GST M1 AND T1 GENE VARIANTS DEFICIENCY WITH PASSIVE MATERNAL SMOKING DURING PREGNANCY MAY INCREASE ASTHMA RISK IN INDIAN CHILDREN

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ABSTRACT

It has been evident that Glutathione S-transferase (GST) gene deficiency may contribute to the development of asthma. Therefore, we aimed to assess the association of GSTT1 and GSTM1 deficiency with passive maternal smoking (MS) during pregnancy with asthma in north Indian children. It was a hospital based case-control study, 175 bronchial asthma cases and 175 controls, aged 1 year -15 years were recruited. Cases included were those children presenting symptoms of asthma according to EPR 2007 and excluded were subjects with other respiratory diseases. Non asthmatic children were enrolled as controls. Increased risk of asthma was found in children lacking the GST M1 (OR= 6.37, 95%CI= 1.29-31.42, p= 0.024) and GST T1 (OR= 4.84, 95%CI= 1.68-13.71, p= 0.003) gene variants whose mother's were exposed to smoking during pregnancy. We concluded that the GST gene variants(GSTT1 and GSTM1) deficiency with passive maternal smoking during pregnancy may increase asthma risk in North Indian children.

Keywords: *Glutathione, Asthma, Polymorphism, Children*

No. of Tables: 4 **No. of References: 24**

INTRODUCTION

Bronchial asthma is a complex inflammatory disorder characterized by reversible airflow obstruction. Adrian (2012),both genetic and environmental factors are associated with asthma pathogenesis. Overall, it affects nearly 155 million people worldwide. Hoffjan& Ober., (2002) In India, the prevalence of asthma in adults is 2.38% Aggarwal et al., (2006)and in children it varies from 6 to 31%.ISAAC Steering Committee,(1998); Paramesh.,(2002). Glutathione-S-transferase (GST) enzymes, which play an important role in detoxification processes, may therefore influence risk of asthma. Genetic studies of the GST system have mainly focused on GSTM1 and GSTT1gene variants that code for the enzymes GSTM and GSTT, respectively. Kabesch et al., (2004)GSTM1 is broadly studied as compared toGSTT1 because it is polymorphic with a common null allele that produces a complete lack of the enzyme. However its association with asthma development is not well established. Several studies have demonstrated an increased asthma risk in subjects with GSTM1 null genotype Ivaschenko et al., (2002), Islam et al., (2002), Sadat et al., (2004),Gilliand et al., (2002),Holla et al., (2006),Karam et al., (2012), Lima et al., (2010), Liang et al., (2013); Li et al., (2013). whereas other studies havereported no association between themPiacentini et al., (2012), Reddy et al., (2012),Piacentiniet al.,(2012). Gene environment relationship is nowadays an important issue; advances in asthma management are likely to depend on their better understanding. It is equally believed that the increase childhood asthma involve changes in specific environmental exposures among the genetically susceptible population Lee et al., (2007).Maternal smoking either passive or active during pregnancy are associated with childhood asthma Le Souëf P. N., (2000).However, gene environment relationship of GST gene with passive maternal smoking has not yet been studied in north India. Therefore, we aimed to assess the association of GST gene variants with asthma as compared to controls having passive maternal smoking exposure during pregnancy.

MATERIALS AND METHODS

Study design and setting The study group consisted of 175 asthmatic cases and 175 controls were enrolled from Department of Pediatrics, King George's Medical University, Lucknow, a tertiary care centre in northern India. All the patients were diagnosed according Expert Panel Report (EPR) 2007.(National Institute of Health 2007)The study design was approved by the institutional ethics committee (IEC). Before enrollment in the study, a written informed consent was obtained from the parent/guardian of each participant.Inclusion criteria for cases were 1-15 years with symptoms of bronchial asthma. The diagnosis of asthma was based on following criteria: (i) Current presence of wheeze with a history of more than one episode of documented wheeze or use of bronchodilator in the preceding 12 months or (ii) with first episode of wheeze along with positive family history of asthma in parents or sibling. Subjects having pneumonia, disseminated bronchiectasis, bronchiolitis, pneumo-thorax, pyothorax, immune-compromised status or malignancy were excluded. Controls included were children of age 1-15 years with no present and past history of asthma or other respiratory disease and without family history of asthma in mother, father or sibling.

