

Variants in genes coding for Glutathione S-Transferases and asthma outcomes in children

Steve Turner MD<sup>1</sup>, Ben Francis PhD<sup>2</sup>, Nuha Wani<sup>1</sup>, Susanne Vijverberg PhD<sup>3,4</sup>,

Maria Pino-Yanes PhD<sup>5-7</sup>, Somnath Mukhopadhyay MD<sup>8,9</sup>, Roger Tavendale PhD<sup>9</sup>,

Colin Palmer PhD<sup>9</sup>, Esteban G Burchard MD MPH<sup>10,11</sup>, Simon Kebede Merid<sup>12</sup>, Erik Melén<sup>12,13</sup>,

Anke H Maitland-van der Zee PhD<sup>3,4</sup> on behalf of the Pharmacogenomics in Childhood Asthma Consortium

<sup>1</sup>Child Health, University of Aberdeen, United Kingdom

<sup>2</sup>Department of Biostatistics, University of Liverpool, Liverpool, United Kingdom

<sup>3</sup>Department of Respiratory Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

<sup>4</sup>Division of Pharmacoepidemiology and Clinical Pharmacology, University of Utrecht, Utrecht, the Netherlands

<sup>5</sup>CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain

<sup>6</sup>Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna (ULL), Santa Cruz de Tenerife, Spain

<sup>7</sup>Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna (ULL), La Laguna (Tenerife), Spain

<sup>8</sup>Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and Sussex Medical School, Brighton, United Kingdom.

<sup>9</sup>Population Pharmacogenetics Group, University of Dundee, United Kingdom

<sup>10</sup> Department of Bioengineering and Therapeutic Sciences and Medicine, University of California, San Francisco, USA

<sup>11</sup> Center for Genes, Environment and Health, University of California, San Francisco, USA

<sup>12</sup> Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

<sup>13</sup> Sachs' Children's Hospital, Södersjukhuset, Stockholm, Sweden

Short title: GST variants and asthma outcomes in children

## **ABSTRACT**

Our hypothesis was that children with mutations in genes coding for Glutathione S-transferases (GST) have worse asthma outcomes compared to children with active type genotype. Data were collected in five populations. The rs1695 single nucleotide polymorphism (*GSTP1*) was determined in all cohorts (3692 children) and *GSTM1* and *GSTT1* null genotype were determined in three (2362 children). *GSTT1* null (but not other genotypes) was associated with a minor increased risk for asthma attack and there were no significant associations between *GST* genotypes and asthma severity. Interactions between *GST* genotypes and SHS exposure or asthma severity with the study outcomes were non-significant. We find no convincing evidence that the *GST* genotypes studied are related to asthma outcomes.

## INTRODUCTION

The Glutathione S-transferases (GST) are a family of enzymes which are important for the antioxidative defences of many organs in the body including the respiratory system[1][2]. Asthma is a very common chronic respiratory condition characterised by increased oxidative stress[3,4], and in adults plasma GST concentration are elevated in asthma[5]. The oxidative burden is further increased during an acute attack of asthma[6], and this may indicate an inadequacy of host antioxidative properties.

There are polymorphisms in the genes coding for M, P and T members of the GST family which are common and may be relevant to asthma and asthma outcomes. Approximately 50% of the population is null for *GSTM1* (i.e. individuals with the null variant have no gene product), 20% is null for *GSTT1* and 15% is homozygous for the Ile-Val single nucleotide polymorphism (rs1695)[7]. The GST variants may be relevant to asthma causation[8-10] and the mechanism may involve interactions with oxidative exposures including antenatal[11,12] or postnatal[13,14] second hand smoke exposure and air exposures[15-17].

There is some evidence that GST genotype may be associated with altered risk for asthma attacks and severity of asthma is poorly understood, and that interactions with inhaled exposures may also be present[7,18]. Among asthmatic children there is evidence of interaction between GST genotype and second hand smoke (SHS) for reduced lung function[19] and between GST genotypes and ozone exposure for wheeze[20]. One study of 341 families found an association between a GST genotype and increased asthma severity score (unpublished data presented in a systematic review[8]) but this was not confirmed in two small case-control studies [21,22]. No study has linked GST genotypes to asthma attacks.

