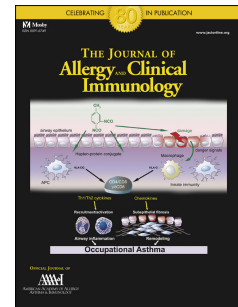


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Traffic-related air pollution exposure is associated with allergic sensitization, asthma and poor lung function in middle age

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Title: Traffic-related air pollution exposure is associated with allergic sensitization, asthma and poor lung function in middle age.

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Declaration and conflict of interest: We declare that we have participated in the conception and design, or analysis and interpretation of data and drafting the article or revising it critically for improvement intellectual content. We have all seen and approved of the final version. We also declare that we have no conflict of interest in connection with this paper.

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Abstract

Background: Traffic-related air pollution (TRAP) exposure is associated with allergic airway diseases and reduced lung function in children, but evidence concerning adults, especially in low pollution settings, is scarce and inconsistent.

Objectives: To determine if exposure to TRAP in middle age is associated with allergic sensitization, current asthma and reduced lung function in adults, and whether these associations are modified by variants in *Glutathione S-Transferase* genes.

Methods: The study sample comprised the 5th decade follow-up of the Tasmanian Longitudinal Health Study. Mean annual residential nitrogen dioxide (NO₂) exposure was estimated for current residential addresses using a validated land-use regression model. Associations between TRAP exposure and allergic sensitization, lung function, current wheeze and asthma (n = 1,405) were investigated using regression models.

Results: Increased mean annual NO₂ exposure was associated with increased risk of atopy (aOR 1.14; 95% CI 1.02, 1.28 per one IQR increase in NO₂ [2.2 ppb]) and current wheeze (aOR 1.14; 1.02, 1.28). Similarly, living < 200 m from a major road was associated with current wheeze (1.38; 1.06, 1.80) and atopy (1.26; 0.99, 1.62), and was also associated with having significantly lower pre- and post-BD FEV₁ and pre BD FEF_{25-75%}. We found evidence of interactions between living <200m from a major road and *GSTT1* polymorphism for atopy, asthma and atopic asthma. Overall, carriers of the *GSTT1* null genotype had an increased risk of asthma and allergic outcomes if exposed to TRAP.

Conclusion: Even relatively low TRAP exposures confer an increased risk of adverse respiratory and allergic outcomes in genetically susceptible individuals.

Capsule summary: This study provides evidence that even very low levels of ambient air pollution exposure is associated with allergic sensitization, asthma and lower levels of lung function in middle age adults. These elevated risks are pronounced in carriers of *GSTT1* null.

Key Messages: Relatively low levels of traffic-related air pollution exposure is associated with increased risk of allergic sensitization, asthma and lower levels of lung function, and carriers of *GSTT1* null may be at a greater risk for these outcomes.

Key words: Air pollution; allergic sensitization; asthma; *Glutathione S-Transferase* genes; and respiratory function

Abbreviations: DAG, Directed Acyclic Graph; DEP, Diesel Exhaust Particles; *GSTs*, *Glutathione S-Transferase* genes; *GSTT1*, *Glutathione S-Transferase theta1*; *GSTM1*, *Glutathione S-Transferase mu1*; *GSTP1*, *Glutathione S-Transferase pi1*; HDM, House Dust Mite; LUR, Land-Use Regression;

- 33 PAH, Polycyclic Aromatic Hydrocarbons; ROS, Reactive Oxygen Species; SPT, Skin Prick Test;
34 TAHS, Tasmanian Longitudinal Health Study; TRAP, Traffic-Related Air Pollution

Introduction

While numerous studies have demonstrated associations between Traffic-Related Air Pollution (TRAP) exposure and childhood allergic respiratory diseases (1, 2), few have investigated this association in adults. Most adult studies investigating associations between TRAP and allergic respiratory outcomes have been based on time series analyses assessing the effect of short-term exposure to higher levels of air pollution on acute exacerbations of asthma or asthma ED/hospital admissions (3). Further, little is known about regions with low levels of TRAP exposure (below the National Air Quality standards) and risk of adult allergic respiratory diseases. Recent reviews, based on a limited number of population studies investigating TRAP exposure and adult allergic respiratory diseases (4) and lung function have highlighted inconsistent results (3, 4). These inconsistencies may be due to a range of factors, including differing genetic susceptibility to the effects of TRAP between study populations.

Air pollution-induced asthma has recently been identified as an important phenotype, although the mechanisms behind air pollution induced asthma onset and exacerbations remain unclear (8). TRAP exposure has been suggested to cause oxidative damage to the airways, leading to inflammation, remodelling and increased risk of allergic sensitization (4). Previous findings from both animal and *in vitro* experiments have shown that TRAP can enhance allergic reactions (4, 5). Although allergic sensitization is known to be a strong risk factor for asthma, there is no consistent evidence that TRAP exposure is associated with allergic asthma.

The constituents of TRAP, including nitrogen dioxide (NO₂) and particulate matter, are strong oxidants (6, 7). They cause oxidative stress in exposed individuals by producing reactive oxygen species (ROS) (8). Under ideal circumstances ROS are buffered by the endogenous antioxidant system (9), which includes enzymes produced by *Glutathione S-Transferase* genes (*GSTs*). Variants of the *Glutathione S-Transferase pi1*, *mu1* and *theta1* (*GSTP1*, *GSTM1* and *GSTT1*, respectively) genes are of considerable interest, given that these genes code for phase II detoxification enzymes, which play an important role in modulating inflammatory responses and are triggered by ROS (10). Previous studies, conducted predominantly in children, have demonstrated that carriers of the *GSTM1* null variant and/or *GSTP1* variant are at increased risk of allergic diseases when exposed to TRAP (11). Very few adult studies have reported on the interaction between polymorphisms in the *GST* genes and TRAP exposure on the risk of asthma, reduced lung function and allergic sensitization (11).

