ONLINE DATA SUPPLEMENT

Ozone, Oxidant Defense Genes and Risk of Asthma during Adolescence

Talat Islam, M.B.B.S., Ph.D.

Rob McConnell, M.D.

W. James Gauderman, Ph.D.

Ed Avol, M.S.

John M. Peters, M.D.

Frank D. Gilliland, M.D., Ph.D.

SUBJECTS AND MATERIAL

Initially in 1993, 3,681 students from fourth-, seventh-, and tenth-grade classrooms were enrolled into the study from 12 communities in southern California (selected on the basis of different ambient pollution levels). This includes five more children than has been reported previously (E1). Another 497 students who were in the same classrooms as participants were recruited in 1994. In 1996, an additional 2,081 fourth-grade children from the same communities and schools were recruited. All students were followed until their high school graduation. Thus, the first tenth-, seventh- and fourth-grade cohorts were followed until 1995, 1998 and 2001 respectively. The 1996 cohort was followed until 2004.

Parents or guardians of each participating student provided written informed consent and completed a self-administered questionnaire, which provided demographic information, characterized history of prior respiratory illness and its associated risk factors, lifetime tobacco-smoke exposure information and household characteristics. At study entry and in each subsequent year until high school graduation, children completed questionnaires that included asthma symptoms, diagnoses of asthma and secondhand-smoke exposure, and participated in a private interview about current respiratory health, asthma status, inhaler use and cigarette smoking. Children who did not complete the questionnaires or interview due to absence during visits at schools were interviewed by telephone by trained staff using the same instruments. Children who moved from a study school area during a school year were interviewed by telephone to collect information for the year that they moved and were not interviewed in subsequent years. Children who dropped out of the study were censored at the date of their last interview year. Children

who reported asthma at any interview were censored at t he midpoint of the year of diagnosis. The study protocol was approved by the Institutional Review Board for human studies at the University of Southern California, and written informed consent was provided by a parent or legal guardian for all participants.

Analyses were restricted to children of Hispanic (N=576) or non-Hispanic White ethnicity (N=1,125) due to insufficient representation of other races (Asian (N=106), African-American (N=89), and others (N=99)) in the cohort.

Race and Ethnicity

The race/ethnicity categories were based on parental response to the race and ethnicity questionnaire of their children. We did not collect information regarding the parents' race or ethnicity in all subjects. We did collect this information in a case control study. Both parents of Hispanic and non-Hispanic White children were overwhelmingly of the same ethnicity as the child. Unlike the studies in the East coast populations, we have very few Caribbean Hispanics in our study (<2%), and they were not included in this analysis.

Air-Pollution Data

The study was based on a quasi-factorial design that focused on O_3, PM_{10}, strong acid and NO_2 as the pollutants of primary interest. Regions of southern California were selected with the aim of maximizing the variability and minimizing the correlations in these four pollutants, based on historic routine air monitoring data and specialized monitoring studies, interpolating from more distant monitoring sites if no local data were available. In regions with pollution patterns of interest, cities or neighborhoods with stable, largely middle-income populations that were ethnically representative of southern

California as a whole, were identified from 1990 census data. To address community-level sources of variability, we sought to maximize the number of participating communities within existing financial constraints. Ultimately, 12 communities were selected. Four of these, in which public school officials declined to participate, were replaced by nearby communities with similar populations and air quality.

Assessment of Past Exposures

The approach used to estimate past exposures to air pollutants was based on data collected from 1986-1990 by monitoring stations existing prior to our own study. This allowed estimates of O_3, PM_{10}, NO_2 and acid vapor with spatial interpolation as needed, particularly for acid vapor. These data provided the estimates of relatively recent exposure upon which the communities were selected. We have also obtained estimates of lifetime exposure, based on residence histories and routine measurements nationwide.

