Online Data Supplement

Transforming Growth Factor-β1 C-509T Polymorphism, Oxidant Stress, and Early Onset Childhood Asthma

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METHODS

Study design and subjects

Children of this study participated in the CHS, which has been described previously (E1, E2). In brief, a total of 6,259 children attending public school in 12 southern California communities were recruited in two cohorts in 1993 and 1996 for the CHS. Mean age at enrollment was 12.1 years (standard deviation (SD) 2.4 years) for cohorts recruited in 1993 and was 9.6 years (SD, 0.4 years) for cohort recruited in 1996. Beginning in 1998, we started collecting buccal samples and by May 2006, we collected and genotyped samples from 3,825 (61.1%) of children. Parents provided informed consent for participants below 18 years of age whereas participants over 18 years of age gave their informed consent. Children who were African-American (n = 163), Asian (n = 166) or belonged mixed race/ethnicity (n = 192) were excluded from the analysis due to insufficient sample sizes for stratified analyses and concern for population stratification. We also excluded 28 children for whom we did not have race/ethnicity information. Sixty-two children with unknown asthma status and 61 children with early transient wheezing were also excluded.

Of the 3,153 non-Hispanic and Hispanic white children with available buccal samples, we were unable to obtain genotype results for the two SNPs in the TGFβ1 gene for 130 (4.1%) children. Therefore, our final sample included 3,023 children who were either Hispanic (n = 927) or non-Hispanic (n = 2,096) white with known asthma status and not with early transient wheezing. The University of Southern California Institutional Review Board approved the study.

Exposure assessment

Residential distance from nearest freeway was assigned to each child based on his/her residence at study entry. Residence addresses were standardized and their locations geocoded by use of the TeleAtlas database and software (Tele Atlas Inc., Menlo Park, CA, USA, http://www.na.teleatlas.com). We used ERSI ArcGIS version 8.3 (ESRI, Redland, CA, USA, http://www.esri.com) software to calculate the distance from each residence to the nearest freeway, defined as an interstate freeway, US highway, or restricted-access high-way, and to the nearest major non-freeway road, which included other types of highways and large roads.

Distance to the nearest major road (m) 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 labeled 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 through A7) covered local roads, neighborhood road, rural roads, vehicular trails, roads with special characteristics, and other thoroughfares.

To estimate vehicle counts near homes, annual average daily traffic volumes were obtained from the California Department of Transportation (CALTRANS) Highway Performance Monitoring System for the year 2000. The traffic volumes were transferred from the CALTRANS roadway network to the TeleAtlas networks using previously described methods (E3). The hourly traffic volumes on weekdays and weekend days were estimated from the annual average daily traffic

volumes and the average diurnal and day-of-week freeway and non-freeway traffic variations observed in Southern California. These data were used to calculate the daily average number of vehicles traveling within 150 meters of each residence, weighted by inverse distance from the home to each road. This local traffic density was expressed as traffic volume per square meter.

Buccal Cell Collection and Processing

Children were provided with two toothbrushes and instructed to brush their teeth with the first one. They were instructed to gently brush the buccal mucosa with the second toothbrush. The brush was then placed in a leak proof container that was filled with an alcohol-based fixative. Children then swished liquid throughout their mouths and expelled the fluid into a container. The majority of buccal cell specimens were collected at school under the supervision of study staff. The remaining specimens were collected at home and sent to us by mail.

Buccal cell suspensions were centrifuged at 2,000g on the day they were received in the laboratory. The pellets were stored frozen at –20°C until used for DNA extraction, at which time they were resuspended and incubated in 600 μl of lysis solution from a PUREGENE DNA isolation kit (cat #D-5000; GENTRA, Minneapolis, MN) containing 100 μg/ml proteinase K overnight at 55°C. DNA extraction was performed according to manufacturer's recommendations. The DNA samples were resuspended in aqueous solution and stored at –20°C.