We sought to quantify the association between current TRAP exposure and allergic sensitization, asthma and lung function in adults using data from Tasmanian Longitudinal Health Study (TAHS). We also aimed to determine whether these associations are modified by variants in the *GSTT1*, *GSTM1* and *GSTP1* genes.

Methods

Study population

The study sample consisted of participants in TAHS. The design of this study has been previously described (12). Subjects included in this analysis were adults who participated in the TAHS 5th decade laboratory study. Briefly, TAHS started in 1968 by recruiting 8,583 Tasmanian children aged seven years. Participants of the baseline survey were known as ‘proband’ s. At the baseline survey, parents of the children completed a respiratory health questionnaire and probands underwent clinical examination and lung function measurements. Subsequent follow-up surveys were completed in 1974, 1981 and 1992 at the ages of 13, 20 and 31 years, respectively. In 2004, when the probands were 44 years old, 7,562 (88.1%) participants of the original 1968 cohort were invited to participate in a postal survey and responses were obtained for 5,729 (78.4%) participants (12). On the basis of participation in previous follow-ups and history of asthma, a subgroup of participants (n = 2,387) was invited to undergo a detailed laboratory study, which was undertaken between 2005 and 2008 (5th decade laboratory study). At the laboratory visit, participants underwent lung function tests, Skin Prick Tests (SPTs) for allergens and provided blood samples. In addition to laboratory tests, participants completed a detailed interviewer-administered questionnaire. Of the 2,387 probands invited, 1,405 (59.2%) participated in the laboratory study, while 1,396 (58.9%) had valid SPTs (Figure 1). The 5th decade follow-up study was approved by the Human Research Ethics Committee at the University of Melbourne (HREC No. 040375). Written informed consent was provided by all participants when they attended the laboratory.

Exposure assessment

Address information was collected from all participants who attended the laboratory study (n = 1,405) and were successfully geocoded for 1,367 (97.3%). The distance from participants’ residences to the nearest major road was calculated in ArcGIS 10.1 (ArcGIS 10.1, Redlands, CA: Environmental Systems Research Institute). Major roads were defined as Public Sector Mapping Agencies (PSMA) Australia transport hierarchy code 301 and 302 for states of Victoria, Tasmania, Queensland and New South Wales. Major roads included roads carrying ‘massive traffic’, categorised as freeways, highways, arterial roads and sub-arterial roads (13). Participants were classified as living in close proximity to a major road if their residential address was < 200m in straight-line distance from a major road. This cut-point was chosen based on the known rate of decay observed in levels of major traffic pollutants downwind (14). A satellite-based Land-Use Regression (LUR) model was used to assign mean annual exposure to NO₂ (15). Briefly, this LUR model used satellite observations of tropospheric NO₂ columns in combination with land use, roads and other predictors to estimate ground-level NO₂ across Australia. The model captured 81% of the spatial variation in annual NO₂ levels between 2006 and 2011 with a cross-validated prediction error of 19% (15). Mean annual

residential exposures to outdoor NO₂ were estimated based on the participants' geocoded addresses. The selection of proximity roads and NO₂ as TRAP markers were based on previous findings showing a strong relationship of decaying NO₂ with increasing distance from major roads (16).

Outcome Definitions

Skin Prick Tests (SPTs)

SPTs were performed for eight aeroallergens including *Dermatophagoides pteronyssinus*, cat, *Cladosporium cladosporioides*, *Alternaria tenuis*, *Penicillium mix*, *Aspergillus fumigatus*, perennial ryegrass and mixed grasses (which included Kentucky bluegrass, orchard, redtop, Timothy, sweet vernal grass and meadow fescue) (Hollister-Stier, Spokane, WA, USA) using histamine as a positive control and employing a standard technique (17). A wheal size of ≥ 3 mm more than the negative control was regarded as an indication of sensitization. Sensitization was categorized into: atopy (sensitized to any of the aeroallergens tested e.g., *Dermatophagoides pteronyssinus*, cat, *Cladosporium cladosporioides*, *Alternaria tenuis*, *Penicillium mix*, *Aspergillus fumigatus*, perennial ryegrass and mixed grasses); and sensitization to any mould allergens tested (e.g., *Cladosporium cladosporioides*, *Alternaria tenuis*, *Penicillium mix* and *Aspergillus fumigatus*).

Lung Function Tests

Spirometry was performed using the EasyOneTM Ultrasonic Spirometer (Ndd, Medizintechnik, AG, Switzerland). Detailed methods have been published elsewhere (18). Briefly, spirometry was conducted according to the joint American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines (19). Spirometry was repeated 10 minutes after 200µg of salbutamol was administered via spacer. Reference values from the Global Lung Initiative 2012 were used derive z-scores (20). Z-scores describe the deviation from the mean predicted value and are expressed as standard deviations.

Asthma Definition

Current asthma was defined as having asthma or wheezy breathing within the last year. *Current atopic asthma* was defined as asthma or wheezy breathing within the last year and sensitization to any allergen tested. *Current non-atopic asthma* was defined as asthma or wheezy breathing within the last year and not sensitized to any allergen tested. *Current wheeze* was defined as wheezing or whistling in the chest without having a cold, in the last 12 months.