Assessment of Current Exposures

An objective of the ambient air-monitoring program established by our study team was to obtain continuous measurements of O_3, NO_2 and PM_{10} with hourly averaging in all communities. An additional objective was to obtain integrated (2-wk average) measurements of $PM_{2.5}$ (particulates less than 2.5 μm in diameter) (total mass, as well as nitrate, sulfate and ammonium ion concentrations) and gaseous nitric, hydrochloric, formic and acetic acids for determination of seasonal and annual average concentrations. Ambient air quality monitoring was established in all 12 communities by adding instruments to seven pre-existing facilities operated by air pollution control agencies and by constructing five new stations. All stations monitored hourly O_3 using ultraviolet photometers, hourly NO_2 (actually total NO_X minus NO) using chemiluminescent

instruments and hourly PM_{10} mass using tapered element oscillating microbalance (TEOM) instruments. The TEOM PM_{10} measurements were adjusted by 2 to 25% to account for losses from volatilization based on analysis of collocated TEOM and HiVol PM_{10} measurements in four of the 12 communities. A new aerosol/acid sampler was developed to collect 2-wk integrated samples of the aforementioned $PM_{2.5}$ and gaseous acid components at each station.

Average pollutant levels in the 12 study communities varied substantially between communities, but there was little year-to-year variation in levels within community (E2). Average ozone levels showed low correlation with any of the other monitored pollutants (Table E1). Substantial correlation was observed between each of the other study pollutants monitored in this study. Thus, $PM_{2.5}$, NO_2, acid vapor, PM_{10} and elemental and organic carbon were a correlated package of pollutants with a similar pattern of variation across the 12 communities. The ranking of the communities into higher (six communities) and lower (six communities), based on annual average of ozone or any of the non-ozone package of pollutants, remained consistent throughout the calendar years between 1994 and 2003.

We calculated long-term mean pollutant levels (from 1994 through 2003) for subsequent statistical use. Communities defined as higher or lower based on any of the non-ozone 'package' of pollutants were the same for all of the correlated pollutants. Communities were classified as higher or lower by numerically ranking the 12 communities for each ambient pollutant of interest and dividing the list in half. The ozone levels (10 a.m.- 6 p.m.) ranged from 46.5-64.9 ppb in the higher ozone communities (mean=55.2ppb) and 28.6-45.5 ppb in the lower ozone communities

(mean=38.4 ppb). As the residence of the students was not selected based on the location of the monitoring site, those were randomly distributed in respect to the monitoring site. Thus, the assessed air-pollution exposure provides a community-specific exposure, and the measurement error is randomly distributed in respect to the monitoring sites. Due to this non-differential measurement error, any observed effect of air pollution is not likely to be biased but could be underestimated.

Sociodemographic and Medical History Information

Ethnicity was defined based on parental response to the baseline questionnaire. 'Hispanicity' was based on parental response to the question, "Is your child of Hispanic or Spanish descent?" Parents who reported that their children were Whites and not of Hispanic descent were considered non-Hispanic Whites. In the CHS cohort, only 0.6% Hispanics were of Puerto Rican or Cuban origin, and these were excluded from this analysis as they might have a different overall risk of asthma compared to Hispanic Whites.

Selected aspects of children's medical histories and family histories of asthma and allergy were collected at study entry. Personal history of allergy included any history of hay fever, allergic rhinitis, allergies to food or medicine, inhaled dusts, pollen, molds, animal fur or dander, or skin allergies not including poison ivy and oak. Family history of asthma was defined as any biological parent having been diagnosed with asthma. During annual school visits, students' height and weight were measured and recorded using standard protocols. Children's exposure to smoking *in utero* and SHS exposure at home was determined from questionnaire data completed by the parent/guardian at the time of study entry. Children also provided information regarding personal smoking as well as

exposure to secondhand smoke at home annually during the private interview. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). We categorized BMI into age- and sex-specific percentiles based on the Centers for Disease Control (CDC) BMI growth charts using one-month age intervals. Participants with BMI at or above the 85th percentile were classified as overweight/at risk of overweight. Children's propensity to be exposed to outdoor pollution was measured using questionnaire responses about the time each child spent outdoors after school hours (over five consecutive days prior to the annual activity questionnaire) and participation in team sports. Time spent outdoors was dichotomized into 'high' and 'low' by the median value. These household and indoor exposures, as well as air pollution, were also considered as potential risk factors.

Based on prior knowledge, we examined pets (dogs, cats, birds and other furry animals), pests, air conditioning, use of a gas stove, household water damage, mold and mildew on household surfaces, house plants, carpeting in bedrooms, heating source, age of the home and humidifier use as potential confounding covariates for household and indoor exposures.

