The demographic study has been performed to focus almost every possible aspect in asthma occurrence with immense support of statistical data. Worldwide statistics emphasise the mortality rate due to this infirmity and according to World Health Organization (WHO) by 2020 asthma along with Chronic Obstructive Pulmonary Disease (COPD) may become the third leading cause of death (WHO statistics, 2008). Indian demography reveals the accountability of asthma which is also severe and increasing in a significant rate. India has reported about 10 to 15 million asthmatics and 57,000 deaths annually (WHO statistics, 2010). Along with long term exposure to certain chemicals, smoke, irritants or even climate changes that have consequential impact on the diseased state, certain molecular intervention also discloses asthmatic disposition with the involvement of variegated functional genes. Research from identical ethnic group along with statistical computation supports the evidence for predisposition of disease from expansive range of risk factors.³ The impact of environmental changes with respective geographic distribution helps in finding disease incidence. Asthma is also influenced by genetics. Having a parent with asthma doubles a child's risk in experiencing the disease and having two affected parents increases the risk fourfold.⁴ The greater concordance of asthma among monozygotic twins as compared with dizygotic twins further supports this genetic influence.⁵ Previous genetic association study on asthma has reported 43 replicated genes, where the most frequently asthma associated genes are *TNF α* , *IL4*, *FCERB*, *ADAM 33*, and *GSTP1*.⁶ Other genes identified are *DPP10*, *GPR154* and *PHF11* by linkage and fine mapping and *ORMD3*, *IL1RL1*, and *PDE4D* by genome-wide association studies, most of which are associated with inflammation or a shift of the immune system toward a Th2 response, while others are surrogate biomarkers of inflammation.^{2,7} None alone is sufficient to predict or explain asthma, and there is a high degree of heterogeneity in the association of these genotypes among affected individuals or populations. These findings suggest that asthma genes interact in a complex manner to regulate the risk and severity of the disorder and that genetics alone is insufficient to fully explain inter-individual or inter-population variations of the disease. The missing explanation could reside in gene-environment interactions, which are now believed to be mediated even by epigenetic mechanisms.⁶ Hyper responsiveness can be triggered by activation of immune cells by allergen-specific IgE.^{2,7} Processing and presenting of certain allergens by antigen

presenting cells (APCs) are key phenomenon in hypersensitivity. Cooperation of APC with T and B lymphocytes kindle responses and is enhanced by several cytokines. One of these small cytokines is known as chemokines that primarily deals with attraction and regulation of leukocyte trafficking into the tissues by binding certain receptors. A seven membrane spanning G-protein coupled receptor has been found to play a significant role in this cellular pathway, known as Chemotaxis. Two notable chemokines are involved in asthma pathogenesis- CC chemokines having two adjacent cysteine residues and CXC chemokines having two cysteine separated by one amino acid residue.⁸ IL-8 and IFN- induced protein10 (IP-10) comes under CXC chemokines and primarily aim the neutrophil; but the CC- chemokines (MCP1 to MCP4, MIP α and MIP β) are known to target monocytes, T-cells and eosinophils. Asthma pathogenesis follows a two step phenomenon involving sensitization to an aeroallergen with a preferential development of antigen specific Th2 cells and targeting the Th2 driven allergic inflammation to the lower airways, regulated by a complex network of mutually interacting cytokines, chemokines and growth factors.⁹ The Monocyte chemotactic protein-3 (MCP-3) is a well known secreted chemokine located on chromosome 17q11.2-q12. MCP-3 along with MCP-1 and MCP-2 evokes inflammatory responses by attracting T cells of CD4⁺ and CD8⁺ phenotypes. It is a CC chemokine structurally related to another prototypic CC chemokine MCP1, but with a distinct receptor usage and spectrum of action. It binds to CCR1, CCR2 and CCR3 receptors resulting in an overlapping interaction, though is distinct from that of MCP1 which binds CCR2 alone.¹⁰ MCP3 activates mononuclear phagocytes, T cells, NK cells and basophils as MCP1 does but it also activates eosinophils and dendritic cells which are not affected by MCP1.¹¹ Literatures review that 17q region has great significance in asthma predisposition. Study done by Batra et al., 2011 on North/Northwest Indian population reveals an association of two SNPs in MCP-3 gene promoter MCP3(-1381T/C) [dbSNP: rs3091318] and second intron MCP3 (+563C/T) [dbSNP: rs3091321] with asthma. Whereas, an early study based on Korean population did not report any remarkable association of MCP3 gene with asthma.^{8,12} A study by Fitzpatrick et al., 2010 to discriminate severe from moderate asthma in pediatric population (n=53 asthmatic children, severe = 31 children) and 30 non-smoking adults, undergoing bronchoscopy evaluated for 23 cytokines and chemokines measured in the bronchoalveolar lavage (BAL) fluid and alveolar macrophage (AM) lysate collected from them reported no significant elevation in the concentration of chemokines (MCP3, MCP4) in the asthma group.¹³ The broad spectrum of action of MCP3 and expression of other chemokines (RANTES, MIP1 α , MIP1 β , eotaxin, MCP1 and Nitric oxide synthase) by 17q11-21 region has been reported to be of significance for asthma predisposition in Indian population. This prompted us to evaluate the association of MCP3 gene and asthma by investigating promoter polymorphism (-1381T/C) and intronic variants (+563C/T) of MCP3 gene using molecular tools. Hence this cohort study aims to evaluate the

