

Microsomal epoxide hydrolase, glutathione S-transferase P1, traffic and childhood asthma

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Asthma is the most common chronic disease in children and remains a significant public health concern. An emerging body of evidence suggests that traffic-related pollution near home increases the risk of asthma^{1 2} and reduces lung growth³ in children. In addition, in utero and second hand exposures to smoking have been associated with an increased risk of asthma in children.^{4 5} Incomplete combustion of constituents of tobacco and fossil fuels generates complex mixtures that include high levels of polyaromatic hydrocarbons (PAHs).^{1 2 4} PAH exposure, which results in oxidative stress, has been associated with asthma and wheeze.^{6–8}

Enzymes that metabolise xenobiotics appear to play a role in the pathogenesis of asthma.⁵ Although epidemiological studies have examined the associations between a spectrum of genes encoding enzymes involved in xenobiotic metabolism, such as glutathione S-transferase (GST) M1, P1 and T1,^{9–15} the contribution of another enzyme encoded by the microsomal epoxide hydrolase (EPHX1) gene to childhood asthma has not been extensively investigated.

The potential role of EPHX1 and GSTs in asthma stems from the function of the encoded enzyme in several important xenobiotic metabolic pathways and the subsequent oxidant stress-mediated tissue damage that may contribute to the pathogenesis of asthma (fig 1). EPHX1 is involved in the detoxification of reactive epoxides from metabolically activated PAHs to generate trans-dihydrodiols, whereas GSTs detoxify PAHs through formation of glutathione conjugates. In one metabolic pathway trans-dihydrodiols are metabolised to semiquinones, which may produce oxidative stress through catalytic generation of reactive oxygen species (ROS).¹⁶

Two single nucleotide polymorphisms (SNPs) in the EPHX1 gene that affect enzyme activities have been studied

Background: Microsomal epoxide hydrolase (EPHX1) metabolises xenobiotics including polyaromatic hydrocarbons (PAHs). Functional variants at this locus have been associated with respiratory diseases. The effects of EPHX1 variants may depend upon exposures from tobacco smoke and traffic emissions that contain PAHs as well as variants in other enzymes in the PAH metabolic pathway such as glutathione S-transferase (GST) genes. A study was undertaken to investigate associations of variants in EPHX1, GSTM1, GSTP1 and GSTT1 with asthma and the relationships between asthma, EPHX1 metabolic phenotypes and exposure to sources of PAHs. **Methods:** Odds ratios (ORs) and 95% confidence intervals (CIs) were computed to estimate the associations of genetic variants and exposures with asthma phenotypes using data from 3124 children from the Children's Health Study.

Results: High EPHX1 activity was associated with an increased risk for lifetime asthma (OR 1.51, 95% CI 1.14 to 1.98) which varied by GSTP1 Ile105Val genotype and by residential proximity to major roads (p for interaction = 0.006 and 0.03, respectively). Among children with GSTP1 105Val/Val genotype, those who had high EPHX1 phenotype had a fourfold (95% CI 1.97 to 8.16) increased risk of lifetime asthma than children with low/intermediate EPHX1 phenotype. Among children living within 75 metres of a major road, those with high EPHX1 activity had a 3.2-fold (95% CI 1.75 to 6.00) higher lifetime asthma risk than those with low/intermediate activity. The results were similar for current, early persistent and late onset asthma. Children with high EPHX1 phenotype, GSTP1 Val/Val genotype who lived <75 metres from a major road were at the highest asthma risk.

Conclusion: EPHX1 and GSTP1 variants contribute to the occurrence of childhood asthma and increase asthma susceptibility to exposures from major roads.

extensively. In an in vitro study, a tyrosine to histidine substitution at codon 113 in exon 3 (ie, Tyr113His) reduced enzyme activity by $\geq 50\%$ whereas a histidine to arginine substitution at codon 139 in exon 4 (ie, His139Arg) increased enzyme activity by $\geq 25\%$.¹⁷ These variant alleles, however, exerted modest effects on EPHX1 enzyme activity in in vivo studies.¹⁸ Based on the differences in EPHX1 activity by these two SNPs, EPHX1 metabolic phenotypes have been formulated using genotypic combinations.^{19 20} Among GSTs, homozygous deletion of GSTM1 and GSTT1 genes results in complete absence of enzyme activity,^{21 22} whereas an isoleucine to valine substitution at codon 105 (ie, Ile105Val) in exon 5 of the GSTP1 gene affects enzyme activity in PAH metabolism and results in increased oxidant stress.^{8 23}

Because EPHX1 and GSTs affect metabolism of potentially toxic xenobiotics such as PAHs, it is likely that the risk of asthma in children from these sources of PAH exposure may depend on a combination of EPHX1 and GST genotypes. Although an earlier study found joint effects of EPHX1 and GSTP1 on chronic obstructive pulmonary disease,²⁴ the joint effects of these genes and exposures to important sources of PAHs (smoking and traffic emissions) on the occurrence of childhood asthma is largely unknown.