Buccal Cell Collection and Processing

Children were provided with two toothbrushes and instructed to brush their teeth with the first and gently brush the buccal mucosa with the second toothbrush. The second 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 returned 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 an aqueous solution and stored at –20°C.

Catalase

Genotyping for CAT-62C>T and CAT-844C>T of the human Catalase gene was performed using the TaqMan Allelic Discrimination (AD) assay (Applied Biosystems, Foster City, CA). A fragment containing the associated polymorphism was amplified using primer pairs and MGB probes listed in Table E1. The TaqMan Genotyping reaction was amplified on a GeneAmp PCR system 9600, and fluorescence was detected on an ABI PRISM^{TM} 7700 Sequence Detector (Applied Biosystems). The main cycling parameters 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. The results from TaqMan PCR were validated by using PCR/RFLP methods and automatic sequencing (BigDye version 3.1, 377XL DNA sequencer, Applied Biosystems).

Manganese Superoxide Dismutase

The MNSOD Ala-9Val (rs4880) was genotyped. DNA from participants' buccal cells was extracted using a DNA isolation kit (PUREGENE, Minneapolis, MN). Samples were analyzed in an ABI PRISM 7700 Sequence Detector (Applied BioSystems) using Applied BioSystems-Sequence Detection Systems 1.9.1 software to

discriminate allelic genotypes. Each reaction was prepared by TaqMan PCR, which contained QuantiTec™ probe PCR Master Mix (QIAGEN), a pair of forward and reverse primers (designed by ABI Primer Express software), a MGB probe for each allele and genomic DNA as a template. PCR was conducted on a prepared sample plate in the following manner: 95˚C for 15 min (AmpliTaq® Gold DNA Polymerase activation), followed by 40 cycles of 92˚C for 15 s (denature) and 60˚C for 1 min (anneal/extend). All primer and probe sequences are listed in Table E1. Assays were repeated on 10% of the samples for quality control. The SNP assay was validated using a second genotyping method, Restriction Fragment Length Polymorphism (RFLP).

Hemeoxygenase-1

To determine the length of the $(GT)_n$ repeats in the HO-1 promoter region, PCR products were generated using the fluorescence-labeled forward primer and the unlabeled reverse primer (Table E1). To reduce non-specific PCR product, the Touchdown PCR method was applied. In the Touchdown PCR method, the annealing temperature was initially set at 69 °C then gradually decreased over a period of 16 cycles to 57 °C. Finally, PCR was performed over 30 cycles of 45 s at 95 °C, 1 min at 56 °C and 1 min at 72 °C. The size of the $(GT)_n$ repeats in each fluorescence-labeled PCR product was determined with GeneScan Analysis Softrware (Applied Biosystems). Five known HO-1 $(GT)_n$ repeats were run in each experiment as positive controls (Table E2). Genotypes of 12 homozygous samples were confirmed by automatic sequencing (BigDye version 3.1, 377XL DNA sequencer, Applied Biosystems).

Characterizing LD and Selecting Haplotype

We defined the block structure of HMOX1, Catalase and MNSOD by means of Haploview version 3.3 (download from http://www-rcf.usc.edu/~stram/*tagSN*Ps.html) using the resequencing data of sample size 71 for each ethnicity (Hispanic and non-Hispanic White) from the genetic data base of the well characterized Multi Ethnic Cohort population (E3).

The ethnic-specific haplotype blocks (Figures E3-6) were defined based on the method using the confidence intervals of D' proposed by Gabriel *et a.l* (E4) (with the upper confidence interval as 0.97 and the lower confidence interval as 0.70). The squared correlation (R_h^2) between the true haplotypes (h) and their estimates were then calculated, and the calculation of R_h^2 is described in detail by Stram *et a.l* (E5). htSNPs were then chosen using TagSNPs, a program that implements an expectation maximization (EM) algorithm approach by finding the minimum set of SNPs (within a block), which would have $R_h^2 \geq 0.85$ for all haplotypes with an estimated frequency of ≥ 5%. Missense SNPs were included as htSNPs before minimizing the number of htSNPs.