Genetic data

Blood samples for genetic analysis were collected at age 44 years. Genotyping of *GSTM1* and *GSTT1* was performed using a multiplex PCR technique (21), and for all experiments positive primers for

beta-globin were included as a positive PCR control. Individuals were classified as either *GSTT1* null (homozygous for the *GSTT1**0 allele) or *GSTT1* non-null (homozygous or heterozygous for the *GSTT1**1 allele) and as either *GSTM1* null (homozygous for the *GSTM1**0 allele) or *GSTM1* non-null (possessing at least one functional *GSTM1* allele). A customised Illumina GoldenGate Genotyping Assay (www.illumina.com) was used to genotype the *GSTP1* (rs1695 A→G: *Ile105Val*) polymorphism. Individuals were categorized as having *GSTP1*-AA, *GSTP1*-AG or *GSTP1*-GG genotype.

Statistical analysis

The associations of both NO₂ exposure and proximity to major roads with the outcomes of allergic sensitization, asthma and wheeze were assessed using logistic regression models. The association between the exposure variables and lung function (FEV₁, FVC, FEV₁/FVC and FEV_{25-75%}) was analyzed using linear regression models. The lung function test results are normally distributed. Hence, regression with no transformation of the lung function tests has been performed. Covariates were selected for the models after considering alternative causal models using a Directed Acyclic Graph (DAG) in DAGitty software (22). Socio-economic status (defined using education), smoking status, gas cooking, gas heating, keeping windows open more than 1 hour per week and rural or urban status (using Accessibility/Remoteness Index of Australia 2006) were included in the regression models. In all models, NO₂ exposure was entered as a continuous variable, while living < 200 m from a major road was treated as a dichotomous exposure.

Initially, associations were examined between the exposure variables and allergic sensitization, asthma, wheeze and lung function. To investigate the potential effect modification role by genetic polymorphisms, categorical variables of *GST* genotypes were then added as an interaction term to the regression models. For all three genes an autosomal dominant genetic model was assumed, with genotypes entered as binary variables. For interactions, $p \leq 0.10$ was considered as significant and stratified analyses were subsequently performed. All associations were assessed by including the same confounders that were identified in the initial DAG. STATA version 13.1 (Stata corporation, College Station, Texas, USA) was used to perform all the statistical analyses. Results for NO₂ exposure were scaled to an interquartile range increase (IQR) in mean annual NO₂, which in this sample was 2.2 ppb.

Results

The mean ($\pm$ SD) age of the cohort at participation was 44.8 ± 1 years and 49% were male (Table 1). Over half (55.8%) of the cohort was sensitized to at least one of the aeroallergens tested. Sensitization to house dust mite (HDM) was the most prevalent type of allergic sensitization (41.3%), followed by perennial rye grass (32.0%) and mixed grasses (30.7%). The prevalences of current asthma and wheeze were 23.6% and 28.5%, respectively. Study characteristics of the participants without a geocoded residential address (2.7%) were similar to those of participants who had geocoded residential address, other than slight difference in gender (male gender = 63%) (data not shown).

Of all participants, 27.4% lived < 200 m from a major road (Table 1). The mean annual NO_2 exposure ($\pm$ SD) of the cohort was 5.1 ppb (± 2.6). The correlation between distance to major roads and mean annual NO_2 at the home address was modest ($r = -0.40$). More than half of the participants had at least one *val* allele in *GSTP1* (60.10%), while 16.15% had a null allele in *GSTT1* (Table 1).

Main environmental effects

Each IQR increase in mean annual NO_2 exposure (2.2 ppb) was associated with increased risk of atopy (adjusted Odds Ratio [aOR] = 1.14, 95% CI: 1.02, 1.28), cat (aOR = 1.31, 95% CI: 1.15, 1.49) and HDM sensitization (aOR = 1.20, 95% CI: 1.08, 1.34). Similarly, there was a significant association between living < 200 m from a major road and HDM sensitization (aOR = 1.33, 95% CI: 1.04, 1.70), and trend towards a significant association between living < 200 m from a major road and atopy (aOR = 1.26, 95% CI: 0.99, 1.62) (Tables 2 & 3). Mean annual NO_2 exposure was associated with atopic asthma (aOR = 1.14, 95% CI: 1.00, 1.30), but not with non-atopic asthma. Both measures of exposure variables were associated with an increased risk of wheeze (NO_2 aOR = 1.14, 95% CI: 1.02, 1.28; < 200 m aOR = 1.38, 95% CI: 1.06, 1.80) (Tables 2 & 3).

Living < 200 m from a major road was associated with significant reduction in both pre- and post-BD FEV_1 and pre-BD $\text{FEF}_{25-75\%}$ (β : -0.14, 95% CI: -0.28, -0.01; β : -0.13, 95% CI: -0.26, 0.00, and; β : -0.14, 95% CI: -0.27, -0.01, respectively). Similar but non-significant trends were observed between NO_2 exposure and the same lung function outcomes (Tables 2 & 3). The ratio FEV_1/FVC was not associated with either TRAP exposure.

Main genetic effects

Gene variants in *GSTT1*, *GSTM1* or *GSTP1* were not associated with sensitization, respiratory outcomes or lung function except for one isolated finding (Online Supplement-Table 1), where carriers of *GSTM1* null had a reduced risk of cat allergen sensitization (aOR 0.70 95%CI 0.51, 0.97).