Determination of haplotype block structure

We determined the haplotype block structure of the TGFβ1 gene in non-Hispanic and Hispanic whites using genotyping data on subjects from the Multiethnic Cohort (MEC) (E4) who were representative of the racial/ethnic groups included in the present study. We used 15 SNPs that

were in Hardy-Weinberg equilibrium for identifying the common haplotypes in 66 non-Hispanic Whites and 65 Latinos (Hispanic whites) using the software Haploview (version 3.3, available at http://www.broad.mit.edu/mpg/haploview/index.php). We excluded SNPs from determining haplotypes if the minor allele frequency was less than 1%. In order to have a complete coverage of the TGFβ1 gene including its 5′ and 3′ untranslated regions (UTRs), 3 SNPs (ie, rs2241714, rs11666933, and rs4803457) that were located upstream to the TGFβ1 promoter region were genotyped. These SNPs were in the MGC4093 gene, which encodes for hypothetical protein LOC80776. In the 3′ UTR region, 2 SNPs (ie, rs2241718 and rs3810173) that were in the MGC20255 gene were genotyped. This gene encodes for hypothetical protein LOC90324. Haplotype block structure was defined using Gabriel's criteria (E5).

Genotyping

Genomic DNA was extracted from buccal mucosal cells by using PUREGENE™ DNA purification kit (Gentra Systems, Minneapolis, MN). Samples were analyzed in ABI prism 7700 Sequence Detector (Applied BioSystems, Foster City, CA) using Applied Biosystems-Sequence Detection Systems 1.9.1 software to discriminate allelic genotypes. Each reaction contained genomic DNA, QuantiTect probe (Qiagen, Valencia, CA), both forward and reverse primers (designed using ABI Primer Express software, Applied BioSystems), and a minor groove binding (MGB) probe for each allele.

The DNA fragment containing the SNP C-509T of the TGFβ1 gene was amplified using forward (5′-GCTCAGTAAAGGAGAGCAATTCTTACA-3′) and reverse

(5′-GGTAGGAGAAGGAGGGTCTGTCA-3′) primers. TaqMan MGB probe for C and T alleles were labeled with reporter dyes FAM (5′-CCATCC<u>C</u>TCAGGTGT-3′) and VIC (5′-TTCCATCC<u>T</u>TCAGGTGT-3′), respectively.

The DNA fragment containing the SNP rs4803457 of the TGFβ1 gene in the 5'flanking region was first amplified by using forward (5′-GCAGCCAAAGTGATCTTTCC-3′) and reverse (5′-CTGCCATCCATGTTCATGTC-3′) primers, and the PCRs were run for 35 cycles in the following manner: 95°C for 1 minute, 56°C for 1 minute and 72°C for 2 minute. The first-step PCR products were used as templates for the TaqMan PCR, which was amplified using forward (5′-GGTAACCATCATGGGCCTTGTC-3′) and reverse (5′-GGCAAAGCTATGGAAGGAGAG-3′) primers. TaqMan MGB probe for C and T alleles were labeled with FAM (5′-CTGCTC<u>C</u>CTTTGGG-3′) and VIC (5′-CTGCTC<u>T</u>CTTTGGGC-3′), respectively. The main cycling parameters of TaqMan PCR included: 50 ˚C for 2 min, 95 ˚C for 10 min, 35 cycles of 92˚C for 15 s and 60˚C for 1 min.

Each SNP assay was validated in selected samples using a second genotyping method, either the Automatic Sequencing (BigDye version 3.1, 377XL DNA sequencer) or digestion with an allele-specific restriction endonuclease followed by electrophoresis on agarose gels (RFLP). Genotyping was done in plates with 81 samples per batch with 10% of repeat samples within each plate. To minimize error between plates, inter-batch control samples were also placed. In the presence of intra- or inter-plate genotype discrepancy, the entire plate was re-genotyped. If genotype discrepancy could not be resolved, then we labeled the result as "unavailable" and removed the subject from data analyses.

TABLE E1. SNPs used to determine TGFβ1 haplotypes in White and Latino subjects of the

Multiethnic Cohort.