Ambient air pollution, genes and asthma

To assess the effect of ambient air pollution on the relationship between genes and new onset asthma, we needed to consider the possible effect of the communities as all children within a community had the same exposure levels. To address this issue we fitted two-stage models to this time dependent data (E6). Letting λ (t) be the expected hazard rate for asthma in this population and $\lambda_{bs/sex\text{-}age}$ (t) be the sex- and age-specific baseline hazard, then the first stage proportional hazard model has the following form:

Stage 1: $\lambda(t) = \lambda_{bs/sex\text{-}age}(t) \exp\{b_{cLF}Z + \gamma W\}$ ……(1)

where b_{cLF} corresponds to 12 community-specific slopes of genes on asthma risk. This model is further adjusted for different individual level covariates, W (such as race/ ethnicity).

The first stage model is followed by an ecologic regression model in the form:

$b_{cLF} = \delta_0 + \delta X_c + \eta_c$ …….....……(2)

The parameter δ_0, the mean of the within-community slopes b_{cLF}, serves as an aggregated-effect estimate of lung function across communities. However, our parameter of interest is δ, which characterizes the modifying effect of the long-term average pollution levels (X_c) on the relationship between lung function and asthma. Note that the second-stage model (2) accounts for heterogeneity in the community-specific slopes via η_c. The second-stage "ecologic" regression is weighted by the inverses of the variances of b_{cLF}.

Using this framework, we fitted separate models for pollutants; O_3 (10 a.m.–6 p.m. daily average ozone), and averages of PM_{10}, $PM_{2.5}$ and NO_2. Town-specific averages of the pollutants were used as continuous variables in these models. The averages were based on available daily (or bi-weekly) pollutant levels from 1994 to 2003.

REFERENCES

E1. Peters JM, Avol E, Navidi W, London SJ, Gauderman WJ, Lurmann F, Linn WS, Margolis H, Rappaport E, Gong H, Thomas DC. A study of twelve Southern California communities with differing levels and types of air pollution. I. Prevalence of respiratory morbidity. *Am J Respir Crit Care Med* 1999; 159:760-7.

E2. Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J. The effect of air pollution on lung development from 10 to 18 years of age. *N Engl J Med* 2004; 351:1057-67.

E3. Kolonel LN, Henderson BE, Hankin JH, Nomura AM, Wilkens LR, Pike MC, Stram DO, Monroe KR, Earle ME, Nagamine FS. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am J Epidemiol* 2000; 151:346-57.

E4. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science* 2002; 296:2225-9.

E5. Stram DO, Haiman CA, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE, Pike MC. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. *Hum Hered* 2003; 55:27-36.

E6. Berhane K, Gauderman W, Stram D, and Thomas, DC. Statistical issues in studies of the long term effects of air pollution: The Southern California Children Health Study (with discussion). *Stat Sci* 2004;19:414-449.

FIGURE LEGEND

Figure E1: Distribution of $(GT)_n$ repeats of HO-1 polymorphism in non-Hispanic Whites (light shade) and Hispanic (dark shade) children.

Figure E2: The distribution of the bi-allelic average of $(GT)_n$ repeats of HO-1 is presented for non-Hispanic Whites (light shade) and Hispanic (dark shade) children.

Figure E3: LD pattern of Catalase gene among non-Hispanic children

Figure E4: LD pattern of Catalase gene among Hispanic Whites

Figure E5: LD pattern of HMOX1 gene among non-Hispanic children

Figure E6: LD pattern of HMOX1 gene among Hispanic Whites

Table E1. Correlation of mean air-pollution levels from 1994 through 2003 across the 12 communities*

Pollutant	NO_2	Acid Vapor	PM_{10}	$PM_{2.5}$	Elemental Carbon	Organic Carbon
	R values					
Ozone (10a.m.-6p.m.)	-0.10	0.36	0.17	0.15	-0.04	0.12
NO_2		0.86	0.67	0.81	0.94	0.64
Acid Vapor†			0.79	0.86	0.88	0.75
PM_{10}				0.95	0.85	0.97
$PM_{2.5}$					0.92	0.90
Elemental Carbon						0.88

* Unless otherwise noted, values are the 24-hour average pollution levels. NO_2 denotes nitrogen dioxide and $PM_{2.5}$ and PM_{10} particulate matter with an aerodynamic diameter of less than 2.5 μm and less than 10 μm, respectively. All pairwise correlations were significant except between ozone and other pollutants.

† Acid vapor is the sum of nitric, formic and acetic acid.