We have undertaken a study to test the following three hypotheses:

Abbreviations: AKR1 aldo-keto reductase family 1, ; EPHX1, epoxide hydrolase; GST, glutathione S-transferase; PAH, polyaromatic hydrocarbon; ROS, reactive oxygen species; SNP, single nucleotide polymorphism

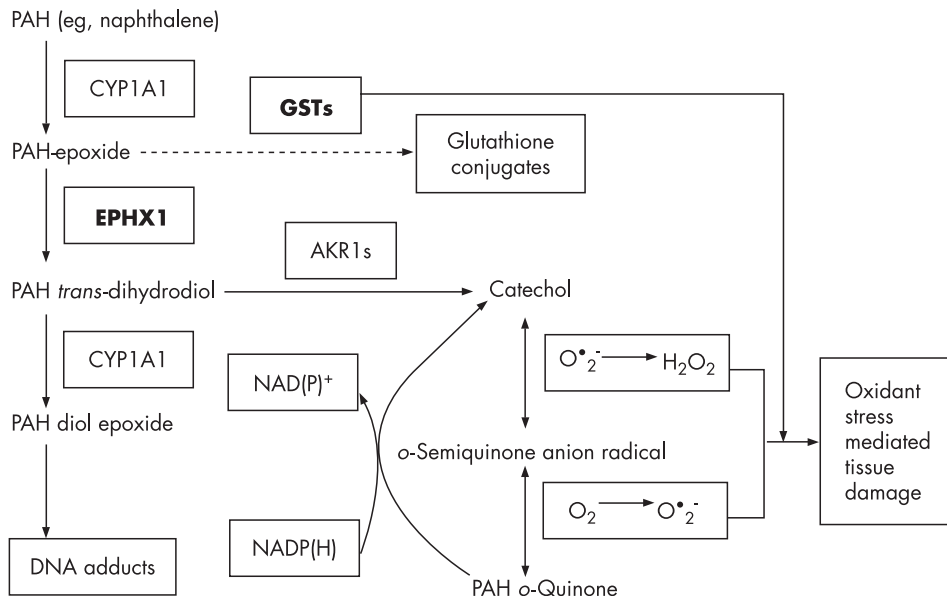


Figure 1 Genes involved in metabolism of polyaromatic hydrocarbons (PAHs) and putative mechanism of asthma occurrence from oxidant stress mediated tissue damage. Genes with functional polymorphisms that are studied are shown in bold. Glutathione S-transferase (GST) P1 105Val/Val genotype increases oxidant stress mediated tissue damage and reduces catalytic activity of the enzyme (broken arrow), resulting in an increased PAH-epoxide pool. Subsequently, higher catalytic efficiency of epoxide hydrolase (EPHX1) variants (ie, high EPHX1 phenotype) increases the formation of *trans*-dihydrodiol which subsequently undergoes redox cycling leading to the formation of reactive oxygen species. AKR1, aldo-keto reductase 1; NADP, nicotinamide adenine dinucleotide phosphate; CYP1A1, cytochrome P1A1; H₂O₂, hydrogen peroxide.

- EPHX1 polymorphisms, phenotypes and diplotypes are associated with asthma.
- Genetic variants in GSTP1, GSTM1 and GSTT1 modify the relationship of EPHX1 phenotypes with asthma.
- Associations between EPHX1 phenotype and asthma depend upon exposure to smoking (in utero and second hand smoke) and residential proximity to major roads.

METHODS

Study design and subjects

Study subjects were drawn from the larger Children's Health Study population which has been described previously.^{25, 26} The Institutional Review Board of the University of Southern California approved the study. The present analysis included 3124 non-Hispanic and Hispanic white children with known asthma status who attended schools in 12 southern California communities. Children were 4th, 7th and 10th grade students of average age 10, 13 and 16 years, respectively, and were enrolled during 1993–6. Details of the study design and subjects are given in the online supplement available at <http://thorax.bmj.com/supplemental>.

Genotyping

Details concerning buccal sample collection, processing, primer and probe information (table E1) and genotyping methods are given in the online supplement available at <http://thorax.bmj.com/supplemental>. The genotyping for the GSTP1 Ile105Val and EPHX1 Tyr113His and His139Arg was performed using the TaqMan Allelic Discrimination (AD) assay. Genotyping for the GSTT1 (null/present) and GSTM1 (null/present) genotypes were determined by real-time PCR.

Exposure assessment

Distance to the nearest major road (in metres) from each child's home was calculated with the ArcGIS 9.1 NEAR function (Redlands, California, USA). We defined road size using the TeleAtlas Geographic Data Technology (Boston, Massachusetts, USA) feature class codes (FCC) and labelled roads with an FCC code in groups A1, A2 and A3 as major roads. These codes represented primary highways with limited access, primary roads without limited access, and secondary and connecting roads, respectively. Lower classifications (A4–A7) covered local

roads, neighbourhood road, rural roads, vehicular trails, roads with special characteristics and other thoroughfares. Information was collected on maternal smoking during pregnancy, lifetime history of second hand smoke exposure and number of smokers (0, 1, 2 or more) at home upon cohort entry using a standardised questionnaire.^{25, 26}

Determination of EPHX1 phenotypes and diplotype

We used methods described by Benhamou¹⁹ for determining the EPHX1 phenotypes (table E2 in online supplement available at <http://thorax.bmj.com/supplemental>). The EPHX1 diplotype frequencies in non-Hispanic and Hispanic white children were imputed separately using the SAS macro code written by Dr Daniel Stram (available at <http://www-rcf.usc.edu/~stram/tagSNPs.html>). This imputation technique uses the partition-ligation expectation-maximum algorithm and provides the maximum likelihood estimates of the haplotype frequencies assuming Hardy-Weinberg equilibrium.²⁷ Haplotype frequency data imputed for each racial/ethnic group were used for analysis in the overall sample.

