Traffic-related air pollution exposure over a 5-year period is associated with increased risk of asthma and poor lung function in middle age

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TRAP is associated with increased risks of asthma and reduced lung function in middle-aged adults
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ABSTRACT Current evidence concerning the impact of exposure to traffic-related air pollution (TRAP) on adult respiratory morbidity mainly comes from cross-sectional studies. We sought to establish more robust measures of this association and potential gene–environment interactions using longitudinal data from an established cohort study.

Associations between measures of TRAP (nitrogen dioxide (NO_2) and distance to major roads) and wheeze, asthma prevalence and lung function were investigated in participants of the Tasmanian Longitudinal Health Study at 45- and 50-year follow-ups. Generalised estimating equations were used to quantify associations and the potential modifying effect of glutathione *S*-transferase gene variants.

Living <200 m from a major road was associated with increased prevalence of current asthma and wheeze, and lower lung function. The association between living <200 m from a major road and current asthma and wheeze was more marked for carriers of the *GSTT1* null and *GSTP1 val/val* or *ile/val* genotypes. Over the 5-year period, higher NO_2 exposures were associated with increased current asthma prevalence. Higher NO_2 exposure was associated with lower forced vital capacity for carriers of the *GSTT1* null genotype.

TRAP exposures were associated with increased risk of asthma, wheeze and lower lung function in middle-aged adults. The interaction with the *GSTT1* genotype suggests that deficient antioxidant mechanisms may play a role in these adverse health effects.

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Introduction

Asthma in adults is a common and important public health problem. It is a heterogeneous disease that may appear at any age and shows variable activity over time [1]. The rapidly increasing prevalence of asthma after the second half of the twentieth century strongly suggests that environmental exposures play a major role. In particular, the role of traffic-related air pollution (TRAP) exposures in exacerbating or causing asthma has attracted substantial interest [2].

The role of short-term (timescales of days to months) TRAP exposure and respiratory health in adults has been the subject of multiple investigations. However, few studies have investigated the effects of long-term (over years to decades) TRAP exposure on asthma risk [3–5] and reduced lung function [6, 7]. The studies that have been conducted have produced inconsistent results. A multicentre analysis of the European Study of Cohorts for Air Pollution Effects (ESCAPE) found no association between exposure to nitrogen dioxide (NO_2), particular matter <2.5 μm ($PM_{2.5}$) or particular matter <10 μm (PM_{10}) and longitudinal change in lung function [6]. In contrast, a US study found that living <100 m from a major road and increased $PM_{2.5}$ exposure were both associated with greater lung function decline [7]. A nationwide study of US women reported that long-term $PM_{2.5}$ exposure was associated with asthma [3]. The Swiss Study on Air Pollution and Lung Disease in Adults (SAPALDIA) found that reduction in PM_{10} over 12 years was associated with reduced wheeze [8]. Conversely, a study of Australian women similarly found no association between NO_2 and asthma [4].

It is possible that differences in genetic susceptibility may, at least in part, explain these apparently inconsistent findings. Components of TRAP, such as NO_2 and $PM_{2.5}$, cause oxidative stress and subsequent damage to the airways, leading to inflammation and lung remodelling [9]. Oxidative stress generated by exogenous sources is buffered by enzymatic and nonenzymatic antioxidants [10]. Variants of the glutathione *S*-transferase (GST) π1, μ1 and θ1 genes (*GSTP1*, *GSTM1* and *GSTT1*, respectively) are of considerable interest, given that these genes control enzymes involved in the regulation of oxidative stress by detoxifying reactive oxygen species (ROS) [11]. Our previous cross-sectional study found that mean annual NO_2 exposure increased the risk of current asthma, wheeze and allergic sensitisation, and that these effects were modified by *GSTT1* polymorphisms, with null carriers at increased risk [12]. SAPALDIA found that decreased PM_{10} exposure over 11 years was associated with an attenuated decline of forced expiratory flow at 25–75% of forced vital capacity (FVC) in carriers of *GSTP1 val/val*. However, they did not find significant interactions with *GSTM1*, *GSTT1* or other lung function outcomes [13].

In this study, our aim was to investigate the effects of TRAP exposure measured using NO_2 and living closer to major roads in two follow-ups over a 5-year period on the prevalence of middle-age current asthma, current wheeze and lung function measured in both follow-ups. We also investigated whether these associations were modified by GST gene polymorphisms.