Interactions with *GSTT1*, *GSTM1* and *GSTP1*

We examined if there was any interaction between the *GST* polymorphisms and air pollution exposure assessed using either living <200m from a major road or NO₂ levels, and the results are shown in Table 4. There were significant interactions between *GSTT1* polymorphism and living <200m from a major road for several outcomes including atopy, HDM sensitization, current wheeze, current asthma and current atopic asthma. For all outcomes there was a greater risk for carriers of the *GSTT1* null. There was also trend towards significant interactions between *GSTT1* polymorphism and NO₂ levels for atopy, cat sensitization and HDM sensitization. For these outcomes there were significantly increased risks in both the *GSTT1* null and non-null groups with increasing exposure to NO₂. However, the odds ratios were higher in those with the *GSTT1* null.

No significant interaction between either *GSTM1* or *GSTP1* and either of the air pollution exposure measures was observed for any allergic sensitization or respiratory outcomes. No significant interaction between any of the *GST* polymorphisms and either of the air pollution exposure measure was observed for any of the lung function outcomes.

Discussion

Our study provides evidence that current TRAP exposure is associated with an increased risk of allergic sensitization, asthma and wheeze, and some measures of lung function in adults. The majority of our findings are consistent for both the measures related to TRAP exposure we assessed: annual mean NO₂ estimated by a satellite-based LUR model and living < 200 m from a major road. The mean annual NO₂ exposure in our study is very low compared with other TRAP exposure studies, which have been conducted only in Europe and North America (2). Nevertheless, even at very low concentrations of TRAP exposure we observed associations with allergic airway disease. Polymorphisms in *GSTT1* modified the associations between TRAP exposure and atopy, and these polymorphisms also modified the associations between distance to major roads and allergic airway disease.

Only four previous studies have investigated associations between TRAP exposure and adult allergic sensitization, all of which were conducted in Europe and North America (23-26). Only two of these reported an increased risk of allergic sensitization associated with exposure to high traffic counts (25) and/or living < 50 m from a major road (26). Exposure misclassification may have attenuated effects and contributed to the null findings in the remaining two studies, as one used self-reported traffic intensity (24) and the other used data derived from a central monitoring station (23). None of these studies looked at interactions with *GSTs*.

Australia is a country with low levels of air pollution (3) and in our study the mean annual residential NO₂ level was 5.1± 2.6 ppb, which was substantially lower than the national air quality standard (mean annual NO₂ = 30 ppb). Nevertheless, we were able to demonstrate that even low levels of TRAP are associated with allergic respiratory diseases.

The evidence from human and animal studies supports our findings of an association between current air pollution exposure and current allergic sensitization. Both animal and human challenge studies have found that the immune responses to new antigens in the presence of Diesel Exhaust Particles (DEP) are altered towards an IgE response rather than an IgG response (27). In addition, in mice, co-exposures to HDM and DEP promote a mixed T_H2/T_H17 response resulting in increased severity of allergic asthma (5). As with mice models, when humans sensitized to ragweed are exposed to DEP and ragweed, a higher ragweed-specific IgE levels and a skewed T_H2 response has been observed (28). Furthermore, when humans are exposed to components of TRAP such as Polycyclic Aromatic Hydrocarbons (PAHs) and DEP, there is a suppression of T regulatory cell (Treg) function leading to exacerbation of asthma (29, 30). Additionally, in human subjects PAHs have been shown to produce epigenetic changes resulting in suppression of Treg function and a shifting towards T_H2 responses (4, 29).

Variants of the *GSTs* that are linked with decreased oxidative capacity have been reported by both epidemiological and controlled human exposure studies to modify the association with TRAP-induced allergic diseases (11, 31). Variants in *GSTM1* and *GSTT1* are characterized by presence or absence of a homozygous allele deletion, known as null genotype. *GSTT1*, *GSTM1* and *GSTP1* act through a common mechanism involved in managing oxidative stress, where conjugating ROS with glutathione enables detoxification and ultimately protects tissues against oxidative damage (32). Past literature has shown effect modification by *GSTs* of the effect of TRAP exposure on allergic disease outcomes (33, 34). However, very few studies have investigated the effect modification by *GSTs* on the association between TRAP exposure and allergic sensitization (35, 36). All of these studies have focused on children and have investigated only interactions with *GSTP1* variants (35, 36). Our study is the first to demonstrate effect modification by *GSTs* on the association between TRAP exposure and allergic sensitization in adults.

There is evidence of interaction between *GSTT1* and some environmental pollutants on asthma and airway obstruction. A study of children aged between 9 and 11 years, reported environmental tobacco smoke exposure to be associated with prevalence of current asthma and wheeze, but only for children with *GSTT1* null deletion (37). The few studies that examined interactions between *GSTT1* variants and TRAP exposure on asthma/lung function found no evidence (11, 38). We found that *GSTT1* null carriers had a higher risk of atopy compared with carriers of *GSTT1* non-null when living <200m from a major road and that they had increased current wheeze, asthma and atopic asthma, but not with NO₂ exposure. Similar to our results, a study investigating interaction of *GSTT1* with the association of NO₂ exposure and asthma in adults found no significant effect (38). Another study investigating interaction of *GSTT1* with the association of particulate matter exposure and peak expiratory flow in children found no significant interaction (39) but its limited sample size (n = 43) may have precluded its ability to detect such associations. In support of our findings, a recent controlled human experimental study reported augmented allergen-induced allergic airway inflammation and IL-5 response when exposed to DEP for a short period of time. These effects were enhanced in individuals carrying *GSTT1* null genotype (40), supporting the hypothesis that TRAP exposure may exacerbate inflammation in genetically susceptible individuals with existing allergic conditions.