frequency of asthma, age, lifestyle and correlate with genetic analysis.

MATERIALS AND METHODS

This study was performed with a questionnaire for data gathering. IRB approvals were obtained from University Human Ethical Committee, VIT University, Vellore and from Nalam Hospital Ethical Committee, Nalam Medical Centre and Hospital, Vellore (VIT/UHEC-5/No.1/27.08.2012). Informed and written consent from participants was obtained.

Inclusion criteria: All patients attending the allergy and asthma Out Patient Department of Nalam Medical Centre & Hospital, Vellore, Tamil Nadu who had clinical symptoms and showed reversibility of airway obstruction in pulmonary function tests were included.

Exclusion criteria

1. All patients who had symptoms suggestive of asthma but in whom confirmation could not be done on pulmonary function tests.
2. Patients who had coexistent chronic diseases and acute exacerbation.

A total of 119 patients were recruited. Age of onset, occupation, risk factors and medical history of these asthma patients was recorded in the questionnaire. Peripheral blood sample (5ml) was collected from the patients in EDTA vacutainer by venipuncture. DNA was extracted from leukocytes using the method of Sneha, 2012 standardized in the Biomedical Genetics Research Laboratory (BMGRL), VIT University, Vellore, India.¹⁴ The Optical Density (OD) for all the isolated

DNA samples was checked by using Biophotometer (Eppendorf®). Primers were designed (using Primer Blast) to amplify MCP3-1382T/C and MCP3+563C/T regions and analyse for the presence of two known SNPs in asthma patients. All the PCR products were checked in 1% Agarose Gel Electrophoresis (AGE). To check the presence of SNPs of interest or any other variation in both the amplified promoter and intronic region of MCP3 gene, Single Strand Conformation Polymorphism (SSCP) was performed by following the protocol of Sneha, 2012.¹⁴ DNA sequencing was carried out at Genotypic Technology Pvt. Ltd., Bengaluru, India using Sanger sequencing following di-deoxy chain termination method. The results were analyzed using Finch TV software® for identification of targeted polymorphisms.

Statistical analysis

The demographic characteristics were analysed for the age groups, prevalence of risk factors, frequency of symptoms occurring and occupational exposure in these patients by statistical analysis. The mean, one and two sample t-test were performed by Microsoft excel® 2010 and Minitab software.

RESULTS

Statistical analysis for all 119 patients was carried out based on age group, occupation, exposures to environmental risk factors and medical symptoms. A boxplot of gender wise analysis of all the subjects showed the mean age for females to be near 34 years, whereas for males to be relatively late onset, with a mean age of 42 to 43 years (Fig I).