The nature of the relationship between GST genotypes and asthma outcomes is subject to methodological issues including publication bias, false positive reporting, small sample size and multiple testing and the role of genotypes in the genes coding for GST results in disease modification

is still uncertain[7]. The Pharmacogenomics In Childhood Asthma (PiCA) consortium has come together to address these methodological issues[23-25], and our aim was to use data from a number of patient populations within the PiCA consortium and to explore interactions between GST genotypes and asthma outcomes. Our hypothesis is that children with variants in the genes coding for GST are at increased odds for attacks and for more severe asthma within a population of children with asthma. In recognition of previous work[13,14], an additional hypothesis was that any association between GST genotype and asthma outcomes would be modified by exposure to second hand smoke.

## **SUBJECTS AND METHOD**

**Cohorts.** Four cohorts of children with asthma provided individual patient data (IPD) and summary statistics were provided from a fifth cohort. The four cohorts were BREATHE (no acronym), recruited from primary and secondary care in North East Scotland[19]; Pharmacogenetics of Asthma medication in Children: Medication with Anti-inflammatory effects (PACMAN), recruited from children attending community pharmacies in the Netherlands[26]; the Paediatric Asthma Gene Environment Study (PAGES), recruited from primary and secondary care across Scotland[27]; and in the Swedish population birth cohort BAMSE (Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology), asthmatic children were included[28]. Summary statistics were provided from a fifth study, the Genes-environment and Admixture in Latino Americans study (GALA II), consisting of children diagnosed with asthma in the US with four grandparents of Latino ancestry[29]. More details of the inclusion criteria for the five cohorts are presented in the supplement.

**Genotyping.** The genotypes of interest were the *GSTM1*, *GSTT1* null genotype and homozygous genotype for the substitution of isoleucine with valine at the 105 position of the gene coding for *GSTP1* (rs1695 single nucleotide polymorphism). For PACMAN, BREATHE and PAGES, saliva for DNA extraction was collected using the Oragene system (DNA Genotek ref. OG-250) and specimens were

processed following the manufacturer's protocol. *GSTM1* and *GSTT1* null mutations were determined by polymerase chain reaction and *GSTP1* polymorphism by an allelic discrimination assay as previously described<sup>6</sup>. For GALA II, DNA was extracted from whole blood before an array (Axiom<sup>®</sup> LAT1, World Array 4, Affymetrix, Santa Clara, CA) was used to determine genome wide-genotype data. In BAMSE, *GSTP1* was genotyped by matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry (Sequenom)[28].

**Definition of outcomes.** In PACMAN, the definition of attacks was reported use of oral prednisolone and/or asthma-related ER visits in the previous 12 months. For GALA II, the definition of attack was at least one of the following in the previous 12 months: oral prednisolone treatment, receiving emergency care or admission to hospital for asthma symptoms. BREATHE and PAGES used the same attack definition, which was at least one of the following in the previous six months: receipt of oral corticosteroid treatment, absence from school or hospital admission for asthma. For BAMSE an attack was defined as an asthma related emergency room and/or asthma-related hospital visit in the previous 12 months.

Asthma severity was determined by treatment step as described in the British Thoracic Society/Scottish Intercollegiate Guidelines Network [BTS/SIGN] treatment step[30]. There were very few children on step 5 treatment and a composite step 4/5 group was created.

Children with at least one parent who smoked were categorised as smoke exposed. Smoking exposure was validated by salivary cotinine in 139 participants of the PAGES cohort where median (standard error of mean) cotinine for children with no, one and two resident smokers was 0.5 ng/ml (0.04), 0.58 ng/ml (0.16) and 1.39 (0.95) respectively, Kruskal Wallance test  $p < 0.001$ [27].

**Statistical analysis and power calculation.** IPD analysis was undertaken for the PACMAN, BREATHE, PAGES and BAMSE cohorts. Age (measured in years), gender (female coded as 1 and male coded as 0), exposure to second hand smoke (SHS - coded 1 for presence of or 0 for absence of as per definitions

above), cohort of origin (PACMAN coded as 1, BREATHE coded as 2 and PAGES coded as 3). Genotypes for *GSTM1* and *GSTT1* were coded 1=null and 0=active type. For *GSTP1*, the homozygous valine genotype was coded 1 while the heterozygous and homozygous isoleucine rs1695 genotypes were combined and coded 0. To explore the effect of second hand smoke (SHS) on the relationship between genotype and attack, where associations between outcomes and genotype were significant, an interaction term between the genotype and SHS was calculated.

A logistic regression model was used to investigate the factors affecting risk of attacks. A multinomial regression model was used to investigate the relationship between BTS/SIGN treatment step (with BTS/SIGN treatment step 1 as the reference group).