Outcome assessment

We defined lifetime history of asthma based on parental report of physician-diagnosed asthma at enrolment to the Children's Health Study. A total of 476 children (15.5%) had a diagnosis of asthma. Based on the age at asthma onset and symptom persistence, we divided lifetime asthma into three subgroups: early transient, early persistent and late onset as described by Martinez *et al.*²⁸ Early transient asthma cases (n = 60) were diagnosed before the age of 3 years but had no asthma symptoms or medication use after first grade. Early persistent asthma was defined as a diagnosis before the age of 3 years and at least one episode of wheeze or asthma medication use after starting first grade (n = 175). Children diagnosed with asthma after the age of 3 years were classified as having late onset asthma (n = 241). Current asthma was defined based on parental response to the question: "Which best describes the child's current level of asthma symptoms?" We defined current asthma based on parental response that described asthma symptoms in the previous 12 months which may have required asthma medication on an occasional or regular basis and/or hospital admission.

Power calculation

The study had 80% power to detect ORs of 0.70, 1.47 and 1.37 for EPHX1 Tyr113His, His139Arg and GSTP1 Ile105Val genotypes for early persistent asthma (asthma phenotype with the lowest number of cases), respectively (table E3 in the online supplement available at <http://thorax.bmj.com/supplemental>). Because other asthma phenotypes had higher sample sizes, less strong ORs for these asthma phenotypes could be detected with 80% power. For testing the interactions, the study had >80% power to detect ORs of 1.62 and 2.30 for the modifying effects of GSTP1 Ile105Val genotype and residential distance <75 metres from a major road, respectively. For the joint effects of EPHX1 phenotype, GSTP1 Ile105Val genotype and residential location <75 metres from a major road, the study had 80% power to detect an OR of 7.2 (table E4 in the online supplement available at <http://thorax.bmj.com/supplemental>).

Statistical analysis

We tested our hypotheses in the combined Hispanic and non-Hispanic white population as well as separately within these two racial/ethnic groups. Because the associations between EPHX1 and asthma were similar by racial/ethnic group (no effect modification by race/ethnicity), the results are presented for the combined population. Because early and late onset asthma may have different pathobiologies, the results were presented by asthma phenotype. To assess the relationship between asthma and EPHX1 (genotypes, phenotypes and

diplotypes) and GSTM1, GSTP1 and GSTP1 Ile105Val genotypes, logistic regression models were used to compute odds ratios (ORs) and 95% confidence intervals (CI). Models were adjusted for age, sex, race/ethnicity, in utero exposure to smoking, number of smokers at home, parental history of asthma, parent/guardian education, health insurance coverage and community of residence. Adjusting for the atopic status of the child (based on a parental report of allergy and/or hay fever) did not change the ORs by 5% and EPHX1 phenotypes were not associated with atopy ($p = 0.20$). We therefore did not include the atopic status of the child in the models. Because the EPHX1 113His and 139His alleles are associated with low EPHX1 activity, we computed the ORs for carrying one copy of 113Tyr-139His (high-low), 113His-139Arg (low-high) and 113Tyr-139Arg (high-high) diplotypes compared with those carrying two copies of the 113His-139His (low-low) diplotype using an additive genetic model.

The relationship between EPHX1 phenotypes and asthma in children who inherited specific functional polymorphisms in GSTM1, GSTP1 and GSTT1 was studied. We also examined whether residential location near major roads, in utero exposure to maternal smoking, exposure to second hand smoke, atopic status of the child, parental history of asthma, race/ethnicity and sex modified any associations between EPHX1 and asthma using likelihood ratio tests. Because children who had intermediate EPHX1 activity were not at increased risk of asthma, the low and intermediate EPHX1

Table 1 Selected characteristics of the study participants*

	No asthma		Lifetime asthma		p Value‡
	N†	(%)	N†	(%)	
Sex					
Girls	1454	(54.9)	211	(44.3)	<0.001
Boys	1194	(45.1)	265	(55.7)	
Age (years)					
≤ 10	1458	(55.1)	255	(53.6)	0.37
11–12	512	(19.3)	85	(17.9)	
> 12	678	(25.6)	136	(28.5)	
Ethnicity					
Non-Hispanic white	1823	(68.8)	338	(71.0)	0.35
Hispanic white	825	(31.2)	138	(29.0)	
Parental history of asthma					
No	2075	(83.1)	265	(58.6)	<0.001
Yes	422	(16.9)	187	(41.4)	
Child's atopic status§					
No	1687	(68.3)	131	(31.2)	<0.001
Yes	782	(31.7)	289	(68.8)	
In utero exposure to maternal smoking					
No	2149	(83.0)	380	(80.7)	0.23
Yes	441	(17.0)	91	(19.3)	
Number of smokers at home					
0	1819	(71.0)	313	(67.7)	0.26
1	502	(19.6)	96	(20.8)	
≥ 2	240	(9.4)	53	(11.5)	
Annual family income (\$)					
< 15000	317	(13.9)	49	(11.7)	0.15
15000–49999	969	(42.5)	167	(39.9)	
≥ 50000	994	(43.6)	207	(48.4)	
Parent/guardian education					
< 12th grade	320	(12.4)	33	(7.0)	0.005
12th grade	494	(19.2)	95	(20.2)	
Some college	1160	(45.0)	242	(51.5)	
College	268	(10.4)	46	(9.8)	
Some graduate	336	(13.0)	54	(11.5)	
Health insurance coverage					
No	400	(15.3)	37	(7.9)	<0.001
Yes	2208	(84.7)	430	(92.1)	