SNP (rs number)	Minor allele frequency		Gene and SNP location
	White	Latino	
rs2241714	0.28	0.38	MGC4093 exon 3 (Met3Ile)
rs11666933	0.39	0.43	MGC4093 intronic
rs4803457	0.39	0.43	MGC4093 intronic
rs1800469	0.28	0.39	TGFβ1 5′-UTR -509
rs1982073	0.38	0.43	TGFβ1 Exon 1 (Leu10Pro; T869C)
rs1800471	0.11	0.04	TGFβ1 Exon 1 (Arg25Pro; G915C)
rs2241715	0.27	0.41	TGFβ1 intronic
rs11466321	0.11	0.03	TGFβ1 intronic
rs2241716	0.01	0.02	TGFβ1 intronic
rs2278422	0.43	0.37	TGFβ1 intronic
rs8105161	0.17	0.15	TGFβ1 intronic
rs8179181	0.24	0.15	TGFβ1 intronic
rs11551225	0.00	0.01	TGFβ1 Exon 7 (Phe321Phe)
rs2241718	0.19	0.10	MGC20255 intron 4
rs3810173	0.01	0.07	MGC20255 exon 7

TABLE E2. Associations between TGFβ1 C-509T and rs4803457 polymorphisms, and -509T/ rs4803457T diplotypes and asthma by ethnic group.

	No Asthma	Lifetime 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)[*]
Non-Hispanic whites							
rs1800469 (C-509T)							
CC	813	137	1.0 (Ref.)	52	1.0 (Ref.)	85	1.0 (Ref.)
CT	799	121	0.89 (0.67-1.19)	49	0.90 (0.58-1.40)	72	0.84 (0.59-1.19)
TT	189	37	1.23 (0.80-1.91)	23	1.82 (1.03-3.23)	14	0.72 (0.38-1.34)
rs4803457 (C>T)							
CC	646	116	1.0 (Ref.)	45	1.0 (Ref.)	71	1.0 (Ref.)
CT	856	122	0.75 (0.55-1.01)	50	0.73 (0.47-1.16)	72	0.70 (0.48-1.01)
TT	299	57	1.09 (0.75-1.60)	29	1.31 (0.77-2.24)	28	0.84 (0.51-1.38)
Number of rs4803457T/-509T diplotypes							
0			1.0 (Ref.)		1.0 (Ref.)		1.0 (Ref.)
1			0.88 (0.66-1.17)		0.89 (0.57-1.38)		0.83 (0.58-1.18)
2			1.25 (0.81-1.94)		1.85 (1.04-3.27)		0.73 (0.39-1.37)
Hispanic whites							
rs1800469 (C-509T)							
CC	215	25	1.0 (Ref.)	10	1.0 (Ref.)	15	1.0 (Ref.)
CT	411	60	1.31 (0.77-2.24)	23	1.16 (0.51-2.62)	37	1.42 (0.73-2.77)
TT	188	28	1.89 (0.94-3.83)	13	1.82 (0.61-5.42)	15	2.06 (0.85-5.01)
rs4803457 (C>T)							
CC	252	31	1.0 (Ref.)	13	1.0 (Ref.)	18	1.0 (Ref.)
CT	412	63	1.24 (0.70-2.22)	25	1.03 (0.42-2.53)	38	1.30 (0.64-2.65)
TT	150	19	1.97 (1.01-3.85)	8	2.11 (0.76-5.86)	11	1.90 (0.81-4.45)
Number of rs4803457T/-509T diplotypes							
0			1.0 (Ref.)		1.0 (Ref.)		1.0 (Ref.)
1			1.35 (0.79-2.32)		1.23 (0.54-2.78)		1.45 (0.74-2.83)
2			1.93 (0.96-3.90)		1.89 (0.64-5.62)		2.08 (0.86-5.07)

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, *in utero* exposure to maternal smoking, number of smokers at home, insurance status, residential distance from nearest freeway, and community of residence.

TABLE E3. Effect of residential distance from nearest freeway on asthma, stratified by TGFβ1

C-509T genotypes

	TGFβ1 C-509T		P-value for interaction[†]
	CC/CT	TT	
	OR[*] (95% CI)	OR[*] (95% CI)	
Lifetime asthma			
>1500m	1.0 (Ref.)	1.0 (Ref.)	0.02
1001-1500m	0.98 (0.64-1.50)	0.86 (0.23-3.15)	
500-1000m	0.89 (0.58-1.35)	2.65 (0.91-7.75)	
<500m	0.89 (0.56-1.41)	4.85 (1.36-17.32)	
Early persistent asthma			
>1500m	1.0 (Ref.)	1.0 (Ref.)	0.18
1001-1500m	1.73 (0.95-3.14)	2.15 (0.37-12.46)	
500-1000m	1.23 (0.67-2.27)	1.45 (0.24-8.76)	
<500m	0.88 (0.41-1.86)	6.98 (0.98-49.64)	

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family

income, parental education, *in utero* exposure to maternal smoking, number of smokers at home,

health insurance status, and community of residence.