Our study has strengths and limitations. It is a cross-sectional analysis nested within a well-characterized cohort. The cohort has been well characterized with objective measures of allergy and lung function. Although frequently followed up, TRAP exposure from prior follow-ups could not be evaluated due to lack of exposure and address data, so we were unable to assess longitudinal effects on respiratory and allergic outcomes. This manuscript investigated only whether current TRAP exposure was related to current allergic sensitization, asthma, wheeze and lung function, but not whether TRAP exposure has initiated these conditions. Therefore, we cannot draw any conclusions on the role of TRAP in allergic aetiology of these outcomes. However, our results demonstrate that

current TRAP exposures may well exacerbate existing disease conditions. We used modelled NO₂ exposure from a national LUR model that allowed us to use a single model for all participants regardless of location and which has been found to predict annual NO₂ exposure with relatively low error of 19% (15). We cannot ascribe our findings to NO₂ specifically, as it is only a single proxy for the complex pollutant mixtures that characterize TRAP. Limitations of the proximity variable used in our study have been previously discussed, such as not considering wind directions, traffic volume and composition (41). The predictive ability of the NO₂ model in urban areas may be better than in rural or remote areas where the resolution and variability in predictor variables are greater (15). This could introduce exposure misclassification. On the other hand, the consistency between the results for NO₂ and living < 200 m from a major road suggest that both are good surrogates for TRAP exposure (16). In the current study, we did not correct for multiple comparisons and it is possible that at least some of the observed associations are due to type 1 error. However, we have examined only pre-suggested and biologically plausible hypotheses, and our selection of genes was based on previously reported strong associations. It is possible that we have not detected some gene and TRAP interactions due to limited power given the sample size of our study. Therefore, we highlight the overall pattern of results related to interactions, rather than individual significant associations, as evidence for modification of the association between TRAP exposure and the relevant outcomes by the *GST* gene polymorphisms.

In conclusion, TRAP exposure during middle age, even at relatively low levels of exposure, is associated with increased risk of allergic sensitization, asthma and lower levels of lung function. Carriers of *GSTT1* null may be at greater risk for these outcomes. These findings have important public health implications, particularly for individuals who are genetically susceptible. Current air quality standards in Australia may not fully protect the population. In addition, further studies should be carried out to help elucidate the biological mechanisms underpinning the relationship between *GST* polymorphisms, TRAP exposure and allergic sensitization.

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Figure Legends:

Figure 1: Overview of the Tasmanian Longitudinal Health Study (TAHS) follow-ups

457 **Table 1:** Study characteristics for participants who had a geocoded residential address (N=1367)

Variable		N
Male sex		669 (49.0%)
Socio Economic Status	Grade 1 to 9	90 (6.6%)
	Grade 10 or 12	525 (38.5%)
	Trade/ apprenticeship	489 (35.8%)
	University degree or higher	261 (19.1%)
Smoking status	Never smoked	574 (42.1%)
	Past but not current	410 (30.1%)
	Current	378 (27.8%)
Keep windows open more than 1 hour per week		1027 (75.6%)
Gas cooking		234 (17.1%)
Gas home heating		113 (8.3%)
Lived in rural areas		245 (17.9%)
Allergen sensitization	Atopy	759 (55.8%)
	cat	227 (16.7%)
	House dust mite	568 (41.3%)
	<i>Cladosporium cladosporioides</i>	63 (4.6%)
	<i>Alternaria tenuis</i>	118 (8.7%)
	<i>Penicillium mix</i>	41 (3.0%)
	<i>Aspergillus fumigatus</i>	57 (4.2%)
	Any mould	173 (12.7%)
	Mixed grass & rye grass	473 (34.8%)
Gene frequencies	<i>GSTT1</i> null	188 (16.15%)
	<i>GSTM1</i> null	635 (54.41%)
	<i>GSTP1</i> val/val+val/ile	701 (60.10%)
Respiratory outcomes	Current asthma	323 (23.6%)
	Atopic asthma*	229 (16.8%)
	Non-atopic asthma	94 (6.9%)
	Current wheeze	390 (28.5%)
Residential address <200m for a major road at time of laboratory testing		374 (27.4%)
NO ₂ exposure	NO ₂ quartile	ppb
	1 st	3.5
	2 nd	4.3
	3 rd	5.8

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 459 * Atopic asthma was defined as asthma or wheezy breathing within the last year and sensitization to
 460 any allergen tested
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Table 2: The association between mean annual NO₂ exposure at residential address and middle-age allergic sensitization, wheeze, asthma and lung function. Estimates expressed as ORs and β per 1 IQR increase in mean annual NO₂ exposure (2.2 ppb)