*Participants include non-Hispanic and Hispanic white children of the Children's Health Study with and without asthma with available EPHX1 Tyr113His and His139Arg genotypes obtained from buccal samples collected during 1998–2006.

†Totals differ due to missing data.

‡p values from Pearson χ^2 tests comparing children with asthma with those without asthma.

§Child's atopic status was determined based on parental report of allergy and hay fever.

phenotypes were combined (ie, low/intermediate) for testing the interaction between EPHX1 phenotypes and GSTP1 Ile105Val and distance of residence from major roads. For GSTP1 Ile105Val we used a co-dominant coding for testing the interactions. We have previously observed that children living within 75 metres of a major road were at a higher risk of asthma.¹ We therefore examined whether the relationship between EPHX1 phenotypes and asthma differed in children living <75 metres and ≥75 metres from a major road. All tests were two-sided at a 5% significance level. SAS V.9.1 (SAS Institute Inc, Cary, North Carolina, USA) was used for all analyses.

RESULTS

Compared with children with no asthma, children with physician-diagnosed asthma were more likely to be male (asthma prevalence 18.2% in boys, 12.7% in girls; $p < 0.0001$), to have a parental history of asthma ($p < 0.0001$) and to have health insurance coverage ($p < 0.0001$) (table 1). Children with atopy were at an increased risk of asthma ($p < 0.0001$); however, EPHX1 and GSTP1 genotypes and EPHX1 phenotypes were not associated with atopy (all $p > 0.20$). Although children with and without asthma had significant differences in the distribution of parent/guardian education, they did not differ by year of cohort entry, age, ethnicity or annual family income. Children with early persistent asthma were more likely to be exposed to two or more smokers at home ($p = 0.04$) and to maternal smoking in utero ($p = 0.06$) than children with no asthma diagnosis. The EPHX1 Tyr113His and His139Arg and the GSTP1 Ile105Val genotypes were in Hardy-Weinberg equilibrium in the overall cohort and in non-Hispanic and Hispanic white children without asthma (table E5 in the online supplement available at <http://thorax.bmj.com/supplemental>).

Although EPHX1 Tyr113His polymorphism was not significantly associated with lifetime asthma, statistically significant association was found for late onset asthma (table 2). Compared with children with the Tyr/Tyr genotype at the Tyr113His locus, those with the His/His genotype were 49% less likely to have late onset asthma (95% CI 0.29 to 0.88). Children heterozygous for the 139His allele had significantly increased risk of lifetime, current and late onset asthma) than children with no 139His allele. The GSTP1 Ile105Val polymorphism was associated with asthma, and the association was stronger for children with early persistent asthma. Compared with children

homozygous for the Ile allele, those with one and two Val alleles had a 1.37 (95% CI 0.95 to 1.96) and 1.95 (95% CI 1.19 to 3.18) times increased risk of early persistent asthma, respectively. Variants in GSTM1 and GSTT1 were not associated with asthma phenotypes in this sample (table E6 in the online supplement available at <http://thorax.bmj.com/supplemental>).

The EPHX1 phenotypes and diplotypes were significantly associated with asthma (table 3). Children with the high EPHX1 phenotype had a 1.51 times higher risk of lifetime asthma (95% CI 1.14 to 1.98) than children with the low EPHX1 phenotype, and a statistically significant trend of increasing asthma risk with higher EPHX1 activity was observed ($p = 0.007$). The results were similar (not shown) when another genotype-derived EPHX1 phenotype classification described by Smith and Harrison was used.²⁰ In the combined sample the diplotype frequencies for 113His-139His (low-low), 113His-139Arg (low-high), 113Tyr-139His (high-low) and 113Tyr-139Arg (high-high) were 0.24, 0.07, 0.58 and 0.10, respectively. The diplotype frequencies did not differ between non-Hispanic and Hispanic white children. Children with one copy of the 113Tyr-139Arg (high-high) diplotype were at 1.32 fold (95% CI 1.04 to 1.67) increased risk of asthma compared with those with two copies of the 113His-139His (low-low) diplotypes. These associations were stronger in children with late onset asthma.