[†] P-values for TGFB1 C-509T by residential distance from nearest freeway were obtained from

likelihood ratio test from non-stratified models with appropriate interaction terms and were based

on 3df.

TABLE E4. Joint effects of TGFβ1 C-509T genotypes and diplotypes and residential distance from nearest freeway on lifetime asthma in long-term residents.

	Residential distance from nearest freeway	No asthma N	Lifetime asthma N	OR (95% CI)
TGFβ1 C-509T				
CC/CT	>1500m	929	49	1.0 (Ref.)
CC/CT	1001-1500m	243	27	1.06 (0.63-1.78)
CC/CT	500-1000m	296	33	1.07 (0.65-1.78)
CC/CT	<500m	236	21	0.93 (0.52-1.67)
TT	>1500m	149	7	0.61 (0.27-1.40)
TT	1001-1500m	37	2	0.53 (0.12-2.39)
TT	500-1000m	50	8	2.18 (0.91-5.25)
TT	<500m	29	3	2.11 (0.57-7.76)
				$P^\dagger = 0.12$
Number of rs4803457T/-509T diplotypes				
0 or 1	>1500m			1.0 (Ref.)
0 or 1	1001-1500m			1.06 (0.63-1.79)
0 or 1	500-1000m			1.06 (0.64-1.76)
0 or 1	<500m			0.93 (0.52-1.67)
2	>1500m			0.61 (0.27-1.42)
2	1001-1500m			0.53 (0.12-2.39)
2	500-1000m			2.37 (0.98-5.72)
2	<500m			2.11 (0.57-7.80)
				$P^\dagger = 0.10$

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, *in utero* exposure to maternal smoking, number of smokers at home, insurance status, and community of residence. Children living in Lake Arrowhead and Lompoc were excluded from the analysis. Models could not be fit for early persistent and late onset asthma phenotypes.

[†] P-values for TGFβ1 C-509T and rs4803457T/-509T diplotypes by residential distance from nearest freeway were obtained from likelihood ratio tests from non-stratified models with appropriate interaction terms and were based on 3df.

TABLE E5. Distribution of Residential distance from a major road and traffic density* by

residential distance from nearest freeway.

	Residential distance from nearest freeway							
	>1500m		1001-1500m		500-1000m		<500m	
	N	(%)	N	(%)	N	(%)	N	(%)
Residential distance from a major road								
≥75m	990	(83.5)	240	(77.9)	335	(85.0)	207	(68.5)
<75m	196	(16.5)	68	(22.1)	59	(15.0)	95	(31.5)
Traffic density								
1st tertile (<0.1)	457	(36.0)	106	(31.6)	138	(32.9)	77	(24.5)
2nd tertile (0.1-15.5)	462	(36.4)	105	(31.3)	146	(34.8)	66	(21.0)
3rd tertile (>15.5)	349	(27.5)	124	(37.0)	135	(32.2)	171	(54.5)

* Traffic volume per square meter within 150m of residence, weighted by inverse distance from

the home to each road (see methods of the online supplement for further details).

TABLE E6. Joint effects of TGFβ1 C-509T genotypes and other traffic measures on asthma.

TGFβ1 C-509T	Traffic measures	Lifetime asthma OR (95% CI)[*]	Early persistent asthma OR (95% CI)[*]
	Residential distance from a major road		
CC/CT	≥75m	1.0 (Ref.)	1.0 (Ref.)
CC/CT	<75m	1.22 (0.85-1.76)	0.91 (0.51-1.62)
TT	≥75m	1.37 (0.89-2.13)	1.45 (0.77-2.72)
TT	<75m	1.20 (0.54-2.70)	2.28 (0.89-5.88)
	Traffic density[†]		
CC/CT	1st tertile (<0.1)	1.0 (Ref.)	1.0 (Ref.)
CC/CT	2nd tertile (0.1-15.5)	0.93 (0.66-1.32)	1.01 (0.61-1.67)
CC/CT	3rd tertile (>15.5)	0.79 (0.56-1.14)	0.66 (0.38-1.17)
TT	1st tertile (<0.1)	1.76 (0.95-3.25)	1.76 (0.71-4.42)
TT	2nd tertile (0.1-15.5)	0.95 (0.48-1.86)	1.01 (0.38-2.67)
TT	3rd tertile (>15.5)	0.85 (0.43-1.69)	1.72 (0.77-3.83)

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, *in utero* exposure to maternal smoking, number of smokers at home, health insurance status, and community of residence.