Methods

Study population

The study sample consisted of participants in the 45- and 50-year follow-ups of the Tasmanian Longitudinal Health Study (TAHS) [14–16]. Briefly, the TAHS included children born in 1961 (n=8583) and schooled in Tasmania, Australia in 1968. In 2002–2005, the majority of the cohort (67%) was retraced and resurveyed. Respondents to this survey who had either participated in past follow-up studies and/or reported symptoms of asthma or cough were invited to participate in a laboratory study in 2005–2008

(n=2387). Of those invited, 1397 (58.6%) took part in a full laboratory visit and completed a questionnaire, and 354 (14.8%) completed a telephone questionnaire or laboratory visit only. All participants who took part in the full laboratory study (n=1397) were invited for another laboratory study during 2010 and 2012. Of those, 794 (56.8%) took part in a full laboratory visit and completed a questionnaire, and 43 (3.1%) completed a telephone questionnaire or laboratory visit only. Although the TAHS started in the state of Tasmania, the study participants are now distributed throughout Australia [16]. The mean±SD age of participants was 44.8±1 and 49.6±0.6 years at the 2005–2008 and 2010–2012 follow-ups, respectively. We investigated the association between TRAP exposure and respiratory health of participants who completed both the 2005–2008 study (referred to as the 45-year follow-up) and the 2010–2012 study (referred to as the 50-year follow-up). Addresses were successfully geocoded for 780 out of the 837 (93.2%) eligible participants. The 45- and 50-year follow-ups were approved by the Human Research Ethics Committee of the University of Melbourne, Melbourne, Australia (040375.1). Written informed consent was provided by all participants.

TRAP exposure assessment

Living <200 m from a major road

The distance from each participant's residence to the nearest major road at age 45 and 50 years was calculated using ArcGIS version 10.1 (Environmental Systems Research Institute, Redlands, CA, USA). Major roads were defined using Australian transport hierarchy codes supplied by the Public Sector Mapping Agencies [17]. Codes 301 and 302 were used to identify major roads in the states where participants lived (Victoria, Tasmania, Queensland and New South Wales). Participants were categorised into two groups based on the observation that most components of TRAP decay towards near-background levels within ~200 m downwind of major roads [18]: 1) living <200 m and 2) living >200 m from a major road.

NO_2 exposure

Mean annual NO_2 exposures were assigned using a satellite-based land-use regression (LUR) model [19]. Briefly, this LUR model estimated mean annual NO_2 levels based on tropospheric NO_2 columns observed by satellite in combination with other predictors, such as land use and roads, to estimate ground-level NO_2 across Australia. The previously published satellite-based LUR model used in this study was internally cross-validated using 68 monitoring sites across Australia, including all states and territories (apart from the sparsely populated Northern Territory). The model captured 81% of spatial variability in annual NO_2, with a cross-validated prediction error of 19% [19]. We also conducted an external validation for our LUR model; it captured 66% of annual NO_2 at a completely independent set of 98 urban background and near-traffic validation sites across Australia. Mean prediction bias was low (−0.2 ppb) and prediction error was comparable to our initial cross-validation results (19% *versus* 25% in the original and validated results, respectively) [12]. The spatial resolution of our LUR model was variable, as the LUR predictors also varied in resolution. In practice, this resulted in an approximate resolution of 150–200 m in urban areas and 1 km in nonurban areas. The spatial variability of NO_2 is greater in urban areas and ~80% of the TAHS participants in this study lived in "significant urban areas", as defined by the Australian Bureau of Statistics [20]. Mean annual residential exposures to outdoor NO_2 at age 45 and 50 years were estimated and assigned separately based on participants' geocoded addresses. Therefore, each participant had separate annual average NO_2 values at age 45 and 50 years. Consequently, the exposure (mean NO_2 in the year leading up to respiratory measurement) was measured and recorded before the outcome at both time-points.

Pre-bronchodilator spirometry

Pre-bronchodilator spirometry at both time-points was performed with the EasyOne Ultrasonic Spirometer (ndd Medizintechnik, Zurich, Switzerland) using the same methods. Spirometry was conducted according to the guidelines of the American Thoracic Society and European Respiratory Society [21]. Subsequently, Global Lung Initiative 2012 reference values were used to derive z-scores [22]. The z-scores represent the deviation from the age-, sex-, height- and race-adjusted population mean in standard deviation units.

Definitions of current asthma and wheeze

Current asthma at 45 and 50 years was defined as having asthma or wheezy breathing within the last 12 months. Current wheeze at both follow-ups was defined as wheezing or whistling in the chest without having had a cold in the last 12 months.