The association between EPHX1 phenotypes and asthma varied by GSTP1 Ile105Val genotype (table 4). In children with GSTP1 105Val/Val genotypes, those with high EPHX1 activity had a 4-fold (95% CI 1.97 to 8.16) increased risk of lifetime asthma compared with children with low/intermediate EPHX1 activity phenotypes. High EPHX1 activity was not statistically significantly associated with an increased risk of asthma in children with GSTP1 105 Ile/Ile or Ile/Val genotypes. The results were similar for early persistent, late onset and current asthma. The GSTM1 and GSTT1 null/present genotype did not modify the relationship between EPHX1 and GSTP1 Ile105Val and asthma (not shown).

The associations between EPHX1 phenotypes and asthma depended on the proximity of a child's home to a major road (table 5). Among children who lived within 75 metres of a major road, those who had high EPHX1 activity had a 3.24-fold (95% CI 1.75 to 6.00) increased risk of lifetime asthma (p for interaction = 0.03) compared with children with low/intermediate EPHX1 activity. High EPHX1 activity was not

Table 2 Associations between the EPHX1 (Tyr113His and His139Arg) and GSTP1 (Ile105Val) polymorphisms and asthma phenotypes

	No asthma		Lifetime asthma		Current asthma		Early persistent asthma (diagnosis by 3 years)		Late onset asthma (diagnosis after 3 years)	
	N	N	OR* (95% CI)	N	OR* (95% CI)	N	OR* (95% CI)	N	OR* (95% CI)	
EPHX1 Tyr113His										
Tyr/Tyr	1253	241	1.0	154	1.0	89	1.0	126	1.0	
Tyr/His	1112	189	0.86 (0.69 to 1.07)	124	0.87 (0.67 to 1.12)	62	0.81 (0.57 to 1.15)	99	0.83 (0.62 to 1.10)	
His/His	283	46	0.79 (0.55 to 1.13)	30	0.79 (0.51 to 1.22)	24	1.21 (0.74 to 1.97)	16	0.51 (0.29 to 0.88)	
EPHX1 His139Arg										
His/His	1817	290	1.0	186	1.0	112	1.0	140	1.0	
His/Arg	741	174	1.42 (1.14 to 1.76)	114	1.45 (1.12 to 1.89)	57	1.25 (0.89 to 1.78)	96	1.58 (1.19 to 2.10)	
Arg/Arg	90	12	0.78 (0.41 to 1.47)	8	0.81 (0.38 to 1.76)	6	0.94 (0.38 to 2.31)	5	0.68 (0.26 to 1.73)	
GSTP1 Ile105Val†										
Ile/Ile	1043	171	1.0	116	1.0	57	1.0	93	1.0	
Ile/Val	1229	230	1.17 (0.94 to 1.47)	146	1.08 (0.82 to 1.41)	85	1.37 (0.95 to 1.96)	116	1.08 (0.81 to 1.46)	
Val/Val	343	65	1.31 (0.94 to 1.81)	38	1.14 (0.76 to 1.70)	29	1.95 (1.19 to 3.18)	26	0.93 (0.58 to 1.49)	

*ORs adjusted for age, sex, race/ethnicity, in utero exposure to maternal smoking, number of smokers at home, community of residence, parental education, health insurance and parental history of asthma.

†Data on GSTP1 Ile105Val genotype were unavailable for 43 children.

Table 3 Associations between EPHX1 metabolic phenotypes, EPHX1 haplotypes and asthma phenotypes

	No asthma		Lifetime asthma		Current asthma		Early persistent asthma (diagnosis by 3 years)		Late onset asthma (diagnosis after 3 years)	
	N	N	OR* (95% CI)	N	OR* (95% CI)	N	OR* (95% CI)	N	OR* (95% CI)	
EPHX1 metabolic phenotypes†										
Low	1061	171	1.0	113	1.0	67	1.0	80	1.0	
Intermediate	1116	193	1.07 (0.85 to 1.35)	123	1.06 (0.80 to 1.41)	70	0.92 (0.64 to 1.33)	98	1.21 (0.88 to 1.66)	
High	471	112	1.51 (1.14 to 1.98)	72	1.50 (1.08 to 2.09)	38	1.28 (0.83 to 1.98)	63	1.84 (1.28 to 2.64)	
p for trend			0.007		0.004		0.38		0.001	
EPHX1 diplotypes‡										
113His-139His (low/low)			1.0		1.0		1.0		1.0	
113Tyr-139His (fast/low)			1.17 (0.97 to 1.41)		1.19 (0.95 to 1.50)		1.11 (0.84 to 1.47)		1.26 (0.97 to 1.62)	
113His-139Arg (low/fast)			1.42 (0.87 to 2.33)		1.61 (0.91 to 2.87)		1.85 (0.94 to 3.65)		1.09 (0.53 to 2.25)	
113Tyr-139Arg (fast/fast)			1.32 (1.04 to 1.67)		1.33 (1.00 to 1.77)		1.07 (0.73 to 1.55)		1.61 (1.19 to 2.18)	

*ORs adjusted for age, sex, race/ethnicity, in utero exposure to maternal smoking, number of smokers at home, community of residence, parental education, health insurance and parental history of asthma.

†See table E2 in the online supplement (available at <http://thorax.bmj.com/supplemental>) for determinations of the EPHX1 phenotypes based on Tyr113His and His139Arg polymorphisms. Low and fast within parentheses represent in vitro catalytic efficiency of the two EPHX1 polymorphisms in the order the alleles are presented before the parentheses.