[†] Traffic volume per square meter within 150m of residence, weighted by inverse distance from the home to each road (see methods of the online supplement for further details).

TABLE E7. Effect of *in utero* exposure to maternal smoking on early persistent asthma, stratified by TGFβ1 C-509T genotypes

In utero exposure to maternal smoking	TGFβ1 C-509T		$P^{\dagger}$
	CC/CT	TT	
	OR* (95% CI)	OR* (95% CI)	
No	1.0 (Ref.)	1.0 (Ref.)	0.11
Yes	0.97 (0.57-1.66)	3.15 (0.81-12.26)	

* ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, *in utero* exposure to maternal smoking, number of smokers at home, health insurance status, residential distance from nearest freeway, and community of residence.

† The *P* value for the *in utero* maternal smoking by TGFβ1 C-509T interaction was obtained from likelihood ratio tests from a non-stratified model with appropriate interaction terms and was based on 1 df.

TABLE E8. TGFβ1 C-509T genotypes and exposure to maternal smoking *in utero* and early persistent asthma in children who had no secondhand smoke during childhood

TGFβ1 C-509T	Exposure to *in utero* maternal smoking	No asthma	Early persistent asthma	
		(N)	(N)	OR[*] (95% CI)
CC/CT	No	1434	82	1.0 (Ref.)
CC/CT	No	98	9	1.19 (0.54-2.63)
TT	Yes	220	15	1.44 (0.77-2.70)
TT	Yes	13	4	8.25 (2.22-30.63)
				$P = 0.06$[†]

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, health insurance status, residential distance from nearest freeway, and community of residence.

[†] The *P* value for the *in utero* maternal smoking by *TGFβ1* C-509T interaction was obtained from likelihood ratio test from a non-stratified model with appropriate interaction terms and was based on 1 df.

TABLE E9. Joint effects TGFβ1 C-509T and residential distance from nearest freeway on lifetime asthma, stratified by *in utero* exposure to maternal smoking.

TGFβ1 C-509T	Residential distance from nearest freeway	*In utero* maternal smoking	
		Yes	No
		OR[*] (95% CI)	OR[*] (95% CI)
CC/CT	>1500m	1.0 (Ref.)	1.0 (Ref.)
CC/CT	1001-1500m	1.61 (0.54-4.85)	0.94 (0.59-1.50)
CC/CT	500-1000m	1.47 (0.47-4.63)	0.76 (0.48-1.21)
CC/CT	<500m	1.50 (0.47-4.78)	0.81 (0.48-1.38)
TT	>1500m	-	0.89 (0.48-1.65)
TT	1001-1500m	10.04 (1.05-95.92)	0.51 (0.16-1.69)
TT	500-1000m	2.57 (0.28-23.52)	1.89 (0.89-4.02)
TT	<500m	10.02 (1.25-80.40)	1.96 (0.67-5.71)

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, number of smokers at home, health insurance status, and community of residence.

TABLE E10. Association between TGFβ1 C-509T genotype and asthma, stratified by history of atopy.

TGFβ1 C-509T	History of atopy					
	No			Yes		
	Lifetime asthma	Early persistent asthma	Late onset asthma	Lifetime asthma	Early persistent asthma	Late onset asthma
	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)
CC	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)
CT	0.98 (0.62-1.57)	1.25 (0.57-2.72)	0.91 (0.51-1.61)	1.02 (0.74-1.42)	1.08 (0.67-1.74)	0.98 (0.66-1.47)
TT	1.20 (0.62-2.32)	2.37 (0.94-5.94)	0.66 (0.24-1.80)	1.44 (0.89-2.34)	1.52 (0.77-2.97)	1.31 (0.71-2.42)

* ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, number of smokers at home, health insurance status, and community of residence.

TABLE E11. Comparison between children who provided buccal samples and those who did not participate in the genetic study.