Gender wise analysis of asthma patients with respect to age group

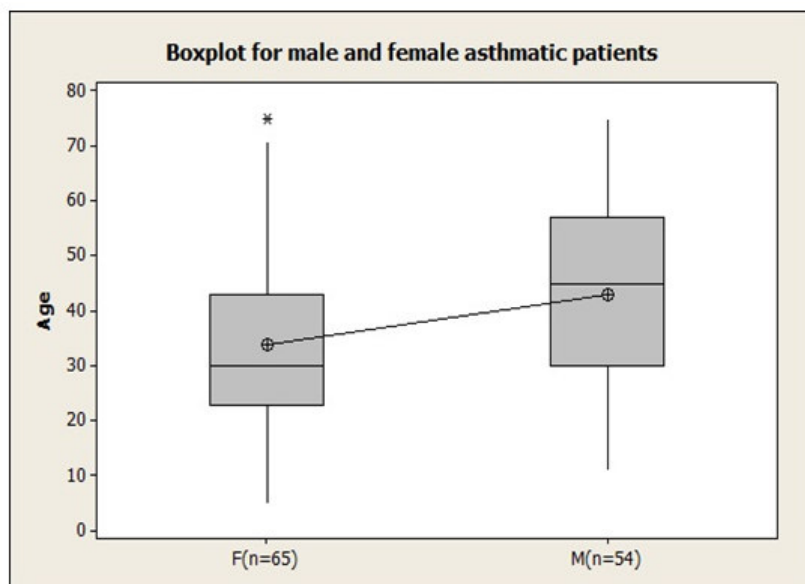


Figure I
Mean age of females is 34 years and males is 42-43 years

The one sample t-test was performed by considering risk factors as a variable parameter and is presented in Fig IIa, b respectively. The major risk factors considered were dust, food, strong odor, animals, alcohol, mold, pollen, climate, chemical, tobacco smoke and wood smoke. The patients habitually exposed to any of these two to three factors were found to be predisposed to asthma (mean value = 2.554 at 95% CI) (Table I).

Effect of environmental risk factors in asthma patients

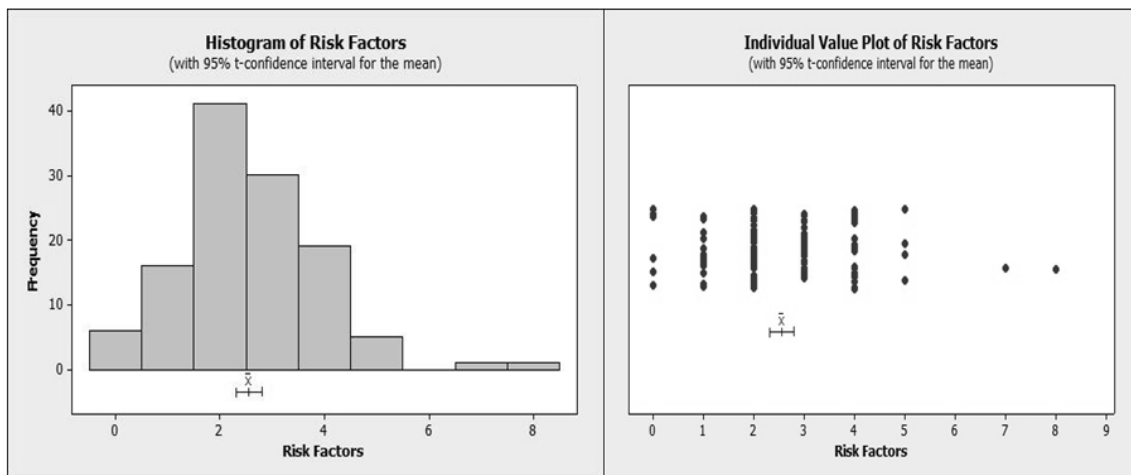


Figure IIa, b
Representation of the frequency of number of asthma patients vs. the number of risk factors (0-9)

Table I
Calculation for risk factors associated with asthma

Variable	N	Mean	Std Dev	SE Mean	95% CI
Risk Factors (RF)	119	2.55462	1.35107	0.12385	(2.30936, 2.79988)

Table I Risk factors calculation

Similarly, all the patients were analyzed for medical history using one sample t-test. A total number of six symptoms were considered as variable with respect to number of patients (Fig IIIa, b). Major symptoms observed were nasal symptoms, shortness of breath, sneezing, wheezing, coughing and other allergic reactions (like cold, itching, eye irritations, skin problems, eczema). The t-test revealed that the patients with two to three symptoms have a high risk of asthma with a mean value of 2.521 at 95% confidence interval (Table II).