Type of allergen/respiratory outcomes	Unadjusted				Adjusted †			
	OR	95% CI	<i>p</i>	n/N	OR	95% CI	<i>p</i>	n/N
Atopy	1.17	1.06, 1.29	<0.01	759/1361	1.14	1.02, 1.28	0.02	748/1348
Cat allergen sensitization	1.35	1.21, 1.51	<0.01	227/1360	1.31	1.15, 1.49	0.00	221/1347
HDM sensitization	1.21	1.10, 1.34	<0.01	568/1361	1.20	1.08, 1.34	<0.01	559/1348
Any mould sensitization	1.15	1.01, 1.30	0.03	173/1361	1.11	0.96, 1.28	0.16	169/1348
Mix grass and rye sensitization	1.14	1.03, 1.25	0.01	473/1361	1.05	0.94, 1.17	0.37	463/1348
Current wheeze	1.04	0.94, 1.15	0.40	390/1367	1.14	1.02, 1.28	0.02	387/1349
Current asthma	1.09	0.99, 1.21	0.09	323/1367	1.10	0.97, 1.24	0.13	320/1349
Current non-atopic asthma*	0.95	0.77, 1.16	0.61	94/1367	0.96	0.76, 1.22	0.75	93/1349
Current atopic asthma*	1.14	1.02, 1.28	0.02	229/1367	1.14	1.00, 1.30	0.05	227/1349
Lung function	Unadjusted				Adjusted †			
	β	95% CI	<i>p</i>	n	β	95% CI	<i>p</i>	n
ZFEV ₁ preBD ^{\$}	0.00	-0.05, 0.05	0.96	1359	-0.05	-0.11, 0.01	0.09	1341
ZFEV ₁ postBD ^{\$}	0.00	-0.05, 0.05	0.88	1338	-0.05	-0.10, 0.01	0.09	1320
ZFVC preBD ^{\$}	0.02	-0.03, 0.07	0.37	1359	-0.02	-0.07, 0.03	0.47	1341
ZFVC postBD ^{\$}	0.00	-0.04, 0.04	0.99	1338	-0.04	-0.09, 0.01	0.14	1320
ZFEV ₁ /FVC preBD ^{\$}	-0.01	-0.06, 0.03	0.57	1358	-0.04	-0.09, 0.02	0.19	1340
ZFEV ₁ /FVC postBD ^{\$}	0.01	-0.04, 0.06	0.67	1337	-0.01	-0.06, 0.04	0.69	1319
ZFEF _{25-75%} preBD ^{\$}	-0.01	-0.06, 0.04	0.62	1359	-0.05	-0.10, 0.01	0.08	1341
ZFEF _{25-75%} postBD ^{\$}	0.01	-0.04, 0.06	0.74	1337	-0.03	-0.09, 0.02	0.22	1319

Adjusted †: adjusted for socio economic status, smoking status, rural/urban location, gas cooking, gas heating and open windows more than 1 hr per week. *Reference group for non-atopic and atopic asthma is never asthma.

^{\$}Values are expressed as the mean (standard deviation) unless otherwise specified (A z-score is the deviation from the mean predicted value, where 95% of normally distributed data lies between -1.96 SD and +1.96 SD)

Table 3: The association between proximity to major roads (living < 200 m from a major road) and middle-age allergic sensitization, wheeze, asthma and lung function.

Type of allergen/respiratory outcomes	Unadjusted				Adjusted †			
	OR	95% CI	p	n/N	OR	95% CI	p	n/N
Atopy	1.23	0.96, 1.56	0.10	759/1361	1.26	0.99, 1.62	0.06	748/1348
Cat allergen sensitization	1.23	0.90, 1.68	0.19	227/1360	1.28	0.93, 1.76	0.13	221/1347
HDM sensitization	1.31	1.03, 1.66	0.03	568/1361	1.33	1.04, 1.70	0.02	559/1348
Any mould sensitization	0.93	0.64, 1.33	0.68	173/1361	0.93	0.64, 1.34	0.68	169/1348
Mix grass and rye sensitization	1.10	0.86, 1.41	0.47	473/1361	1.12	0.87, 1.45	0.38	463/1348
Current wheeze	1.38	1.07, 1.78	0.01	390/1367	1.38	1.06, 1.80	0.02	387/1349
Current asthma	1.24	0.94, 1.62	0.13	323/1367	1.21	0.91, 1.59	0.19	320/1349
Current non-atopic asthma*	1.19	0.75, 1.88	0.47	94/1367	1.21	0.76, 1.94	0.42	93/1349
Current atopic asthma*	1.26	0.92, 1.72	0.15	229/1367	1.21	0.88, 1.66	0.25	227/1349
Lung function	Unadjusted				Adjusted †			
	β	95% CI	p	n	β	95% CI	p	n
ZFEV ₁ preBD ^s	-0.13	-0.26, 0.01	0.06	1359	-0.14	-0.28, -0.01	0.03	1341
ZFEV ₁ postBD ^s	-0.12	-0.24, 0.01	0.08	1338	-0.13	-0.26, 0.00	0.05	1320
ZFVC preBD ^s	-0.10	-0.22, 0.02	0.11	1359	-0.10	-0.22, 0.01	0.09	1341
ZFVC postBD ^s	-0.10	-0.21, 0.02	0.10	1338	-0.10	-0.21, 0.02	0.10	1320
ZFEV ₁ /FVC preBD ^s	-0.07	-0.19, 0.05	0.26	1358	-0.05	-0.18, 0.07	0.37	1319
ZFEV ₁ /FVC postBD ^s	-0.04	-0.16, 0.08	0.53	1337	-0.08	-0.21, 0.04	0.18	1340
ZFEF _{25-75%} preBD ^s	-0.12	-0.25, 0.00	0.06	1359	-0.14	-0.27, -0.01	0.03	1341
ZFEF _{25-75%} postBD ^s	-0.10	-0.22, 0.03	0.15	1337	-0.11	-0.24, 0.01	0.07	1319