Genetic data

Blood samples for genetic analysis were collected at the 45-year follow-up. Genotypes of *GSTM1* null and *GSTT1* null were detected using a multiplex PCR technique [23], and for all experiments positive primers

for β-globin were included as a positive PCR control. Individuals were categorised for *GSTT1* as either *GSTT1* null (homozygous for the *GSTT1**0 allele) or *GSTT1* non-null (homozygous or heterozygous for the *GSTT1**1 allele). For *GSTM1*, individuals were categorised as either *GSTM1* null (homozygous for the *GSTM1**0 allele) or *GSTM1* non-null (possessing at least one functional *GSTM1* allele). A customised GoldenGate genotyping assay (Illumina, San Diego, CA, USA) was used to genotype the *GSTP1* (rs1695 A→G: Ile105Val) polymorphism. Individuals were categorised by genotypes as *GSTP1*-AA (*ile*/*ile*), *GSTP1*-AG (*ile*/*val*) or *GSTP1*-GG (*val*/*val*).

Statistical analysis

TRAP exposure and outcomes at age 45 and 50 years

We investigated associations between NO_2 exposure and living <200 m from a major road at both age 45 and 50 years (selected based on availability of both exposure and outcome data for this cohort) and outcomes measured at the same ages using a multivariable logistic regression model. Since most participants contributed data at two occasions, we fitted the model using generalised estimating equations (GEEs) with an exchangeable correlation structure to account for nonindependence of outcome data due to repeated measures. More formally, the GEE approach assumes a linear relationship between the log odds of the outcome and exposures. However, it uses a "working" correlation matrix to re-weight the contribution of observations during the model-fitting and estimation process to reflect, in this case, the paired nature of the data and to ensure that correct standard errors are obtained. Assuming independence of observations will understate the true standard errors since, due to within-person correlation of outcomes, two sets of measurements on each of n participants has less information than a single set of measurements on each of 2×n participants.

We analysed both z-scores of lung function and raw lung function adjusted for age, sex and height.

In this analysis, we only focused on participants who lived at the same address at both time-points (referred to as "nonmovers"), which included >90% (n=709) of participants. A sensitivity analysis of those who moved (referred to as "movers") showed negligible variation in estimated effects (data not shown).

GST interactions

To investigate potential effect modification by genetic polymorphisms, categorical variables representing GST genotypes were added as interaction terms to the GEE models. For all three genes, an autosomal dominant genetic model was assumed, with genotypes entered as binary variables. Interactions with $p \leqslant 0.10$ were considered significant and stratified analyses were subsequently performed.

In all models, NO_2 exposure was entered as a continuous variable and living <200 m from a major road as a binary variable. Results for categorical outcomes were reported as odds ratios with 95% confidence intervals. The results for NO_2 exposure were scaled to an interquartile range (IQR) increase in mean annual NO_2 at the 50-year follow-up, which in this sample was 2.4 ppb. A directed acyclic graph produced using DAGitty software [24] was constructed to identify possible confounders. This showed what adjustment was required in the regression models in order for the estimated measures of association to have a causal interpretation (under the assumption that the resulting model was correctly specified). Socioeconomic status (defined using educational attainment), smoking status, type of cooking (gas and electric), type of heating (wood/coal, gas room heating and other (electric, ducted heating and reverse cycle air conditioning)) and rural or urban location (using the accessibility/remoteness index of Australia 2006 [21]) were included in the models. The same confounders that were included in the main models were adjusted for in the interaction models. In a sensitivity analysis we included sampling weights and all the other cofounding variables described previously in the model to test whether the findings were influenced by the sampling. Sampling weights were derived by calculating the inverse probability of selection for the 45-year follow-up. All statistical analyses were performed using Stata version 13.1 (StataCorp, College Station, TX, USA).

Results

Study characteristics were, for the most part, similar between participants in the 45- and 50-year follow-ups, with the exception that those in the 50-year follow-up were less likely to be current smokers and less likely to have asthma (table 1). In this cohort, participants used a number of different methods for cooking and heating. At age 50 years, the use of wood/coal for heating was reduced by nearly 10% compared with age 45 years and the use of "clean methods for heating" (*i.e.* electric, ducted heating and reverse cycle air conditioning) increased by 12.4%. From age 45 to 50 years, the use of gas for cooking increased by 6.6% (table 1).

Of the 837 who participated in both the 45- and 50-year follow-ups, 768 (91.2%) had geocoded address data at the two time-points (figure 1). Of those, a similar proportion lived <200 m from a major road at

both follow-ups (table 2). The mean difference between NO_2 exposure in participants living <200 *versus* >200 m from a major road was statistically significant, while the participants living <200 m from a major road had a higher exposure. Similar significant differences were observed at both age 45 and 50 years (*t*-test, p<0.0001) (supplementary table S2).

