

# **Polymorphisms of *Glutathione-S-transferase* genes and asthma: a HuGE systematic review and meta-analysis including unpublished data**

*Protocol for a HuGE association review*

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*Potential conflict of interest*

None.

## Background

Oxidative stress is thought to play a key role in the pathogenesis of asthma<sup>1</sup>, and the antioxidant glutathione is critical to protecting the lungs against oxidant damage. Consequently there has been interest in associations between glutathione-S-transferase (GST) polymorphisms and risk of asthma. A common gene deletion polymorphism of *GSTM1* has been suggested as a susceptibility gene for asthma<sup>2-4</sup> and reduced lung function growth<sup>5</sup> in children. Other studies have examined the relationship of *GSTM1* and *GSTT1* to asthma but reported conflicting findings<sup>2;6-9</sup>. Data on *GSTP1* and asthma are also conflicting. One study reported an association of Val105 with increased risk of asthma<sup>4</sup>. In contrast, Val105 has been reported by one group to be highly protective for asthma, atopy and airway responsiveness in adults<sup>10</sup>, and others have reported no association between *GSTP1* and asthma<sup>2</sup>. However, many of these studies were relatively small or confined to selected populations, and preferential publication of positive studies may have occurred. While a systematic review can help understand the reasons for the heterogeneity of study results, pooling of the results across similar studies in a meta-analysis can overcome the problem of limited statistical power.

Although a meta-analysis on *GST* genes and asthma has recently been published in the Pakistan Journal of Biological Sciences<sup>11</sup>, it only evaluated *GSTM1* and *GSTT1*.

Our aim is to perform an up-dated meta-analysis on asthma risk associated with *GSTM1*, *GSTT1* and *GSTP1*, which would also include new unpublished data on the association of the three genes with asthma phenotypes in approximately 6,000 children and their mothers in the Avon Longitudinal Study of Parents and Children (ALSPAC), a population-based birth cohort. Further unpublished data are also available from 341 UK Caucasian families with at least two affected sibs with asthma<sup>12</sup>.

## Methods

### **Criteria for study inclusion**

#### *Polymorphisms*

*GSTT1*, *GSTM1* and *GSTP1* gene variants

#### *Clinical outcomes*

- Primary outcome: asthma
- Secondary outcomes: wheezing; bronchial hyper-responsiveness; lung function; atopy; total IgE; eczema

#### *Types of studies*

Population-based genetic association studies and family studies, in children and adults

## Search methods

- Electronic search of: MEDLINE, EMBASE, ISI Science Citation Index, HuGENet
- Cross-checking of reference lists
- Direct contact with authors of primary studies and experts in the field to identify other or unpublished studies

## Data extraction

Two reviewers (CM and RG) will independently extract data using a pre-piloted form, with a third reviewer available for arbitration (JWH).

## Quality assessment

Criteria for the evaluation of study quality will be based on the HuGENet guidelines for the reporting of genetic association studies<sup>13</sup>. No quality weights will be used in the meta-analysis models, but sensitivity analyses will be performed to assess the possible influence of quality on the pooled results.

## Data synthesis

The data will be pooled with random effects models using per-genotype odds ratios.

In the absence of *a priori* evidence on what the underlying genetic model might be for the polymorphisms being investigated, we will use a “genetic model-free” approach<sup>14</sup> to pool the results across genotype groups. This approach does not assume a specific genetic model but estimates it from the data, with the only assumption that the unknown genetic model is constant across studies. However, if there is evidence from the data that the genetic model varies across studies, then joint pairwise comparisons of the three genotype groups will be performed, using a bivariate meta-analysis model. The joint modelling of the two risks (mutant homozygotes vs. wild homozygotes, and heterozygotes vs. wild homozygotes), which accounts for their correlation, tends to increase the precision of the two pooled estimates compared with separate meta-analyses. For *GSTT1* and *GSTM1* genes, the majority of the studies have evaluated the polymorphisms only as presence/absence of homozygous deletions. This means that the pooled risk estimate may have to be expressed as a single odds ratio, although the unpublished data from the large ALSPAC cohort does provide information on the three genotype groups.

Between-study heterogeneity will be investigated using the  $Q$  test and the  $I^2$  statistic. The possible presence of publication bias will be assessed graphically using funnel plots, and its asymmetry will be formally evaluated using Begg’s and Egger’s tests.

For each study, deviation from Hardy-Weinberg equilibrium (HWE) will be tested using the exact test, and the magnitude of the departure measured using the inbreeding coefficient. Studies with statically significant and/or large deviations from HWE will be further investigated for methodological problems. However, they will only be excluded from the analysis in the presence of evidence of such problems, including population stratification and genotyping errors<sup>15</sup>.



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