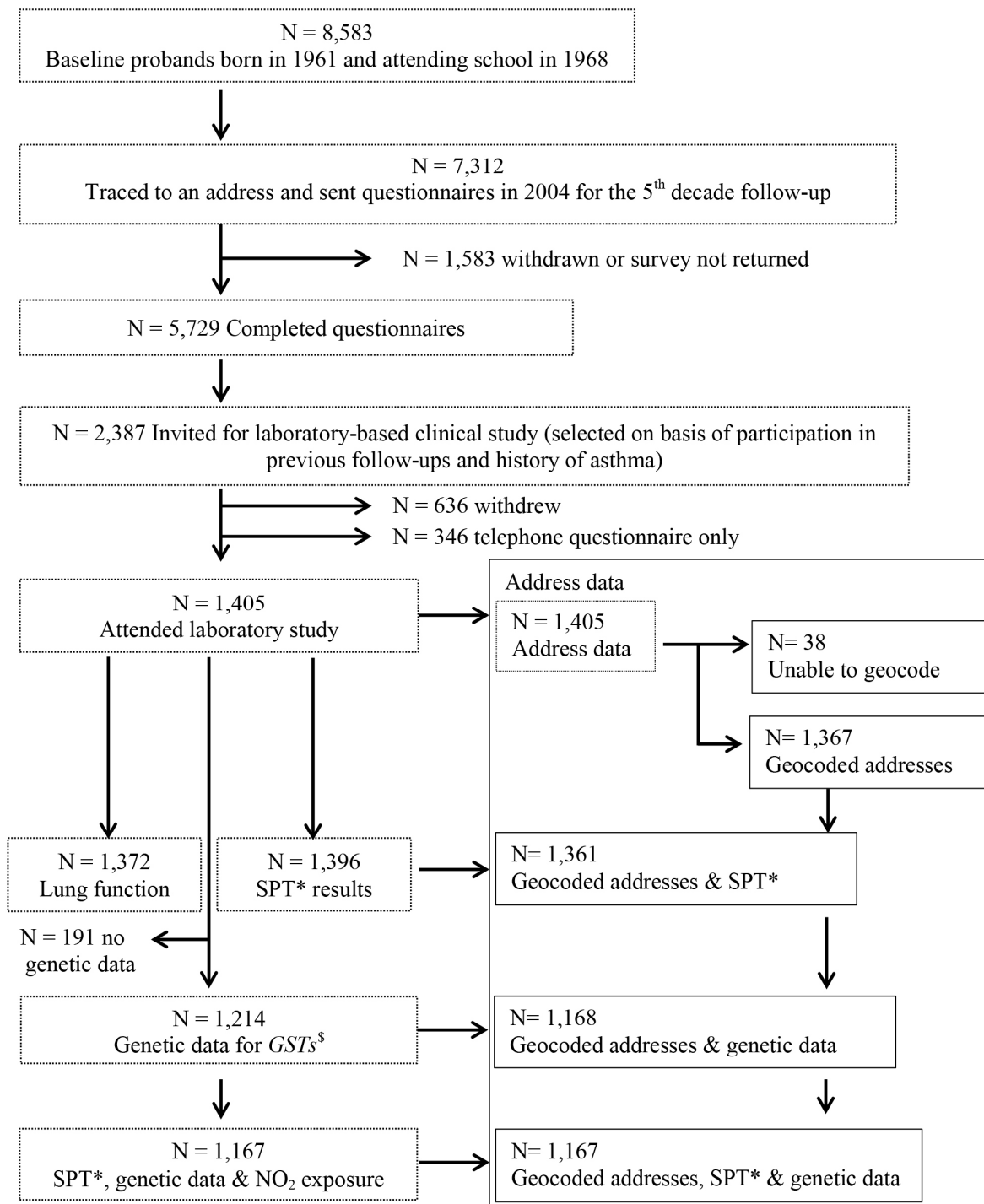
Adjusted †: adjusted for socio economic status, smoking status, rural/urban location, gas cooking, gas heating and open windows more than 1 hr per week. *Reference group for non-atopic and atopic asthma is never asthma.

^sValues are expressed as the mean (standard deviation) unless otherwise specified (A z-score is the deviation from the mean predicted value, where 95% of normally distributed data lies between -1.96 SD and +1.96 SD)

Table 4: Stratified analysis of *GSTT1* interactions for outcomes with an interaction p value ≤ 0.10 (for the associations between exposure to mean annual NO_2 and living <200m from a major road and outcomes of allergic sensitization and respiratory outcomes, results of interactions p value > 0.10 not shown). Estimates for NO_2 exposure expressed as ORs IQR increase in mean annual NO_2 exposure (2.2 ppb)

Outcomes	<i>GSTT1</i> Variant	n/N	NO_2				< 200 m			
			OR	95% CI	p	p_{int}	OR	95% CI	p	p_{int}
Atopy	non-null	535/965	1.10	0.97, 1.25	0.13	0.08	1.18	0.88, 1.58	0.27	0.04
	null	102/188	1.51	1.04, 2.20	0.03		2.66	1.30, 5.43	0.01	
Cat allergen sensitization	non-null	156/965	1.25	1.08, 1.45	<0.01	0.06	-	-	-	-
	null	28/187	1.95	1.27, 2.99	<0.01		-	-	-	
HDM sensitization	non-null	413/965	1.14	1.01, 1.29	<0.04	0.10	1.22	0.92, 1.63	0.17	0.04
	null	73/188	1.58	1.12, 2.24	<0.01		2.59	1.32, 5.05	0.01	
Current wheeze	non-null	273/963	-	-	-	-	1.18	0.85, 1.62	0.32	0.02
	null	56/190	-	-	-		3.00	1.48, 6.10	<0.01	
Current asthma	non-null	219/963	-	-	-	-	1.05	0.75, 1.48	0.77	0.01
	null	47/190	-	-	-		2.92	1.43, 5.95	<0.01	
Current atopic asthma	non-null	156/963	-	-	-	-	1.11	0.76, 1.64	0.60	0.01
	null	33/190	-	-	-		3.53	1.56, 7.98	<0.01	