‡The ORs are per increase of one copy of the given diplotype.

statistically significantly associated with asthma risk in children who lived ≥ 75 metres from a major road. To address the potential for exposure misclassification we restricted the analyses to children who were long-term residents at their home addresses (ie, lived ≥ 1 years before asthma diagnosis or study entry) and found similar results (table E7 in the online supplement available at <http://thorax.bmj.com/supplemental>). In this restricted sample, among children living within 75 metres of a major road, those with high EPHX1 activity had a 4.16-fold (95% CI 1.84 to 9.42) increased risk of asthma (p for interaction = 0.009) compared with children with low/intermediate EPHX1 activity.

The association between EPHX1 high activity phenotype and lifetime asthma varied by GSTP1 Ile105Val and residential location near major roads (p for interaction = 0.04, table 6). Children were at the highest risk if they had high EPHX1 phenotype, GSTP1 105Val allele and lived near major roads. Children with high EPHX1 activity and living within 75 metres of a major road had a 2.61-fold (95% CI 1.22 to 5.58) and 8.91-fold (95% CI 2.40 to 33.12) increased risk of asthma if they had one and two GSTP1 105Val alleles, respectively, compared with children with low/intermediate EPHX1 activity, GSTP1 Ile/Ile genotype who lived ≥ 75 metres from a major road. The

corresponding ORs in long term residents were 2.67 (95% CI 1.06 to 6.73) and 5.50 (95% CI 1.05 to 28.72), respectively (table E8 in the online supplement available at <http://thorax.bmj.com/supplemental>). The results were essentially similar for current, early persistent and late onset asthma (not shown). However, the confidence limits were imprecise for early persistent and late onset asthma because of small sample sizes available for analysis.

Maternal smoking during pregnancy, exposure to second hand smoke, number of smokers at home, race/ethnicity, child's sex, child's atopic status and parental asthma did not modify any of the genetic associations (data not shown).

DISCUSSION

We found that a high EPHX1 metabolic phenotype is associated with an increased occurrence of asthma in children. This association varied by GSTP1 Ile105Val genotypes and by distance of residence from major roads. For early persistent asthma the increased risk associated with a high EPHX1 phenotype was only found in children who also had the GSTP1 105Val allele or lived near major roads. When genes and environmental exposures were considered together, children with high EPHX1 activity and the GSTP1 105Val allele who

Table 4 Association between EPHX1 phenotypes and asthma stratified by GSTP1 Ile105Val genotype

EPHX1 metabolic phenotypes by asthma phenotypes	GSTP1 Ile105Val						p Value‡
	Ile/Ile		Ile/Val		Val/Val		
	N*	OR† (95% CI)	N*	OR† (95% CI)	N*	OR† (95% CI)	
Lifetime asthma							
Low/intermediate	842/135	1.0	1012/181	1.0	297/42	1.0	
High	201/36	1.12 (0.74 to 1.70)	217/49	1.34 (0.92 to 1.94)	46/23	4.01 (1.97 to 8.16)	0.006
Current asthma							
Low/intermediate	842/94	1.0	1012/114	1.0	297/24	1.0	
High	201/22	1.15 (0.67 to 2.00)	217/32	1.31 (0.80 to 2.13)	46/14	4.42 (1.65 to 11.83)	0.03
Early persistent asthma							
Low/intermediate	842/49	1.0	1012/66	1.0	297/19	1.0	
High	201/8	0.65 (0.29 to 1.43)	217/19	1.55 (0.87 to 2.76)	46/10	3.89 (1.32 to 11.43)	0.02
Late onset asthma							
Low/intermediate	842/67	1.0	1012/92	1.0	297/16	1.0	
High	201/26	1.65 (0.99 to 2.75)	217/24	1.28 (0.78 to 2.11)	46/10	4.40 (1.56 to 12.45)	0.06

*Frequencies represent children with no asthma/children with asthma phenotype under study. Children with missing data on GSTP1 Ile105Val were excluded.

†ORs represent odds ratios for asthma outcomes associated with high EPHX1 phenotype within each stratum of GSTP1 Ile105Val genotype and were adjusted for age, sex, race/ethnicity, in utero exposure to maternal smoking, number of smokers at home, community of residence, parental education, health insurance and parental history of asthma.

‡p values for the EPHX1 activity phenotype by GSTP1 Ile105Val genotype interactions were obtained from likelihood ratio tests from a non-stratified model with appropriate interaction terms and were based on 2df.