Effect of NO_2 exposure and living <200 m from a major road from age 45 to 50 years on current asthma, current wheeze and lung function

In the GEE models, living <200 m from a major road and exposure to an IQR increase in average annual NO_2 exposure (*i.e.* 2.4 ppb) was associated with increased current asthma prevalence (adjusted OR (aOR) 1.49, 95% CI 1.09–2.05 and aOR 1.10, 95% CI 0.96–1.27, respectively). Similar results were observed for living <200 m from a major road and increased current wheeze prevalence (aOR 1.61, 95% CI 1.19–2.19). Living <200 m from a major road was also associated with lower forced expiratory volume in 1 s (FEV_1) (β −0.32, 95% CI −0.49–−0.15), FVC (β −0.20, 95% CI −0.35–−0.05) and FEV_1/FVC (β −0.19, 95% CI −0.34–−0.04) (table 3). We observed similar associations with raw lung function adjusted for age, sex and height (supplementary table S1). These findings remained similar in the sensitivity analysis that adjusted for sampling weights (supplementary table S2).

Influence of GST gene polymorphisms on the relationship between TRAP exposure from age 45 to 50 years and current asthma, current wheeze and lung function

Polymorphisms of *GSTT1* were found to modify the associations between TRAP exposure from age 45 to 50 years and respiratory outcomes. NO_2 exposure from age 45 to 50 years was associated with a significant reduction in FEV_1 and a trend towards reduction in FVC only for *GSTT1* null carriers ($p_{interaction}$=0.01 and 0.05, respectively).

TABLE 1 Study characteristics of the 709 participants who had a geocoded residential address at age 45 and 50 years

	Age 45 years	Age 50 years
Male	345 (48.7)	345 (48.7)
Socioeconomic status (education)		
Grade 1–9	38 (5.4)	38 (5.4)
Grade 10 or 12	264 (37.3)	264 (37.3)
Trade/apprenticeship	252 (35.6)	252 (35.6)
University degree or higher	153 (21.6)	153 (21.6)
Smoking status		
Never-smoker	318 (45)	309 (43.9)
Ex-smoker	226 (31.9)	266 (37.8)
Current smoker	163 (23.1)	129 (18.3)
Type of cooking		
Gas	141 (20.1)	185 (26.7)
Electric	562 (79.9)	507 (73.3)
Type of heating		
Wood/coal	320 (45.4)	243 (34.5)
Gas room heater	60 (8.5)	49 (7.1)
Other	325 (46.1)	412 (58.4)
Lived in rural areas	80 (11.3)	80 (11.3)
Respiratory outcomes		
Current asthma	187 (27.1)	164 (23.4)
Current wheeze	209 (30.0)	202 (28.9)
Gene frequencies		
GSTT1 null	94 (15.5)	94 (15.5)
GSTM1 null	338 (55.7)	338 (55.7)
GSTP1 val/val+val/ile	370 (60.1)	370 (60.1)
Lung function		
FEV_1	−0.36±1.08	−0.37±1.12
FVC	0.02±1.01	−0.062±1.00
FEV_1/FVC	−0.63±1.03	−0.56±0.93

Data are presented as n (%) or mean±SD. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity.