Table E2. Primers and probes for genotyping HMOX1,Catalase and MNSOD polymorphisms

GENE		Primer/probe sequences
HMOX1	Microsatellite Polymorphism $(GT)_n$ alleles	Forward primer: 5'- ACCTTCTGGGACGCCTGG-3' Reverse primer: 5'- GGAAACAAAGTCTGGCCATAGG-3' Interpret HO-1 Genescan results: 217 = 30 repeat, 219=31 repeat Inter-plate Controls: 201/217, 203/203,217/219,201/231,203/219
CAT-262C>T rs1001179	C → T	Forward primer: : 5'-GCCTGAAGGATGCTGATAACC-3' Reverse primer: 5'-GCCTGAAGACCGGAGATACC-3' C probe: 5'-(6FAM)CCCGGGATAGCCGA-3' T probe: 5'-(VIC)CCCGGAATAGCCGAA-3'
CAT-844C>T rs769214	C → T	Forward primer: : 5'-TTCTTTAAACACTGGAGAAATCTGCTT-3' Reverse primer: 5'-ATATATTACTAAGTATTTTACTCTTCAACATAGCTTTTT-3' C probe: 5'-(6FAM)AAATTTTACCCCCAGGTAAG-3' T probe: 5'-(VIC)AAATTTTACTCCCAGGTAAG-3'
MNSOD rs4880	T → C	Forward primer:5'-CTGTGCTTTCTCGTCTTCAG-3' Reverse primer:5'-ATGATCTGCGCGTTGATGT-3' T probe: 5'-(VIC) CCCCAAAGCCGGAG-3' C probe: 5'-(6FAM)CCCCAAAACCGGAG-3'

Table E3. Selected characteristics of non-Hispanic White children at study entry, with and without genetic data

	Genetic Data Available		Genetic Data not Available	
	N (1,125)	%	N (479)	%
Age Group				
7-9 Years	585	52.0	172	35.9
10-11 Years	241	21.4	102	21.3
>11 Years	299	26.6	205	42.8
Boys	519	46.1	249	52.0
Overweight/at Risk of Overweight*	113	10.3	51	10.7
Parental History of Asthma	174	16.2	56	12.4
History of Allergy*	258	23.6	96	20.8
History of Allergic Rhinitis*	180	16.3	70	14.8
Humidifier Use	330	30.0	117	25.0
Smokers at Home	190	17.1	131	27.6
Maternal Smoking during Pregnancy	192	17.4	104	22.3
Current Maternal Smoking	109	9.8	83	17.4
Pests of any Kind	929	85.7	342	75.3
Pets at Home	1016	90.3	409	85.4
Health Insurance	1002	90.3	406	86.6
Income†				
≤14,999	74	6.58	53	11.1
15,000-49,999	385	34.2	163	34.0
≥50,000	524	46.6	192	40.1
Highest Parental Education Level†				
Less than High School	58	5.20	36	7.5
College	861	76.5	377	78.7
Graduate	192	17.1	57	11.9

* All the baseline characteristics differed between the two ethnic groups except those marked with the asterisks.

† The numbers do not add up to the total due to missing data.

Table E4. Selected characteristics of Hispanic children at study entry, with and without genetic data

	Genetic Data Available		Genetic Data not Available	
	N (576)	%	N (311)	%
Age Group				
7-9 Years	336	57.9	137	44.1
10-11 Years	120	21.0	62	19.9
>11 Years	120	21.2	112	36.0
Boys	239	41.9	161	51.8
Overweight/at Risk of Overweight*	107	18.6	59	19.2
Parental History of Asthma*	63	10.9	27	9.6
History of Allergy*	70	12.1	43	14.3
History of Allergic Rhinitis*	102	17.7	60	20.1
Humidifier Use*	86	14.9	48	16.4
Smokers at Home	63	10.9	63	20.9
Maternal Smoking during Pregnancy	42	7.29	31	10.5
Current Maternal Smoking*	26	4.51	29	9.6
Pests of any Kind*	353	61.3	180	67.2
Pets at Home*	384	66.6	215	69.1
Health Insurance*	403	70.0	209	69.4
Income†				
≤14,999	115	20.0	84	27.0
15,000-49,999	240	41.7	113	36.3
≥50,000	113	19.6	40	12.9
Highest Parental Education Level †				
Less than High School	167	28.9	122	39.2
College	339	58.8	61	19.6
Graduate	34	5.90	19	6.1

* All the baseline characteristics differed between the two ethnic groups except those marked with the asterisks.