	Participants (N =3,153)		Non-participants (N = 1,840)	
	N*	(%)	N*	(%)
Sex				
Girls	1688	(53.5)	886	(48.2)
Boys	1465	(46.5)	954	(51.8)
Age (years)				
≤ 10	1730	(54.9)	718	(39.0)
11-12	593	(18.8)	382	(20.8)
> 12	830	(26.3)	740	(40.2)
Ethnicity				
Non-Hispanic white	2172	(68.9)	1137	(61.8)
Hispanic white	981	(31.1)	703	(38.2)
Annual family income ($)				
< $15,500	372	(13.7)	346	(23.1)
$15,000 - $49,999	1144	(42.0)	658	(44.0)
≥$50,000	1205	(44.3)	492	(32.9)
Parent/guardian education				
< 12th grade	362	(11.8)	362	(20.3)
12th grade	595	(19.4)	424	(23.7)
Some college	1404	(45.7)	732	(41.0)
College	314	(10.2)	121	(6.8)
Some graduate	397	(12.9)	146	(8.2)
Health insurance coverage				
No	444	(14.3)	338	(18.9)[†]
Yes	2660	(85.7)	1453	(81.1)
Exposure to maternal smoking *in utero*				
No	2553	(82.6)	1366	(76.9)
Yes	537	(17.4)	411	(23.1)
Number of smokers at home				
None	2153	(70.6)	1035	(58.9)
1	598	(19.6)	458	(26.1)
2 or more	298	(9.8)	264	(15.0)
Residential distance from nearest freeway				
>1500m	1580	(58.6)	806	(52.8)
1001-1500m	351	(13.0)	236	(15.5)
500-1000m	437	(16.2)	259	(17.0)
<500m	327	(12.1)	226	(14.8)
Asthma				
Never	2726	(86.5)	1632	(88.7)
Ever	427	(13.5)	208	(11.3)

*Numbers always do not add up because of missing data. Comparisons were restricted to non-Hispanic and Hispanic white children. Children who had early transient asthma and those with unknown asthma status have been excluded from these comparisons to reflect the population studied.

TABLE E12. Distribution of annual family income and parental education by residential

distance from nearest freeway

	Residential distance from nearest freeway							
	>1500m		1001-1500m		500-1000m		<500m	
	N	(%)	N	(%)	N	(%)	N	(%)
Annual family income ($)								
< $15,500	147	(13.6)	32	(10.7)	53	(14.4)	42	(16.4)
$15,000 - $49,999	385	(35.5)	122	(40.9)	167	(45.4)	143	(55.9)
≥$50,000	552	(50.9)	144	(48.3)	148	(40.2)	71	(27.7)
Parent education								
< 12th grade	161	(13.0)	33	(10.1)	43	(10.7)	51	(16.6)
12th grade	228	(18.5)	70	(21.4)	85	(21.2)	72	(23.4)
Some college	529	(42.9)	152	(46.5)	191	(47.6)	138	(44.8)
College	149	(12.1)	29	(8.9)	33	(8.2)	16	(5.2)
Some graduate	167	(13.5)	43	(13.1)	49	(12.2)	31	(10.0)

TABLE E13. Distribution of TGFβ1 genotypes and residential distance from nearest freeway in two 4th grade students recruited in 1993 and 1996

	4th grade students in 1993		4th grade students in 1996	
	N	(%)	N	(%)
rs1800469 (C-509T)				
CC	432	(40.3)	479	(41.7)
CT	504	(47.1)	499	(43.5)
TT	135	(12.6)	170	(14.8)
rs4803457 (C>T)				
CC	348	(32.5)	389	(33.9)
CT	532	(49.7)	526	(45.8)
TT	191	(17.8)	233	(20.3)
Residential distance from nearest freeway				
>1500m	570	(62.6)	581	(58.0)
1001-1500m	106	(11.6)	126	(12.6)
500-1000m	147	(16.1)	165	(16.5)
<500m	88	(9.7)	129	(12.9)

TABLE E14. Associations between TGFβ1 C-509T and rs4803457 polymorphisms, and rs4803457T/-509T diplotypes and asthma in two different 4th grade children recruited in 1993 and 1996