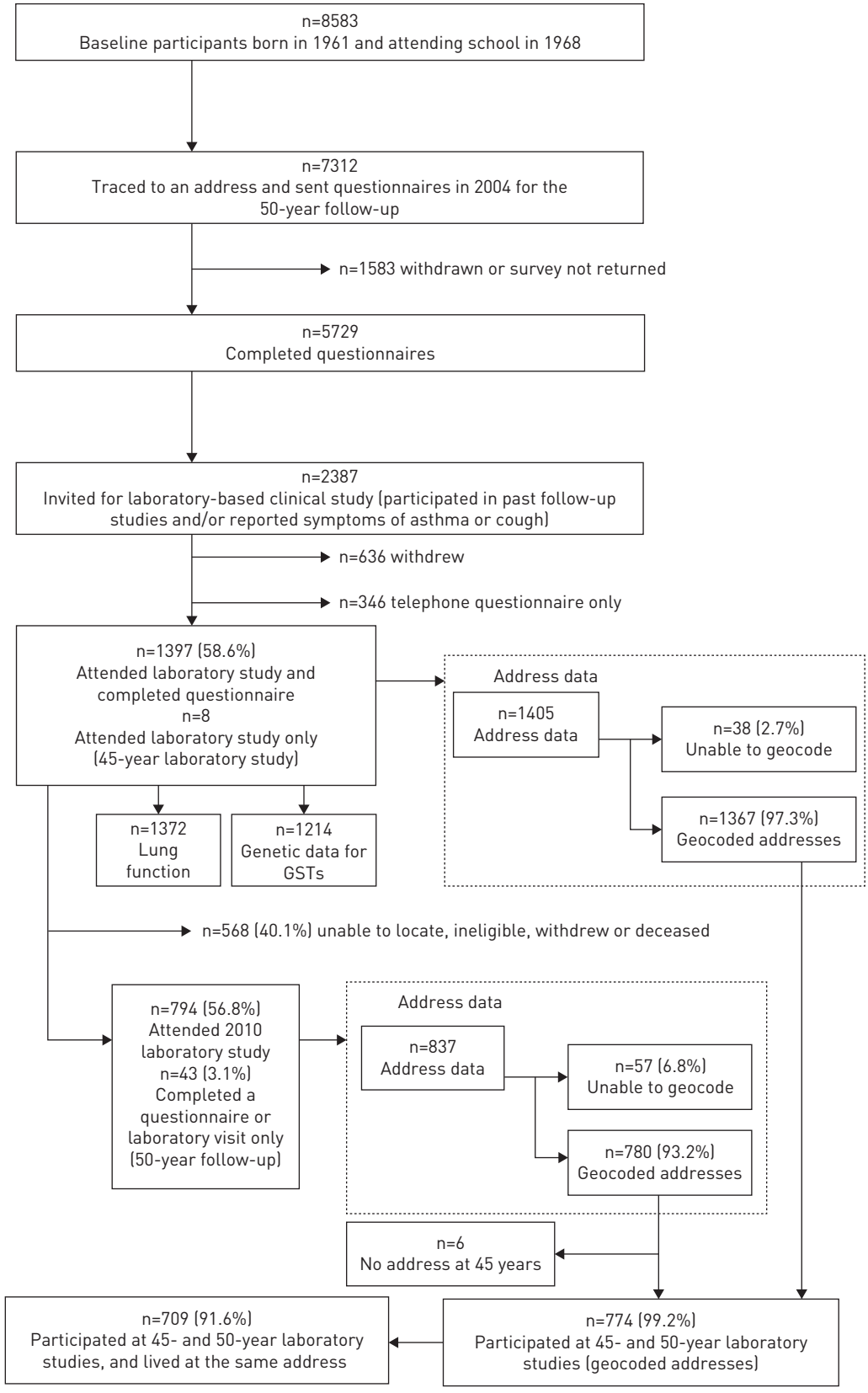

FIGURE 1 Overview of the Tasmania Longitudinal Health Study follow-ups [16].

TABLE 2 Traffic-related air pollution exposure variables for the 709 participants who had a geocoded residential address at age 45 and 50 years

	Age 45 years	Age 50 years
Residential address <200 m from a major road	198 (28.0)	196 (27.64)
Mean annual nitrogen dioxide exposure ppb		
Quartile 1	3.7	3.1
Quartile 2	4.6	3.9
Quartile 1	6.1	5.4
Mean±SD	5.4±2.8	4.6±2.6
Lowest	2.5	1.8
Highest	23.8	22.9

Data are presented as n (%), unless otherwise stated.

GSTT1 polymorphisms were found to significantly modify the association between living <200 m from a major road and risk of current asthma and wheeze. The associations between living <200 m from a major road and increased risk of current asthma and wheeze were more marked in carriers of the *GSTT1* null genotype (aOR 2.84, 95% CI 1.15–7.01 and aOR 3.19, 95% CI 1.15–8.77, respectively).

GSTP1 showed a significant interaction with NO_2 exposure from age 45 to 50 years and current asthma and wheeze prevalence ($p_{interaction}$=0.06 and 0.02, respectively), with carriers of *GSTP1 val/val or ile/val* having an increased current asthma prevalence when exposed to elevated levels of NO_2. There was an unexpected significant interaction observed for *GSTM1* in the association between living <200 m from a major road and FEV_1 ($p_{interaction}$=0.06), with *GSTM1* non-null carriers being at increased risk of lower FEV_1 (table 4).