To utilise all applicable genotype data within the PiCA consortium, meta-analysis of *GSTP1* data from IPD analysis of the BREATHE, PACMAN, PAGES and BAMSE populations with the GALA II population was performed using a random effects inverse variance–weighted model for the attacks outcome, where the individual study ORs are weighted by their confidence intervals. The  $I^2$  statistic was calculated to examine the heterogeneity in the population. This meta-analysis was implemented using the GWAMA software[31]. Details of the power calculation (table 1) and further details of the statistical analysis are presented in the supplement.

## **RESULTS**

### **Study subjects**

Table 1 presents details of the 3,692 individuals included in the analysis. Details of all three GST variants were available in 2197 individuals, including 983 from BREATHE, 719 from PACMAN and 495 from PAGES. Additionally, *GSTP1* details were available in 165 BAMSE and 1,330 GALA II participants.

### **Factors associated with an asthma attack**

Out of 3,692 patients, 1,178 patients (36%) experienced at least one asthma attack as per the definitions described. Individuals who were *GSTT1* null had an increased odds ratio (OR) for an asthma attack (OR 1.20 [1.03, 1.59],  $p=0.02$ ), independent of age, second hand smoke exposure and asthma severity. There was no association between *GSTM1* null (supplement table 2) or being homozygous for Val for rs1695 (supplement table 3) and risk for asthma attack.

The interaction term between *GSTT1* and exposure to SHS approached significance (OR - 1.53 [0.98, 2.38],  $p=0.06$ ) When analysed separately, individuals null for *GSTT1* were at increased risk for an asthma attack when also exposed to SHS (1.60 [1.06, 2.43]  $p=0.02$  whereas those with active *GSTT1* were not at increased risk (1.15 [0.88, 1.51]  $p=0.03$ )

There was no association between *GSTP1* genotype and risk for asthma attack in the meta-analysis. The meta-analysed OR [95% CI] was 0.99 [0.81, 1.21]. An  $I^2$  value of 0 showed no heterogeneity was present in the population. Results from each individual cohort are presented in supplement table 2.

### **Factors associated with severity**

BTS/SIGN treatment step information was available for 3,382 patients, of which 512 (15%), 1,767 (52%), 792 (24%) and 311 (9%) were being prescribed medications that categorised them into BTS/SIGN treatment steps 1, 2, 3 and 4/5 respectively. In the univariate analysis, there was a trend for *GSTM1* null genotype to be potentially associated with the odds of severity ( $p=0.08$ ), this was driven by significantly decreased odds of BTS/SIGN treatment step 2 (OR 0.79 [0.64, 0.98]  $p=0.043$ , see supplement table 4) but this association was clearly non-significant when adjusted for age and exposure to second hand smoke. The interaction between *GSTM1* and SHS exposure showed no evidence of association with severity ( $p=0.90$ ). Neither *GSTT1* ( $p=0.63$ ) or *GSTP1* ( $p=0.43$ ) genotype showed evidence of association with severity.

## DISCUSSION

There is a plausible mechanism whereby variations in the genes coding for GST could modify asthma outcomes in the context of an environment containing an increased oxidative burden (such as SHS exposure), but current understanding is restricted by methodological factors including heterogeneity between populations, power and multiple testing[7,18]. Our study has addressed many of these methodological issues by focussing on populations of children with asthma, demonstrating adequate sample size and by undertaking IPD analysis.

The first finding was that in a multivariable model, *GSTT1* null genotype was associated with an increased risk of attack, however this was of small magnitude and borderline significance. The second notable finding was that children null for *GSTM1* had a borderline significant reduction (and biologically implausible) OR for step 2 treatment in the multinomial model but in not the multivariable model. Our third and final finding was that despite SHS exposure being associated with both outcomes, there were no statistically significant interactions with *GSTT1* for asthma attacks and *GSTM1* for asthma severity. The well-recognised heterogeneity of asthma is thought to be due to disease-modification by within-population variations in genetic and environmental factors[32], but in our study the GST variants studied were not consistently linked to asthma outcomes.