	Lifetime asthma		Early persistent asthma (Diagnosis by 3 years)		Late onset asthma (Diagnosis after 3 years)	
	4th grade students in 1993	4th grade students in 1996	4th grade students in 1993	4th grade students in 1996	4th grade students in 1993	4th grade students in 1996
	OR (95% CI)[*]	OR (95% CI)[*]	OR (95% CI)[*]	OR (95% CI)[*]	OR (95% CI)[*]	OR (95% CI)[*]
rs1800469 (C-509T)						
CC	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)
CT	1.59 (0.99-2.55)	0.95 (0.63-1.43)	1.13 (0.56-2.30)	1.15 (0.64-2.05)	2.25 (1.23-4.15)	0.71 (0.41-1.22)
TT	2.02 (1.04-3.94)	1.84 (1.07-3.17)	3.14 (1.33-7.40)	2.33 (1.10-4.95)	0.90 (0.29-2.72)	1.41 (0.70-2.87)
rs4803457 (C>T)						
CC	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)
CT	1.09 (0.67-1.78)	0.76 (0.50-1.16)	0.77 (0.37-1.61)	0.82 (0.45-1.51)	1.40 (0.75-2.62)	0.62 (0.36-1.09)
TT	1.67 (0.91-3.07)	1.29 (0.78-2.15)	2.28 (0.99-5.21)	1.49 (0.74-3.01)	1.17 (0.50-2.75)	1.05 (0.53-2.06)
Number of rs4803457T/-509T diplotypes						
0	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)
1	1.46 (0.91-2.34)	0.97 (0.64-1.47)	1.02 (0.50-2.07)	1.16 (0.65-2.07)	2.12 (1.16-3.87)	0.74 (0.43-1.26)
2	1.93 (1.00-3.75)	1.93 (1.12-3.32)	2.97 (1.27-6.94)	2.45 (1.15-5.21)	0.86 (0.28-2.60)	1.47 (0.73-2.99)

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, *in utero* exposure to maternal smoking, number of smokers at home, health insurance status, residential distance from nearest freeway, and community of residence.

TABLE E15. Joint effects of TGFβ1 C-509T genotypes and rs4803457T/-509T diplotypes and residential distance from nearest freeway on asthma in two different 4th grade children recruited in 1993 and 1996

	Residential distance from nearest freeway	Lifetime asthma		Early persistent asthma	
		4th grade students in 1993	4th grade students in 1996	4th grade students in 1993	4th grade students in 1996
		OR (95% CI)[*]	OR (95% CI)[*]	OR (95% CI)[*]	OR (95% CI)[*]
TGFβ1 C-509T					
CC/CT	>1500m	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)
CC/CT	1001-1500m	1.52 (0.69-3.36)	1.21 (0.59-2.49)	2.81 (0.88-8.96)	1.79 (0.69-4.61)
CC/CT	500-1000m	0.58 (0.26-1.30)	1.37 (0.69-2.73)	0.82 (0.26-2.58)	1.41 (0.55-3.64)
CC/CT	<500m	1.20 (0.47-3.09)	0.98 (0.44-2.15)	0.44 (0.05-4.28)	0.95 (0.32-2.87)
TT	>1500m	0.71 (0.25-1.99)	1.15 (0.46-2.91)	2.14 (0.62-7.38)	0.94 (0.19-4.65)
TT	1001-1500m	1.75 (0.37-8.32)	0.96 (0.21-4.46)	4.22 (0.73-24.41)	2.76 (0.51-14.84)
TT	500-1000m	4.33 (1.27-14.75)	2.51 (0.83-7.57)	6.62 (1.17-37.30)	2.15 (0.43-10.65)
TT	<500m	-	6.43 (1.97-20.97)	-	9.17 (2.16-38.93)
#TT diplotypes					
0 or 1	>1500m	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)
0 or 1	1001-1500m	1.52 (0.69-3.37)	1.22 (0.59-2.50)	2.81 (0.88-8.95)	1.80 (0.70-4.64)
0 or 1	500-1000m	0.58 (0.26-1.31)	1.35 (0.68-2.68)	0.82 (0.26-2.58)	1.39 (0.54-3.59)
0 or 1	<500m	1.20 (0.47-3.09)	0.98 (0.44-2.16)	0.44 (0.05-4.28)	0.96 (0.32-2.89)
2	>1500m	0.72 (0.26-2.01)	1.17 (0.46-2.95)	2.15 (0.62-7.40)	0.95 (0.19-4.72)
2	1001-1500m	1.75 (0.37-8.33)	0.96 (0.21-4.48)	4.21 (0.73-24.40)	2.79 (0.52-15.00)
2	500-1000m	4.34 (1.28-14.77)	2.86 (0.93-8.75)	6.62 (1.17-37.30)	2.54 (0.50-13.01)
2	<500m	-	6.48 (1.99-21.18)	-	9.31 (2.19-39.64)

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, *in utero* exposure to maternal smoking, number of smokers at home, health insurance status, and community of residence.