Frequency of symptoms associated with asthma patients

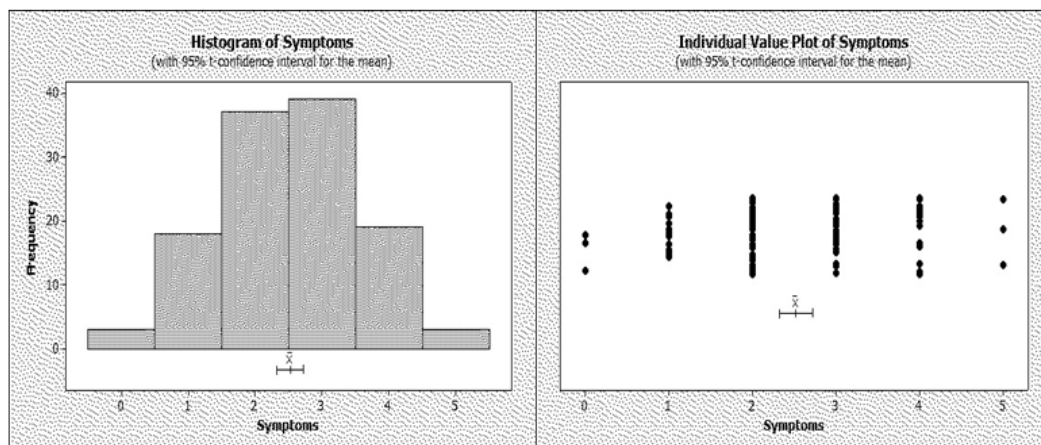


Figure IIIa, b
Representation of the frequency of number of asthma patients vs. number of clinical symptoms

Table II
Calculation for risk factors associated with asthma

Variable	N	Mean	Std Dev	SE Mean	95% CI
Symptoms	119	2.52101	1.08806	0.09974	(2.32349, 2.71853)

Table II Symptoms associated with asthma

Table III presents occupation as the major parameter for all the study subjects; where the patients with sedentary lifestyle was statistically significant and related to increased asthma incidence, $p < 0.05$ at 95% confidence level.

Table III
Occupational influence on asthma

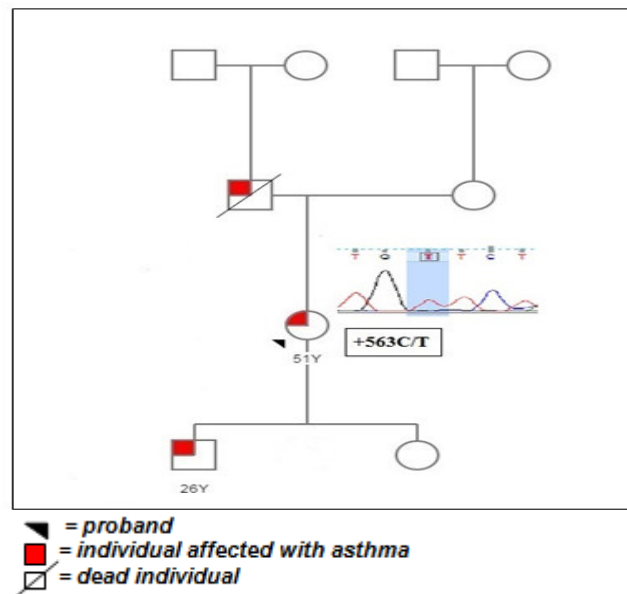
Occupation	X	N	Sample p	95% CI	z-Value	p-Value
Farmer	9	119	0.075630	(0.028125, 0.123136)	-9.26	0.000
Student	16	119	0.134454	(0.073161, 0.195746)	-7.98	0.000
Teacher	8	119	0.067227	(0.022235, 0.112219)	-9.44	0.000
House wife	24	119	0.201681	(0.129587, 0.273774)	-6.51	0.000
Daily wages	16	119	0.134454	(0.073161, 0.195746)	-7.98	0.000
Cook	2	119	0.016807	(0.000000, 0.039903)	-10.54	0.000
Healthcare professional	3	119	0.025210	(0.000000, 0.053376)	-10.36	0.000
Private/Govt. Service	17	119	0.142857	(0.079986, 0.205728)	-7.79	0.000
Business	4	119	0.033613	(0.001231, 0.065996)	-10.18	0.000

Table III Risk calculation for occupational asthma

At the molecular level an altered '+563C/T' intronic locus in one patient was observed (Fig IV). This individual (F/51Y), having the targeted mutation was reported with a history of shortness of breath, chest tightness, eye irritation and severe headache. She was also exposed to dust and was very much allergic to certain food vestiges. Family history unveiled that her father was affected with asthma. Her son (M/26Y) was also affected with the same disease phenotype being allergic to certain chemicals and perfumes and was found to be negative for mutation analysis for MCP3 gene; whereas her daughter and siblings were found to be healthy with no asthmatic symptoms.

Pedigree of patient with family history of asthma and gene mutation

Figure IV
Pedigree analysis of the proband (F/51Y) with MCP3 intronic (+563C/T) mutation.



A two sample t-test was performed as a comparison study that revealed a less significant association of MCP3 polymorphism with asthma in Vellore population with a mean value of 0.0084 (Fig V, Table IV).