There are a number of reasons why there may be no association between the GST variants studied and asthma outcomes. First, there may be no association and factors other than GST are more important to lung antioxidative properties, and publication bias may have led to the literature predominantly containing studies which find an association (either with increased or occasionally reduced[33,34] risk for asthma outcomes) whereas studies which find no association are not submitted or accepted for publication. Second, whilst a power calculation indicates that the population was of adequate size to detect an OR of 1.3 with >92% power for the variants studied, there may be a smaller effect size associated with an interaction with smoking. Third, even though the variants studied here have been linked with asthma causation and some asthma outcomes in other

studies, we may have not studied the most relevant GST variants. Finally, no standard definition of an asthma attack was applied across the five populations studied and this may have weakened any relationship between the genotypes studied and asthma attack, however despite this weakness an association was determined for *GSTT1*.

Whilst variants in the genes coding for GST are thought to be important to disease causation and modification, the underlying mechanism(s) are still not fully understood and our study suggests that the relevance of the GST variants studied to asthma attacks and severity in children is either small or none at all. Plasma GST concentrations are not different between individuals carrying active type or mutations of *GSTM1*, *GTSP1* and *GSTT1*[5,35] but concentrations within the lung correlate with rs1695 status[2] suggesting that genetic polymorphisms are important to GST activity within the lung. It is possible that the reduction in GST activity related to mutations of the three genes studied is either not sufficient to have clinical relevance for children with asthma and/or the populations studied did not experience a burden of oxidative stress sufficient to make the reduced GST activity important.

There are no studies, which are directly comparable to our findings. Rs 1695[13] and *GSTM1* null[14] genotypes have been associated with increased risk for asthma diagnosis, but we find no increased risk for adverse asthma outcomes for these variants within asthmatic populations. Three studies have identified associations between homozygous val genotype rs 1695[15,17,20] and increased risk for asthma symptoms in association with ozone exposure, and we did not record ozone exposure in our study. A third study of 43 children with asthma found that there was no difference daily peak flow measurements between those null for *GSTT1* or *GSTM1* in the context of air pollution exposure[36], and our results are consistent with these findings. A previous report from the BREATHE cohort has described reduced lung function among older children with asthma who were null for *GSTM1* and homozygous for valine for *GSTP1* and who were exposed to second hand smoke[19]; we do not report lung function outcomes in the present paper and no direct comparison can be made.

There are a number of factors which should be considered when interpreting these results. The strengths of our study include the relatively large sample size and the inclusion of more than one population. One potential limitation is that we did not adjust for ethnicity and our cohorts included children of European and Hispanic ethnicity and this introduced a small difference in the distribution of *GSTP1* genotype between populations (table 1), however in the meta analysis there was no heterogeneity in the relationship between this genotype and the outcomes studied and in the IPD for *GSTT1* and *GSTM1* the cohort was included as a covariate which will mitigate against any differences in ethnicity between populations affecting the relationship between genotype and outcome.. Second, we have focussed on only three genotypes and many genes, e.g. *NQO1*, *HMOX*, may be relevant to host antioxidative status either individually or in combination[18]. Third, SHS exposure was by report in four cohorts and thus may be unreliable, but SHS exposure was validated by salivary cotinine in the PAGES cohort[27]. Fourth, we have not related GST variants to objective markers of asthma such as lung function. Finally, in our study the exposure of interest was SHS and we did not consider other exposures, e.g. ozone[20], nitrogen dioxide[37], vitamin C[38], which might have modified the effect of SHS in genetically-susceptible individuals.

In summary, we find no evidence for there being an important association between the GST variants we have studied and adverse asthma outcomes in children. We believe that the associations between the *GSTT1* null variant and asthma attacks and *GSTM1* null variant and asthma severity are false positive findings. Our findings are based on a large number of children from more than one population, meaning that our findings may be generalisable across similar populations[39].

## ACKNOWLEDGEMENTS

Author contribution. ST conceived the idea, and with the assistance of NW wrote the first draft of the paper. ST, SM, CP, EGB, EM and AHMZ obtained funding for the collection of the data. BF undertook the analysis and assisted in the drafting of the paper. SV, MP, RT and SKM were involved in data collection and/or genetic analysis. All authors contributed to the paper.

Conflict of interest. AH Maitland-van der Zee received an unrestricted research grant for research on Pharmacogenomics in childhood asthma from Glaxo Smith Kleine. None of the other authors has a conflict of interest to declare.