Table 5 Association between EPHX1 phenotypes and asthma stratified by distance of residence from major roads

EPHX1 metabolic phenotypes by asthma phenotypes	Distance of residence from a major road				p Value‡
	≥75 metres		<75 metres		
	N*	OR† (95% CI)	N*	OR† (95% CI)	
Lifetime asthma					
Low/intermediate	1540/261	1.0	367/62	1.0	
High	343/72	1.25 (0.93 to 1.69)	66/27	3.24 (1.75 to 6.00)	0.03
Current asthma					
Low/intermediate	1540/175	1.0	367/37	1.0	
High	343/48	1.34 (0.91 to 1.96)	66/16	2.91 (1.25 to 6.77)	0.08
Early persistent asthma					
Low/intermediate	1540/99	1.0	367/22	1.0	
High	343/23	1.10 (0.67 to 1.79)	66/9	3.02 (1.09 to 8.33)	0.18
Late onset asthma					
Low/intermediate	1540/129	1.0	367/30	1.0	
High	343/41	1.44 (0.98 to 2.11)	66/17	4.85 (2.22 to 10.61)	0.02

*Frequencies represent children with no asthma/children with asthma phenotype under study. Children with missing data on distance of residence from major road were excluded.

†ORs represent odds ratios for asthma outcomes associated with high EPHX1 phenotype within each stratum of residential distance from major road and were adjusted for age, sex, race/ethnicity, in utero exposure to maternal smoking, number of smokers at home, community of residence, parental education, health insurance and parental history of asthma.

‡p value for the EPHX1 metabolic phenotypes by distance of residence from major road interactions were obtained from likelihood ratio tests from a non-stratified model with appropriate interaction terms and was based on 1 df.

lived within 75 metres of a major road were at the highest risk of asthma irrespective of age at asthma diagnosis.

Several lines of evidence support a role for variants in EPHX1, GSTP1 and traffic in the pathogenesis of asthma. The first is the role of EPHX1 in xenobiotic metabolism that is relevant to asthma. After EPHX1 metabolises PAHs to *trans*-dihydrodiols, PAH *trans*-dihydrodiols can be further metabolised in at least two pathways. In one pathway, *trans*-dihydrodiols are metabolised to diol-epoxides which lead to electrophilic DNA adduct formation. This mechanism has been proposed as one underlying pathway for tissue damage and chronic health effects.¹⁶ In the second pathway, *trans*-dihydrodiols are metabolised to catechols by dihydrodiol dehydrogenase, the latter belonging to aldo-keto reductase family 1 (AKR1s).¹⁶ Subsequently, auto-oxidation of catechol leads to generation of PAH *o*-quinones and superoxide anion, both of which have been shown to be

cytotoxic and immunotoxic. In addition, PAH *o*-quinones can enter into futile redox cycling,²⁹ thereby generating ROS. It is plausible that high EPHX1 activity producing excessive catechols could overwhelm the conjugation processes. In addition, GSTP1 105Val allele mediated reduced conjugation of some of the PAH metabolites and increased oxidant stress⁸ could lead to inflammation and ROS mediated damage to the airways, resulting in increased asthma in children. Further research into the role of PAHs in asthma should consider functional variants in the AKR1s in addition to those of EPHX1 and GSTP1 genes.

Although exposure to maternal smoking in utero and second hand smoke are sources of PAH exposures and were associated with early persistent asthma in this sample, these exposures did not affect the associations between EPHX1 phenotypes and asthma. In an earlier study of participants in the Children's

Table 6 Joint effects of distance of residence from major road, EPHX1 metabolic phenotypes and GSTP1 Ile105Val on lifetime asthma (N = 2702)

Distance of residence from major road (metres)	GSTP1 Ile105Val	EPHX1 metabolic phenotypes	No asthma (N*)	Lifetime asthma (N*)	OR† (95% CI)
≥75	Ile/Ile	Low/intermediate	589	94	1.0
≥75	Ile/Val	Low/intermediate	720	132	1.19 (0.88 to 1.61)
≥75	Val/Val	Low/intermediate	215	29	0.94 (0.59 to 1.50)
≥75	Ile/Ile	High	141	23	1.03 (0.61 to 1.71)
≥75	Ile/Val	High	158	31	1.35 (0.85 to 2.15)
≥75	Val/Val	High	38	14	2.63 (1.34 to 5.18)
<75	Ile/Ile	Low/intermediate	144	23	1.01 (0.60 to 1.69)
<75	Ile/Val	Low/intermediate	171	28	0.89 (0.54 to 1.44)
<75	Val/Val	Low/intermediate	48	11	1.46 (0.71 to 3.03)
<75	Ile/Ile	High	31	9	1.71 (0.75 to 3.87)
<75	Ile/Val	High	30	12	2.61 (1.22 to 5.58)
<75	Val/Val	High	5	6	8.91 (2.40 to 33.12)
					p=0.04‡

*Children with missing data on distance of residence from major road and GSTP1 Ile105Val were excluded.

†ORs adjusted for age, sex, race/ethnicity, in utero exposure to maternal smoking, number of smokers at home, community of residence, parental education, health insurance and parental history of asthma.

‡p value for EPHX1 activity phenotype by distance of residence from a major road and by GSTP1 Ile105Val genotype interaction was obtained from likelihood ratio test from a non-stratified model with appropriate interaction terms and was based on 7df.