[†] P-values for TGFB1 C-509T by residential distance from nearest freeway for lifetime asthma was statistically significant (P = 0.02)

TABLE E16. TGFβ1 C-509T genotypes and rs4803457T/-509T diplotypes and *in utero* exposure to maternal smoking and early persistent asthma in two different 4th grade children recruited in 1993 and 1996

	In utero maternal smoking	Early persistent asthma	
		4th grade in 1993	4th grade in 1996
		OR (95% CI)[*]	OR (95% CI)[*]
rs1800469 (C-509T)			
CC/CT	No	1.0	1.0
TT	No	2.41 (1.01-5.73)	1.84 (0.83-4.06)
CC/CT	Yes	1.04 (0.39-2.74)	0.68 (0.29-1.59)
TT	Yes	6.21 (1.48-26.01)	2.57 (0.74-9.01)
#TT diplotypes			
0 or 1	No	1.0	1.0
2	No	2.41 (1.01-5.73)	1.94 (0.87-4.31)
0 or 1	Yes	1.04 (0.40-2.74)	0.68 (0.29-1.59)
2	Yes	6.30 (1.50-26.53)	2.59 (0.74-9.07)

[*] ORs adjusted for age, sex, ethnicity, atopic status, parental history of asthma, annual family income, parental education, *in utero* exposure to maternal smoking, number of smokers at home, health insurance status, residential distance from nearest freeway, and community of residence.

TABLE E17. Odds ratios (ORs) and p-values for covariates from the model that tested the modifying effects of traffic distance on lifetime asthma (Table 4 of the manuscript)

Covariate	OR* (95% CI)	P-value	Covariate	OR* (95% CI)	P-value
Age (yrs)			Exposed in utero to maternal smoking	0.88 (0.60-1.29)	0.51
≤ 10	1.0 (Ref.)	0.12			
11-12	1.03 (0.71-1.47)		Health insurance (yes)	1.69 (1.06-2.71)	0.03
> 12	1.37 (1.01-1.84)				
			Parental education		
Sex (boys)	1.58 (1.22-2.04)	0.0006	< 12th grade	1.0 (Ref.)	0.30
			12th grade	1.53 (0.88-2.67)	
Ethnicity (Hispanic white)	1.08 (0.79-1.47)	0.64	Some college	1.32 (0.78-2.25)	
			College	1.11 (0.58-2.15)	
Child's atopic status (yes)	5.16 (3.91-6.80)		Some graduate	0.98 (0.51-1.88)	
Parental asthma (yes)	3.05 (2.31-4.02)	<0.0001	Community		
			Alpine	1.0 (Ref.)	0.32
Annual family income ($)			Upland	0.96 (0.54-1.70)	
< $15,500	1.0 (Ref.)	0.34	Santa Maria	0.88 (0.48-1.63)	
$15,000 - $49,999	0.88 (0.55-1.40)		Atascadero	1.50 (0.89-2.53)	
≥$50,000	1.11 (0.69-1.79)		San Dimas	0.81 (0.45-1.46)	
			Riverside	0.77 (0.42-1.42)	
Numbers of smokers at home			Miraloma	0.94 (0.53-1.67)	
None	1.0 (Ref.)	0.21	Long Beach	0.74 (0.39-1.39)	
One	1.21 (0.85-1.71)		Lancaster	0.79 (0.44-1.42)	
Two or more	1.49 (0.93-2.39)		Lake Elsinore	0.82 (0.46-1.46)	

*ORs are mutually adjusted for all variable presented and the interaction variable for TGFβ1 C-509T genotype and residential distance from nearest freeway, which have been presented in Table 4 of the manuscript.

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