Genotyping:

Genomic DNA was obtained from 2ml peripheral blood of all subjects. *GSTM1* and

GSTT1 gene variants were amplified by multiplex polymerase chain reaction (PCR), including the amplification of a-globin gene fragment used as a control of the DNA sampleArrudaet al., (2001). PCR amplification was conducted in a total volume of 15*µ*L with 20 p mol of each primer. The PCR products were then digested overnight by restriction endo-nuclease at 37◦C. Genotypes were analyzed by electrophoresis on a 2.0% agarose gel.

Data sources/measurement: Demographic, environmental and clinical and physical examination findings were recorded for all subjects included in the present study.Statistical analysis:Data was entered manually in Microsoft office excel 2007. INSTAT 3.0Wass(1998) and SPSS 11.5 (Chicago, *IL*) were used for statistical analyses.Continuous variables were expressed as mean ± S.D. Two-tailed t test was used for categorical variables. Fisher Exact test was applied to avoid type 1 error in the subgroup analyses due to the smaller sample size. P value >0.05 was considered as significant.

RESULTS AND DISCUSSION

This study was conducted from March 2013-Dec 2014. Included were 175 asthmatic cases and 175 controls. Demographical characteristics are represented in Table 1.Screening of GST gene variants (GST T1 and GST M1) was performed for all the recruited subjects. Increased risk of asthma was foundin individuals having GST T1 (OR= 4.84, 95%CI= 1.68-13.71, p= 0.003) and GST M1 (OR= 6.37, 95%CI= 1.29-31.42, p= 0.024) deficiency with passive maternalsmoking during pregnancy (Table 2). Our findings were supported by Rogers et al and Gilliand et al; both were reported an association of GSTM1 null variants with smoking exposure Rogers et al., (2009),Gilliand et al., (2002). As per best of our knowledge no data was available regarding GSTT1 gene variant association with respect to passive maternal smoking either in India or worldwide.ETS exposure is a serioushealth hazard for children; someare even more susceptible to developing asthma when having genetic variations with exposure to either passive or active smoking.GST deficiency and other genetic alterations in detoxificationpathways, yet not studied may add knowledge to the adverse healtheffects caused by smokingLee et al.,(2007).Gene environmental analysis was performed, no statistical significance was observed in children who had GSTM1 or GSTT1 null genotype and were also exposed to passive MS (tables 3 and 4). Frequency of individuals having shortness of breathing and wheeze were high as compared to fever and cough in all the groups.

In this case-control study, increased risk for asthma was found in individuals having garden/ farming field around the house. It could be due to the exposure to allergens from plants in the garden and field. However, we found no such study assessing the association of a garden/farming field around the house with asthma. We also observed preterm birth was significantly associated with asthma.The strength of our study is that the precise care was taken in clinical diagnosis of asthma and lung function test was done in Children age above than 6 years where as small sample size was the drawback.In summary, we found an increased risk of asthma in individuals having GST gene variants (GSTM1 and GSTT1) deficiency with passive maternal smoking exposure during pregnancy. Our findings support the growing literature of gene environment relationship of GST gene and exposure to smoking in asthmatics. Further studies on large population samples should be

performed for better understanding of gene environment relationship.

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Table 1:*Demographic profile of selected population*

Characteristics	Cases (n = 175)	Controls (n = 175)	P value
Age in months (mean ± SD)	74.28±40.89	75.95±36.51	0.686
Weight in kg (mean ± SD)	17.28±7.11	17.11±6.74	0.330
Height in cm (mean ± SD)	109.60±21.14	105.67±22.06	0.090
	N(%)	**N(%)**	
Sex Males	 118(67.4)	 57(32.6)	 0.734
Family Type Joint Nuclear	 104(59.4) 71(40.6)	 70(40.0) 105(60.0)	 0.131
Garden/farming field around the house	73(41.7)	101(57.7)	**0.001**
Preterm Birth	155(88.6)	19(10.9)	**0.029**
Mode of Delivery Normal Caesarean	 135(77.1) 40(32.9)	 39(22.3) 136(87.7)	 0.065
Passive Maternal Smoking	36(20.6)	139(79.4)	0.030
Current Paternal Smoking	133(76.0)	42(24.0)	0.031
Complete Immunization	87(49.7)	9(5.1)	0.507