## REFERENCES

1. Cantlay AM, Smith CA, Wallace WA, Yap PL, Lamb D, Harrison DJ: Heterogeneous expression and polymorphic genotype of glutathione S-transferases in human lung. *Thorax* 49, 1010-1014 (2014).
2. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA: Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 19(2), 275-280 (1998).
3. Nadeem A, Chhabra SK, Masood A, Raj HG: Increased oxidative stress and altered levels of antioxidants in asthma. *Journal of Allergy & Clinical Immunology* 111(1), 72-78 (2003).
4. Sackesen C, Ercan H, Dizdar E *et al.*: A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma. *Journal of Allergy & Clinical Immunology* 122(1), 78-85 (2008).
5. Mak JCW, Ho SP, Leung HCM *et al.*: Relationship between glutathione S-transferase gene polymorphisms and enzyme activity in Hong Kong Chinese asthmatics. *Clinical & Experimental Allergy* 37(8), 1150-1157 (2007).
6. Nadeem A, Raj HG, Chhabra SK: Increased oxidative stress in acute exacerbations of asthma. *Journal of Asthma* 42(1), 45-50 (2005).
7. Minelli C, Wei I, Sagoo G, Jarvis D, Shaheen S, Burney P: Interwild effects of antioxidant genes and air pollution on respiratory function and airway disease: a HuGE review. *Am. J. Epidemiol.* 173(6), 603-620 (2011).
8. Minelli C, Granell R, Newson R *et al.*: Glutathione-S-transferase genes and asthma phenotypes: a Human Genome Epidemiology (HuGE) systematic review and meta-analysis including unpublished data. *Int. J. Epidemiol.* 39(2), 539-562 (2010).
9. Liang S, Wei X, Gong C *et al.*: Significant association between asthma risk and the GSTM1 and GSTT1 deletion polymorphisms: an updated meta-analysis of case-control studies. *Respirology* 18(5), 774-783 (2013).
10. Piacentini S, Polimanti R, Simonelli I *et al.*: Glutathione S-transferase polymorphisms, asthma susceptibility and confounding variables: a meta-analysis. *Mol. Biol. Rep.* 40(4), 3299-3313 (2013).
11. Gilliland FD, Li Y, Dubeau L *et al.*: Effects of Glutathione S-Transferase M1, Maternal Smoking during Pregnancy, and Environmental Tobacco Smoke on Asthma and Wheezing in Children. *American Journal of Respiratory & Critical Care Medicine* 166(4), 457-463 (2002).
12. Murdzoska J, Devadason SG, Khoo S *et al.*: In utero smoke exposure and role of maternal and infant glutathione s-transferase genes on airway responsiveness and lung function in infancy. *American Journal of Respiratory & Critical Care Medicine* 181(1), 64-71 (2010).
13. Lee YL, Lee YC, Guo YL: Associations of glutathione S-transferase P1, M1, and environmental tobacco smoke with wheezing illness in school children. *Allergy* 62(6), 641-647 (2007).