Discussion

Our study provides evidence that long-term TRAP exposure, as measured by NO_2 and living <200 m from a major road, is associated with current asthma. Additionally, living <200 m from a major road over this same period is associated with a lower FEV_1, FVC and FEV_1/FVC in adults aged 45–50 years. *GSTT1* and *GSTP1* polymorphisms modify the effects of living <200 m on the prevalence of current asthma and wheeze, and also modify the effect of changes in NO_2 exposure on FVC, with *GSTT1* null genotype carriers at an increased risk.

TABLE 3 Effect of traffic-related air pollution exposure (nitrogen dioxide (NO_2) exposure and living <200 m from a major road from age 45 to 50 years) on current asthma, current wheeze and lung function (restricted to the 709 nonmovers)

	Unadjusted		Adjusted[#]		Unadjusted		Adjusted[#]	
	OR (95% CI)	p-value	OR (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
NO_2 exposure								
Current asthma	1.07 (0.95–1.21)	0.26	1.10 (0.96–1.27)	0.15				
Current wheeze	0.98 (0.87–1.11)	0.78	1.05 (0.91–1.20)	0.50				
$zFEV_1$[¶]					0.00 (−0.06–0.06)	0.98	−0.02 (−0.09–0.04)	0.47
zFVC[¶]					0.03 (−0.03–0.09)	0.28	0.00 (−0.06–0.07)	0.91
$zFEV_1$/FVC[¶]					−0.04 (−0.10–0.01)	0.14	−0.05 (−0.11–0.02)	0.14
Living <200 m from a major road								
Current asthma	1.48 (1.08–2.02)	0.01	1.49 (1.09–2.05)	0.01				
Current wheeze	1.53 (1.13–2.05)	0.01	1.61 (1.19–2.19)	<0.01				
$zFEV_1$[¶]					−0.27 (−0.44–−0.10)	<0.01	−0.32 (−0.49–−0.15)	<0.01
zFVC[¶]					−0.16 (−0.32–−0.01)	0.04	−0.20 (−0.35–−0.05)	0.01
$zFEV_1$/FVC[¶]					−0.16 (−0.31–−0.01)	0.03	−0.19 (−0.34–−0.04)	0.01

Odds ratio and β are given per interquartile range increase in mean annual NO_2 exposure (*i.e.* 2.4 ppb). FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity. [#]: adjusted for socioeconomic status, smoking status, rural/urban location, type of cooking and type of heating; [¶]: z-scores ($zFEV_1$, zFVC, $zFEV_1$/FVC) represent the deviation from the age-, sex-, height- and race-adjusted population mean in standard deviation units. p-values ⩽0.05 considered significant.

TABLE 4 Associations between traffic-related air pollution exposure (nitrogen dioxide (NO_2) exposure and <200 m from a major road from age 45 to 50 years) and current asthma and current wheeze and lung function, stratified by glutathione *S*-transferase genotype (restricted to the 709 nonmovers)

Respiratory outcome	Gene variation	$p_{interaction}$-value	OR/β[#] (95% CI)	p-value[¶]
NO_2 exposure				
zFEV1[+]	*GSTT1* null	0.05	−0.12 (−0.41–0.02)	0.06
	GSTT1 non-null		0.01 (−0.06–0.09)	0.70
zFVC[+]	*GSTT1* null	0.01	−0.21 (−0.42– −0.01)	0.04
	GSTT1 non-null		0.04 (−0.03–0.12)	0.22
Asthma	*GSTP1 ile/ile*	0.06	0.91 (0.71–1.15)	0.42
	GSTP1 val/val+ile/val		1.21 (1.00–1.46)	0.05
Wheeze	*GSTP1 ile/ile*	0.02	0.82 (0.64–1.05)	0.11
	GSTP1 val/val+ile/val		1.18 (0.97–1.42)	0.10
Living <200 m from a major road				
Asthma	*GSTT1* null	0.09	2.84 (1.15–7.01)	0.02
	GSTT1 non-null		1.40 (0.95–2.05)	0.09
Wheeze	*GSTT1* null	0.10	3.19 (1.15–8.77)	0.02
	GSTT1 non-null		1.44 (1.00–2.08)	0.05
zFEV1[+]	*GSTM1* null	0.06	−0.11 (−0.35–0.14)	0.41
	GSTM1 non-null		−0.44 (−0.71– −0.17)	<0.01

Odds ratio and β are given per interquartile range increase in mean annual NO_2 exposure (*i.e.* 2.4 ppb). FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity. [#]: adjusted for socioeconomic status, smoking status, rural/urban location, type of cooking and type of heating; [¶]: $p_{interaction} \leqslant 0.10$ (for the associations between living over a 5-year period <200 m from a major road and outcomes of asthma and wheeze; results of $p_{interaction} > 0.10$ not shown); [+]: z-scores (zFEV1, zFVC) represent the deviation from the age-, sex-, height- and race-adjusted population mean in standard deviation units. p-values ⩽0.05 considered significant.