Note- Statistically significant values are denoted in bold*

Table 2: *Association of GST T1and GST M1 gene variant deficiency in asthmatics with passive maternal smoking during pregnancy*

	Controls	Cases	OR(95%CI)p value
GST M1 **Null Present**	02(2.7) 70(97.3)	10(13.7) 62(86.3)	6.37(1.29-31.42)**0.024**
GST T1 **Null Present**	05(6.8) 34(93.2)	19(26.0) 20(74.0)	4.84(1.68-13.71)**0.003**

Table 3: *Analysis of gene environment interaction for GSTT1 genotypes versus maternal passive smoking during pregnancy in asthmatic cases*

Outcomes	GSTT1+/MPS+ (n= 27)	GSTT1+/MPS- (n= 109)	GSTT1-/MPS+ (n= 10)	GSTT1-/MPS- (n= 29)	P value
Shortness of Breathing	26(100)	97(89.0)	9(90)	28(93.3)	0.450
Wheeze	18(69.2)	88(80.7)	7(70)	26(86.7)	0.157
Fever	7(26.9)	45(41.3)	2(20)	7(87.5)	0.146
Current cough	16(61.5)	69(63.2)	8(80)	18(60)	0.703

Table 4*: Analysis of gene environment interaction for GSTM1 genotypes versus maternal passive smoking during pregnancy in asthmatic cases*

Outcomes	GSTM1+/MPS- (n=86)	GSTM1+/MPS+ (n=17)	GSTM1-/MPS- (n=53)	GSTM1-/MPS+ (n=19)	P value
Shortness of Breathing	76(89.4)	17(100)	49(90.7)	18(94.7)	0.398
Wheeze	71(83.5)	13(76.5)	43(79.6)	12(63.2)	0.286
Fever	51(60.0)	10(58.8)	36(66.7)	14(73.7)28	0.549
Current Cough	35(41.2)	05(29.4)	17(31.5)	03(15.8)	0.191

REFERENCES

Adrian, S., Chad, W., Chandrika, C., and Mario, C. Mechanisms of Remodeling in Asthmatic Airways. *Journal of Allergy***1**: 1-12,2012

Aggarwal, AN., Chaudhary, K., Chhab'ra, S.K., D'Souza, G.A., Gupta, D., Jindal, S.K., Katiyar, S.K., Asthma Epidemiology Study Group. Prevalence and risk factors for bronchial asthma in Indian adults: a multicentre study. *Indian J Chest Dis Allied Sci***48**:13–22,2006

Arruda, V.R., Lima, C.S., Grignoli, C.R., De Melo, M.B., Lorand-Metze, I., Alberto, F.L., Saad, S.T., Costa, F.F. Increased risk for acute myeloid leukaemia in individuals with glutathione S-transferase mu 1 (GSTM1) and theta 1 (GSTT1) gene defects. *Eur J Haematol*, **66:** 383-8,2001

Gilliland, F.D., Gauderman, W.J., Vora, H., Rappaport, E., Dubeau, L. Effects of glutathione-Stransferase M1, T1, and P1 on childhood lung function growth. *Am J RespirCrit Care Med*. **166**:710–716,2002

Gilliland, F.D., Li, Y.F., Dubeau, L., Berhane, K., Avol, E., McConnell, R., Gauderman, W.J., Peters, J.M. Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. *Am J RespirCrit Care Med.***166:** 457–463,2002

Hoffjan, S. and Ober, C. Present status on the genetic studies of asthma. *CurrOpinImmunol*. **14**:709–717,2002

Holla, I.L., Stejskalova, A., and Vasku, A. Polymorphisms of the GSTM1 and GSTT1 genes in patients with allergic diseases in the Czech population. *Allergy: European Journal of Allergy and Clinical Immunology*. **61:**265–267,2006