14. Kabesch M, Hoefler C, Carr D, Leupold W, Weiland SK, von Mutius E: Glutathione S transferase deficiency and passive smoking increase childhood asthma. *Thorax* 59(7), 569-573 (2004).
15. Hwang B, Young L, Tsai C *et al.*: Fine particle, ozone exposure, and asthma/wheezing: effect modification by glutathione S-transferase P1 polymorphisms. *PLoS ONE [Electronic Resource]* 8(1), e52715 (2013).
16. Bowatte G, Lodge CJ, Knibbs LD *et al.*: Traffic-related air pollution exposure is associated with allergic sensitization, asthma, and poor lung function in middle age. *Journal of Allergy & Clinical Immunology* 139(1), 122-129.e1 (2017).
17. Su MW, Tsai CH, Tung KY *et al.*: GSTP1 is a hub gene for gene-air pollution interactions on childhood asthma. *Allergy* 68(12), 1614-1617 (2013).
18. Yang IA, Fong KM, Zimmerman PV, Holgate ST, Holloway JW: Genetic susceptibility to the respiratory effects of air pollution. *Thorax* 63(6), 555-563 (2008).
19. Palmer CNA, Doney ASF, Lee SP *et al.*: Glutathione S-transferase M1 and P1 genotype, passive smoking, and peak expiratory flow in asthma. *Pediatrics* 118(2), 710-716 (2006).
20. Romieu I, Ramirez-Aguilar M, Sienna-Monge JJ *et al.*: GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. *European Respiratory Journal* 28(5), 953-959 (2006).
21. El Rifai N, Moustafa N, Degheidy N, Wilson M: Glutathione S transferase theta1 and mu1 gene polymorphisms and phenotypic expression of asthma in Egyptian children: a case-control study. *Italian Journal of Pediatrics* 40(1), 22 (2014).
22. Mahmouda MI, Hassem HS, Wahab NH, Saad AA, Moez P: The association between glutathione S-transferase P1 polymorphisms and asthma in Egyptians. *Alexandra J Med* 47, 105-115 (2011).
23. Vijverberg SJ, Tavendale R, Leusink M *et al.*: Pharmacogenetic analysis of GLCCI1 in three north European pediatric asthma populations with a reported use of inhaled corticosteroids. *Pharmacogenomics* 15(6), 799-806 (2014).
24. Vijverberg S, Korster ES, Tavendale R *et al.*: ST 13 polymorphisms and their effect on exacerbations in steroid-treated asthmatic children and young adults. *Clin Exp All* 45, 1051-9 (2015).
25. Turner S, Francis B, Vijverberg S *et al.*: Childhood asthma exacerbations and the Arg16  $\beta$ 2-receptor polymorphism: a meta-analysis stratified by treatment. *J Allergy Clin Immunol* DOI: 10.1016/j.jaci.2015.10.045 (2016).
26. Zuurhout MJ, Vijverberg SJ, Raaijmakers JA *et al.*: Arg16 ADRB2 genotype increases the risk of asthma exacerbation in children with a reported use of long-acting beta2-agonists: results of the PACMAN cohort. *Pharmacogenomics* 14(16), 1965-1971 (2013).
27. Turner SW, Ayres JG, Macfarlane TV *et al.*: A methodology to establish a database to study gene environment interactions for childhood asthma. *BMC Medical Research Methodology* 10, 107 (2010).
28. Melen E, Nyberg F, Lindgren CM *et al.*: Interactions between glutathione S-transferase P1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. *Environ. Health Perspect.* 116(8), 1077-1084 (2008).

29. Pino-Yanes M, Thakur N, Gignoux CR *et al.*: Genetic ancestry influences asthma susceptibility and lung function among Latinos. *Journal of Allergy & Clinical Immunology* 135(1), 228-235 (2015).
30. British Thoracic Society and Scottish Intercollegiate Guidelines Network.: BTS/SIGN guideline on Asthma Management 2012. [https://www.brit-thoracic.org.uk/document-library/clinical-information/asthma/btssign-guideline-on-the-management-of-asthma/2014\(06/12\)](https://www.brit-thoracic.org.uk/document-library/clinical-information/asthma/btssign-guideline-on-the-management-of-asthma/2014(06/12)).
31. Magi R, Morris AP: GWAMA: software for genome-wide association meta-analysis. *BMC Bioinformatics* 11, 288 (2010).
32. Borish L, Culp JA: Asthma: a syndrome composed of heterogeneous diseases. *Annals of Allergy, Asthma, & Immunology* 101(1), 1-8 (2008).
33. Carroll WD, Lenney W, Jones PW *et al.*: Effects of glutathione S-transferase M1, T1 and P1 on lung function in asthmatic families. *Clinical & Experimental Allergy* 35(9), 1155-1161 (2005).
34. Fryer AA, Bianco A, Hepple M, Jones PW, Strange RC, Spiteri MA: Polymorphism at the glutathione S-transferase GSTP1 locus. A new marker for bronchial hyperresponsiveness and asthma. *American Journal of Respiratory & Critical Care Medicine* 161(5), 1437-1442 (2000).
35. Chan-Yeung M, Ho SP, Cheung AHK *et al.*: Polymorphisms of glutathione S-transferase genes and functional activity in smokers with or without COPD. *International Journal of Tuberculosis & Lung Disease* 11(5), 508-514 (2007).
36. Hong Y, Hwang S, Kim JH *et al.*: Metals in particulate pollutants affect peak expiratory flow of schoolchildren. *Environ. Health Perspect.* 115(3), 430-434 (2007).
37. Gref A, Kebede MS, Gruziova O *et al.*: Genome-wide Interaction Analysis of Air Pollution Exposure and Childhood Asthma with Functional Follow-up. *Am J Respir Crit Care Med* DOI 10.1164/rccm.201605-1026OC (2016).
38. Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M *et al.*: Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *American Journal of Respiratory & Critical Care Medicine* 166(5), 703-709 (2002).
39. Hall IP, Blakey JD: Genetic association studies in Thorax. *Thorax* 60(5), 357-359 (2005).