Australia is a continent with relatively low levels of air pollution [25] and the current national air quality standard for annual mean NO_2 is 30 ppb [26]. In our current study, the maximum annual mean NO_2 exposure for participants over the two time-points was estimated at 23.8 and 22.9 ppb at age 45 and 50 years, respectively. The mean±SD NO_2 exposure at age 45 and 50 years for the study population was 5.4 ±2.6 and 4.6±2.6 ppb. Our results indicate that long-term exposure to low levels of NO_2, even levels well below the Australian standards, was associated with increased risk of respiratory diseases. In our previous cross-sectional study, we showed that annual NO_2 exposure or living <200 m from a major road was associated with current asthma, wheeze, allergic sensitisation and lower levels of lung function, and we also found significant interactions with *GSTT1* [27]. This adds to the body of evidence showing that both short- and long-term TRAP exposures, even at low levels of pollution, contribute to adverse effects on health. This is especially the case for genetically susceptible populations.

We observed associations with low NO_2 exposure and asthma. There is evidence from other countries that low levels of NO_2 are associated with respiratory diseases. For example, the SAPALDIA study found a mean annual average of 18.92 ppb (35.6 $\mu g \cdot m^{-3}$) associated with wheeze in smokers [5]. The ESCAPE study, combining six European cohorts, found positive associations between NO_2 exposure and incidence of asthma in adults, where mean annual NO_2 concentrations ranged from 11.6 to 16.4 ppb [28]. It has been shown that air pollution exposure and adverse health outcomes follow a linear dose–response relationship. Therefore, adverse health outcomes associated with lower air pollution levels are to be expected.

Our finding of an association between residing closer to a major road and reduced lung function confirms findings from the Framingham study and a German study on the influence of air pollution on lung function, inflammation and ageing in women (SALIA) [7, 29]. We did not see similar effects with long-term NO_2 exposure, whereas the ESCAPE, SALIA and SAPALDIA studies all reported that this exposure was associated with reduced lung function [6, 29, 30]. The null findings in our study may be due to consistently low levels of NO_2 exposure over the 5-year period (mean±SD NO_2 5.0±2.6 ppb). The three aforementioned studies reported a mean NO_2 exposure between 12 and 20 ppb, which was more than double that observed in our study. Additionally, their larger sample sizes, compared with ours, may have provided more power to detect associations.

There is evidence from human and animal toxicology studies that release of ROS triggered by air pollution exposure is an important step in the pathway leading to oxidative damage and inflammation [31]. However, the biological mechanisms behind long-term air pollution exposure and deteriorating adult lung

function have not been well characterised in humans. Experimental studies provide evidence that short-term exposure to NO_2 and particulate matter can induce endogenous release of ROS in exposed lung epithelial cells. This leads to allergic responses, pulmonary inflammation and pulmonary remodelling [32–34].

Variants of the *GSTP1*, *GSTM1* and *GSTT1* genes act through a common mechanism, protecting exposed tissues from the oxidative damage that may be caused by ROS, by conjugating them with glutathione [11]. Our study is the first to investigate whether GSTs modify the association between long-term air pollution exposure and respiratory or lung function outcomes. Previously, using a cross-sectional study, we showed that living <200 m from a major road for a short period of time was associated with increased risk of current asthma and wheeze for carriers of *GSTT1* null [12]. In the current analysis, we were able to show similar results with asthma and wheeze for those who lived <200 m from a major road.

A recent controlled chamber study of human subjects demonstrated that short-term exposure to diesel exhaust particles (DEPs) augmented allergen-induced markers of both allergic and nonallergic inflammation [34]. The authors found that having *GSTT1* null enhanced those end-points [34]. In our analysis, we did not have data on DEPs, but previous literature has shown that both NO_2 and distance to major roads are proxies for the complex mixture represented by TRAP, which includes DEPs [18]. Thus, similar types of mechanisms are expected to be involved in TRAP exposure and airway inflammation for *GSTT1* null carriers. For the *GSTP1* gene, the identification of the risk allele is not clear. Some studies have shown that risk increases for those with the *ile* allele, while others have shown that *val* allele increases the risk [13, 35]. Similarly, previous studies have shown that *GSTM1* non-null carriers have an increased risk of respiratory diseases [27, 36]. Apart from GSTs, polymorphisms in other oxidative stress genes have been shown to interact with the association of TRAP exposure and asthma. For example, a study by Castro-Giner *et al.* [37] showed that polymorphisms of *NQO1* (NADPH quinone oxidoreductase 1) or *TNFA* (tumour necrosis factor-α) were associated with increased susceptibility to NO_2 exposure and asthma in adults.