Health Study, exposure to in utero smoking was associated with asthma and wheeze in children who had the GSTM1 null genotype.⁵ Possible explanations for not finding a differential asthma risk with EPHX1 activity in children with and without smoking exposures could be that the tobacco smoke contains exposures other than PAHs which are detoxified by other metabolic pathways. For instance, nicotine is a key tobacco component that has been associated with asthma.^{30, 31} However, EPHX1 is not involved in nicotine metabolism³² and we have not considered nicotine metabolising genes such as cytochrome P450 (CYP2A6) or flavin-containing mono-oxygenase 3 (FMO3) in the current investigation. Further studies should consider functional variants in genes in PAH and nicotine metabolism pathways together to identify the genetically susceptible group of children who are at higher risk of asthma from tobacco smoke. We acknowledge that other exposures (eg, particle number concentrations and other particulate constituents such as transition metals) could be correlated with proximity of residence to major roads. However, given the observed associations between the genes involved in PAH metabolism (EPHX1 and GSTP1) and asthma that varied with distance of residence from major roads, it is possible that differences in the distance of residence from major roads represented differential PAH exposure from traffic. Further research is needed to examine this hypothesis.

GSTP1 Ile105Val polymorphism was associated with asthma and also modified the effect of EPHX1 phenotypes on asthma. Although the ORs for GSTP1 Ile105Val and EPHX1 phenotypes were stronger in children with the GSTM1 null genotype (not shown), GSTM1 and GSTT1 null genotypes were not independently associated with asthma. GSTP1 is the predominant enzyme that contributes to the GST enzyme activity in the lungs.³³ This may explain why significant associations were not found between GSTM1 and GSTT1 genotypes and asthma. Furthermore, GSTP1 Ile105Val was associated with early onset asthma but not with late onset asthma, which suggests age related pleiotropy in the effects of GSTP1 Ile105Val genotype on asthma. This differential effect of GSTP1 Ile105Val on age at onset asthma phenotype could explain the observed inconsistencies across studies in the association between GSTP1 Ile105Val and asthma.⁹⁻¹⁴

We found that children with high EPHX1 metabolic phenotype who lived near a major road were at a higher risk of asthma when they carried the variant Val allele in the GSTP1 105 locus. In this population, approximately 19% of children had the high EPHX1 phenotypes and 60% of children had the GSTP1 105 Ile/Val (47%) or Val/Val (13%) genotypes. About 70% of children carried one of these genetic susceptibility factors and about 20% lived within 75 metres of a major road. The percentage of children living near a major road did not vary by the genetic susceptibility factors. Taken together, these data suggest that a considerable proportion of children (approximately 13%) in southern California with functional variants in PAH metabolising genes are at a higher risk of asthma from local traffic related pollution.

The strengths of the present study include selection of SNPs with well documented functional significance, large sample sizes where children without asthma were in Hardy-Weinberg equilibrium for each genotype, use of allelic discrimination assay rather than using restriction fragment length polymorphism (RFLP) method that reduced genotype misclassifications,³⁴ consistent findings in genotype, diplotype and phenotype-based analyses, all main and joint effects in the same directions by race justifying a combined analysis, and biological plausibility for the observed associations.

One concern regarding this study involves the use of physician-diagnosed asthma for our case definition. To address

this problem we independently validated asthma diagnosis in a subset of the Children's Health Study cohort. In a case-control study³⁵ nested in the Children's Health Study, 221 parents provided consent and at least partial information on the physician who made the asthma diagnosis. We were able to obtain medical records of 172 children with asthma and 95.9% of them (n = 165) had either a definite (physician diagnosis of asthma, n = 118) or a probable (physician report of wheeze and steroid and/or β_2 adrenergic agonist use, n = 54) asthma diagnosis. Restricting the analysis to definite and probable asthma showed similar associations to those found in the overall sample.

Children who provided buccal samples differed in socio-economic and demographic factors from those who did not participate in the study, and the proportion of Hispanic white children varied by community (tables E9 and E10 in the online supplement available at <http://thorax.bmj.com/supplemental>). To assess the potential for selection bias we adjusted all analyses for socioeconomic and demographic factors and found relatively little impact of these factors on the associations. To detect any influence of any particular community on the associations observed, we excluded data from one community at a time and repeated the analysis. These sensitivity analyses did not show any particular influence of any single community. We also restricted the analyses to children with health insurance and observed similar results (not shown). In light of these observations, it is less likely that subject recruitment and demographic variations by community could explain the findings. Although we had sufficient power to detect the ORs found, caution should be used in interpreting some of the results which are based on smaller sample sizes.

We decided to conduct the study using personal level traffic data that represented within-community differences in exposure levels and to follow a pathway-based approach for the gene selection; this meant that fewer statistical tests were conducted. Besides, the functional significance of the EPHX1 phenotypes and GSTP1 Ile105Val genotypes have been documented and the analyses were based on specified a priori hypotheses. In this setting it may not be appropriate to disregard prior information on the functional significance of the genetic variants under study and to adjust the p values for testing a set of selected a priori hypotheses.

We conclude that high EPHX1 metabolic activity and the GSTP1 105Val genotype are associated with asthma in children, and the risk is higher in this genetically susceptible group of children who live near major roads. Based on these findings, we hypothesise that specific PAH mediated generation of o-quinones and ROS could be a pathway for the pathogenesis of asthma in childhood. Further research is needed to consider specific PAH exposures and genes in the PAH metabolic pathway in childhood asthma.

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Further details are given in the online supplement available at <http://thorax.bmj.com/supplemental>

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