Adjusted for socio economic status, smoking status, rural/urban, gas cooking, gas heating and open windows more than 1 hr per week. ORs given per IQR increase in mean annual NO_2 exposure (i.e. 2.2 ppb). N = participant who had null or normal genotype; and n = participant who had null or normal genotype and had the condition (allergic sensitization or respiratory out comes)

Figure 1: Overview of the Tasmania Longitudinal Health Study (TAHS) follow-ups

*Skin Prick Test and ^sGlutathione S-Transferase genes

S-Table 1: Associations of *GSTT1*, *GSTM1* and *GSTP1* on allergic sensitization, wheeze, asthma and lung function

Type of allergen/respiratory outcomes	<i>GSTT1</i> (reference group <i>GSTT1</i> non-null)				<i>GSTM1</i> (reference group <i>GSTM1</i> non-null)				<i>GSTP1</i> (reference group <i>GSTP1</i> ile/ile)			
	OR	95% CI	<i>p</i>	n/N	OR	95% CI	<i>p</i>	n/N	OR	95% CI	<i>p</i>	n/N
Atopy	0.94	0.69, 1.29	0.72	641/1159	0.90	0.71, 1.14	0.38	643/1162	1.00	0.79, 1.27	0.99	644/1162
Cat	0.90	0.58, 1.40	0.65	185/1158	0.70	0.51, 0.97	0.03	185/1161	0.89	0.65, 1.23	0.48	185/1161
House dust mites	0.86	0.62, 1.18	0.35	490/1159	0.99	0.78, 1.25	0.91	491/1162	1.02	0.80, 1.29	0.89	492/1162
Any mould	1.14	0.73, 1.80	0.56	148/1159	0.88	0.63, 1.25	0.50	148/1162	1.05	0.74, 1.50	0.79	148/1162
Mix grass and rye	0.87	0.62, 1.22	0.43	387/1159	0.79	0.62, 1.02	0.07	388/1162	0.96	0.75, 1.24	0.76	389/1162
Wheeze	1.09	0.77, 1.55	0.61	330/1159	0.80	0.62, 1.04	0.10	331/1162	0.94	0.72, 1.22	0.62	335/1162
Asthma	1.14	0.80, 1.64	0.47	267/1159	0.86	0.66, 1.14	0.30	268/1162	0.88	0.67, 1.17	0.39	272/1162
Non-atopic asthma	1.20	0.65, 2.21	0.56	77/1159	1.25	0.77, 2.02	0.36	77/1159	0.65	0.41, 1.04	0.08	78/1159
Atopic asthma	1.10	0.72, 1.67	0.66	189/1159	0.76	0.55, 1.04	0.08	190/1159	1.01	0.73, 1.39	0.95	193/1159
<i>Lung function</i>	<i>GSTT1</i> (reference group <i>GSTT1</i> non-null)				<i>GSTM1</i> (reference group <i>GSTM1</i> non-null)				<i>GSTP1</i> (reference group <i>GSTP1</i> ile/ile)			
	β	95% CI	P	n	β	95% CI	P	n	β	95% CI	P	n
ZFEV ₁ preBD	-0.05	-0.22, 0.12	0.55	1153	0.09	-0.04, 0.22	0.17	1156	0.03	-0.10, 0.16	0.65	1156
ZFEV ₁ postBD	-0.06	-0.22, 0.11	0.50	1137	0.07	-0.05, 0.19	0.28	1140	0.04	-0.08, 0.16	0.51	1140
ZFVC preBD	-0.07	-0.23, 0.08	0.36	1153	0.08	-0.04, 0.19	0.18	1156	0.05	-0.07, 0.16	0.44	1156
ZFVC postBD	-0.11	-0.26, 0.04	0.14	1137	0.03	-0.08, 0.14	0.61	1140	0.06	-0.05, 0.17	0.30	1140
ZFEV ₁ /FVC preBD	0.04	-0.12, 0.20	0.60	1152	0.03	-0.09, 0.15	0.62	1155	-0.04	-0.16, 0.08	0.53	1155
ZFEV ₁ /FVC postBD	0.07	-0.09, 0.22	0.40	1136	0.08	-0.03, 0.20	0.17	1139	-0.04	-0.16, 0.07	0.47	1139
ZFEF _{25-75%} PreBD	0.02	-0.14, 0.19	0.77	1153	0.04	-0.08, 0.17	0.49	1156	-0.03	-0.15, 0.10	0.65	1156
ZFEF _{25-75%} post BD	0.06	-0.11, 0.22	0.51	1136	0.12	0.00, 0.24	0.06	1139	-0.02	-0.15, 0.10	0.74	1139

Adjusted for socio economic status, smoking status, rural/urban, gas cooking, gas heating and open windows more than 1 hr per week.