Our study has both strengths and limitations. The TAHS is a well-characterised longitudinal study including repeated measures of lung function and prospectively collected data on a range of risk factors. The average follow-up period for the current analysis was 5 years. Similar studies investigating long-term air pollution exposure have used longer follow-ups [3, 7]. Additionally, in our cohort, address data was collected at age 45 and 50 years, but not the years in between. This may lead to exposure misclassification of long-term exposures, but few participants had moved and NO_2 exposure was very similar between movers and nonmovers. We used modelled NO_2 exposure from a LUR model for all participants regardless of the location. The LUR model has been found to predict annual NO_2 exposure with a relatively low error of 19% [19]. However, we cannot ascribe our findings to NO_2 specifically as it is only one proxy for TRAP. Previous studies investigating long-term TRAP exposure have used various methods to define "long-term exposure". Lazarevic *et al.* [4] used 3-year average NO_2 to investigate asthma symptom prevalence. A study combining six European cohorts estimated NO_2 exposures using a LUR model at 2010 or 2011, although some of the cohorts included in this study were initiated well before the air pollution modelling was conducted [28]. Similarly, three studies (a nationwide cohort of US women, a longitudinal study of the European Respiratory Health Survey and Respiratory Health in Northern Europe (RHINE)) used annual average NO_2 exposure in a single year [3, 38, 39]. In all these studies, exposure measurements were inconsistent when examining long-term exposures. In our analysis we assigned NO_2 exposure separately at two follow-ups, representing actual time-points where the outcome data were collected.

Using distance to major roads as a proxy for TRAP exposure also has limitations. Living closer to a major road alone does not account for complex factors such as wind direction, traffic volume and composition (*e.g.* heavy diesel *versus* light passenger vehicles) that can contribute to TRAP exposure in a certain geographic location. Although there are a number of limitations in using distance to major roads as a proxy for TRAP exposure, our study found consistent and significant associations with living <200 m from a major road. Previous work demonstrates that freshly emitted pollutants and resuspended particles show strong spatial gradients near major roads, with an approximately exponential decay in concentrations to background levels occurring within ~300–500 m [40]. Our selection of a 200 m distance threshold from a major road was consistent with previous studies [41, 42] and informed by the Special Report of the Health Effects Institute [43]. In this study, we observed stronger associations between our outcomes and living near a major road, compared with LUR-based estimates of NO_2. While NO_2 is a useful proxy of combustion sources, distance to a major road may better capture other important pollutants such as black carbon, fine ($PM_{2.5}$) and ultrafine particles, and volatile organic compounds as a whole mixture, even if it is a relatively crude measure of TRAP. Moreover, LUR models are not capable of distinguishing aged NO_2 compared with freshly produced NO_2 from traffic emissions.

Although we investigated long-term associations using GEE models, there is uncertainty in the degree to which exposure precedes outcomes observed in this group of adults. The longer-term associations observed might result from some cumulative exposure over time. There was a high degree of colinearity in TRAP exposures at age 45 and 50 years, and therefore it was difficult to untangle whether the observed outcomes were purely based on long-term or cross-sectional associations. It should also be noted that the current study included a subsample of the 2002–2005 follow-up, a survey of all the cohort members who were traced to an address. The selected subsample from the 2002–2005 follow-up, when participants were 45 years old, was enriched for symptoms of asthma or cough. This subsample was then invited to participate in another laboratory study when they were 50 years old. As a result, this sample has a higher prevalence of asthma. However, in our sensitivity analysis there was no difference in obtained results after controlling for sampling weights. This indicates that the observed associations did not change based on the selection of participants in this study.

In conclusion, in our study, even relatively low levels of NO_2 exposure in adults were found to be associated with increased current asthma prevalence. Similarly, living <200 m from a major road from age 45 to 50 years was found to be associated with increased risk of current asthma, current wheeze and lower lung function. Carriers of the *GSTT1* null genotype may be at a greater risk for asthma and wheeze if they live for a longer period of time closer to a major road (<200 m). This provides evidence at the population level that genetic polymorphisms associated with the antioxidant defence system are important in regulating the harmful effects of TRAP exposure. Our study adds to the existing body of evidence that even relatively low levels of TRAP exposure are associated with asthma and poor lung function in adults.

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