ISAAC Steering Committee. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma an7d Allergies in Childhood (ISAAC). *EurRespir J*. **12**:315–335,1998

Islam, T., Berhane, K., McConnell, R., Gauderman, W.J., Avol, E., Peters, J.M., Gilliland, F.D. Glutathione-S transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. *Thorax*. **64:**197–202,2009

Ivaschenko, T.E., Sideleva, O.G., Baranov, V.S. Glutathione- S-transferase micro and theta gene polymorphisms as new risk factors of atopic bronchial asthma. *J Mol Med***80**:39–43,2002

Kabesch, M., Hoefler, C., Carr, D., Leupold, W., Weiland, S.K., Mutius, E.V. Glutathione S transferase deficiency and passive smoking increase childhood asthma *Thorax*. **59:**569–573,2004

Karam, R.A., Pasha, F.H., El-Shal, S.A., Rahman, H.M., Gad, D.M. Impact of glutathione-S-transferase gene polymorphisms on enzyme activity, lung function and bronchial asthma susceptibility in Egyptian children. *Gene*. **497:**314–319,2012

Le Souëf, P. N. Tobacco related lung diseases begin in childhood. *Thorax*.**55**:1063–1067,2000.

Lee, Y.L., Lee, Y.C., Guo, Y.O. Associations of glutathione S-transferase P1,M1, and environmental tobacco smoke with wheezing illness in school children. *Allergy*. **62:** 641–647,2007

Li, F., Li, S., Chang, H., Nie, Y., Zeng, L., Zhang, X., Wang, Y. Quantitative assessment of the association between the GSTM1-Null genotype and the risk of childhood asthma.*Genetic Testing and Molecular Biomarkers*. **17**: 656–661,2013

Liang, S., Wei, X., Gong, C., Wei, J., Chen, Z., Chen, X., Wang, Z., Deng, J. Significant association between asthma risk and the GSTM1 and GSTT1 deletion polymorphisms: an updated meta-analysis of case-control studies, *Respirology*. **18:**774–783,2013

Lima, C.S.P., Neri, I.A., Lourenco, G.J., Faria, J.C.I., Riberio, D.J. Glutathione S-transferase mu 1 (GSTM1) and theta 1 (GSTT1) genetic polymorphisms and

atopic asthma in children from Southeastern Brazil. *Genetics and Molecular Biology*. **33:**438–441,2010

National Institute of Health – National Heart, Lung, and Blood Institute (NHLBI). Expert Panel Report 3: Guidelines for the diagnosis and management of asthma. Available online at: http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm (accessed December 31, 2009),2007

Paramesh, H. Epidemiology of asthma in India. *Indian J Pediatr***69**: 309–312,2002.

Piacentini, S., Polimanti, R., Moscatelli, B., Re, MA., Manfellotto, D., Fuciarelli, M. Lack of association between GSTM1, GSTP1, and GSTT1 gene polymorphisms and asthma in adult patients from Rome, central Italy. *Journal of Investigational Allergology and Clinical Immunology*. **22:**252–256,2012.

Piacentini, S., Verrotti, A., Polimanti, R., Giannini, C., Saccucci, P., Manfellotto, D. Functional polymorphisms of GSTA1 and GSTO2 genes associated with asthma in Italian children. *Clinical Chemistry and Laboratory Medicine*.**50**:311–315,2012.

Rogers, A., Andersen, B.C., Laza, I.I., Murphy, A., Sharma, S., Klanderman, B.,Raby, B. The interaction of GSTM1 null variants with tobacco smoke exposure and the development of childhood asthma. *ClinExp Allergy*. **39:**1721–1729, 2009.

Saadat, M., Saadat, I., Saboori, Z., Emad, A. Combination of CC16, GSTM1, and GSTT1 genetic polymorphisms is associated with asthma. *Journal of Allergy and Clinical Immunology*. **113:**996–998, 2004.

Wass, J. Software: statistics, fast and easy. *Science*. **282:** 1652, 1998.