Comparison of mutation analysis between two populations

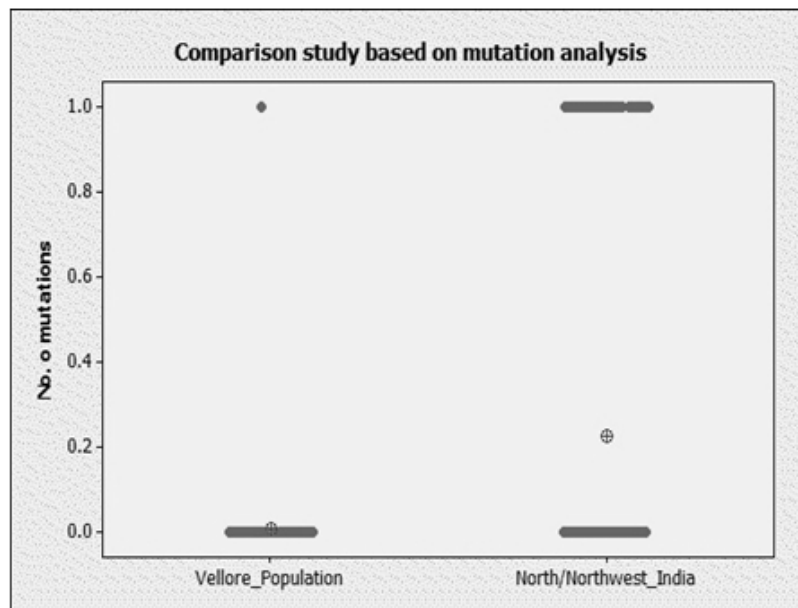


Figure V
Two sample t-test for comparison study between Vellore and North/Northwest population

Table IV
Comparison of mutation analysis between Vellore and North/Northwest Indian population²¹

Population	N	Mean	Std Dev	SE Mean
Vellore	119	0.0084	0.0917	0.0084
North/Northwest_India ²¹	235	0.226	0.419	0.027

Table IV Two sample t-test for Vellore Population vs. North/Northwest Indian population²¹

DISCUSSION

Asthma is a serious global health problem affecting all the age groups, where genetic polymorphisms along with environmental constrains inclines the risk of disease manifestation. The demographic study was performed to identify the environmental influences on asthma occurrence. Dust, food, strong odor, animals, alcohol, mold, pollen, climate, chemical, tobacco smoke and wood smoke were the major risk factors considered in this study. All the risk factors were selected based on reported literatures.^{18,27-36} Alcohol though was not much cited, we included it as a risk factor in triggering asthma phenotype, as it might cause somatic gene alterations.¹⁵⁻¹⁷ Nasal symptoms, shortness of breath, sneezing, wheezing, coughing and other allergic reactions (like cold, itching, eye irritations, skin problems, eczema) were the major symptoms evaluated.³⁷ This study revealed most of the patients were exposed to two to three of the considered risk factors and medical symptoms. Risk calculation for occupational asthma in this study identified patients with sedentary lifestyle to be prone to asthma predisposition as also reported in earlier literatures.^{19,38} Molecular study revealed an alteration in the MCP3 +563C/T intron which is the precise mutation reported in the North/Northwest Indian population too (n=235) but, the presence of genetic polymorphism was found to be less significant in this study population.²¹ However,

similar study done on Korean (n=598), African Americans (n=50) and Caucasian (n=50) population has also not reported any significant genetic evidence of MCP3 associated asthma predisposition.⁸ This indicates that a larger sample size to be included or any other genes might be responsible for the disease occurrence. In addition, in this study, the proband (F/51Y) with asthma phenotype was found to have MCP3 (+563C/T) mutation, whereas her son being affected with the same disease phenotype was not identified with any such alterations. This also indicates a large number of family members to be screened for the mutation in order to identify the pattern of inheritance or which might otherwise confer an unlikely mode of inheritance for this multifactorial disease.¹

CONCLUSION

This study highlights an association between environmental risk factors and asthma with the presence of several clinical symptoms. It also reports a genetic mutation in MCP3 (+563C/T) second intron that was the first of its kind found in Vellore and hence more samples are needed to confirm or refute our findings which will aid to find the genetic association. In view of genetic polymorphism being positive in only one subject, the statistical significance between the symptoms, occupation and polymorphism was not analyzed for this study population.

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CONFLICT OF INTEREST

Declared none.

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