† The numbers do not add up to the total due to missing data.

Table E5. Association of HMOX-1(GT-repeats) and CAT-262C-T genotype with new onset asthma among Hispanic and non-Hispanic White children*

Model	Genes	Model 1	Model 2
		HR (95%CI)	HR (95%CI)
Non-Hispanic White			
Any S	No-S	1	1
	At least one S	0.61(0.39-0.96) †	0.63 (0.38-1.04) ‡
CAT-262C-T (rs1001179)	CC	1	1
	CT or TT	0.84(0.54-1.31)	0.82 (0.52-1.32)
Hispanic White			
Any S	No-S	1	1
	At least one S	0.98(0.50-1.95)	0.85 (0.38-1.91)
CAT-262C-T (rs1001179)	CC	1	1
	CT or TT	1.88(0.96-3.66) ‡	2.13 (1.09-4.18)†

* The adjusted Hazard ratio (HR) and 95% confidence intervals (95% CI) are reported from Cox's proportional hazard model controlling for communities with age- and sex-specific baseline hazard.

Model 1: All Cox models are additionally adjusted for factors that varied between CHS participants with and without available genetic data: overweight/at risk of overweight at study entry, parental history of asthma, history of personal allergy, humidifier use, smokers at home, maternal smoking during pregnancy, maternal smoking at study entry, pets, pests, health insurance and parental income and education.

Model 2: Asthma definition is restricted to asthma cases with inhaler use. Among Hispanic Whites, 16 of the 57 cases were redefined as non-cases and among non-Hispanic Whites, 21 of the 103 cases were re-defined as non-cases.

†p-value <0.01

‡p-value <0.07

Table E6: Effect of HO1 polymorphism on the risk of new onset asthma stratified by time spent outdoors and communities defined by ambient Ozone* level (non-Hispanic Whites)

Time Spent Outdoor	Lower Ozone				Higher Ozone			
	No 'S' allele		'S' allele		No 'S' allele		'S' allele	
	N†	HR(95% CI) ‡	N†	HR(95% CI) ‡	N†	HR(95% CI) ‡	N†	HR(95% CI) ‡
Low	19	1	6	0.45(0.18-2.15)	11	1	11	0.99(0.39-2.57)
High	22	1.30(0.68-2.47)	9	0.57(0.24-1.33)	14	1.23(0.54-2.82)	11	1.19(0.46-3.03)

* Higher- and lower-ozone communities were defined according to average 10 a.m. to 6 p.m. ozone levels.

† Number of children with asthma diagnosis

‡ Relative risk (Hazard ratio) and corresponding 95% CI from Cox's proportional hazard model with stratified baseline hazard for gender and age (in one year intervals) at study entry. All models are adjusted for race/ethnicity and community of residence and stratified by community ozone level.

Table E7. Comparison of selected characteristics at study entry among children in the source population (N=2,998) and those who were lost to follow-up (N=776)

	Lost to Follow-up N (%)	Source Population N (%)
Age Group		
7-9 Years	317(40.9)	1458(48.6)
10-11 Years	189(24.4)	621(20.7)
>11 Years	270(34.8)	919(30.7)
Race		
Non-Hispanic Whites	400(51.5)	1604(53.5)
Hispanic Whites	224(28.9)	884(29.5)
African American	58(7.5)	162(5.4)
Asian	29(3.7)	176(5.9)
Other	65(8.4)	172(5.7)
Boys	364(46.9)	1379(46.0)
Overweight	14(12.9)	392(13.1)
Parental History of Asthma	112(14.4)	365(12.2)
History of Allergy	135(17.4)	552(18.4)
Humidifier Use	150(19.3)	674(22.5)
Smokers at Home*	217(28.0)	543(18.1)
Maternal Smoking during Pregnancy*	157(20.2)	422(14.1)
Maternal Smoking at Study Entry*	136(17.5)	284(9.5)
Pests of any Kind	518(66.8)	2111(70.4)
Pets at Home	551(71.0)	2296(76.6)
Health Insurance	607(78.2)	2419(80.7)
Income*†		
≤14,999	167(21.6)	399(13.3)
15,000-49,999	279(36.0)	1069(35.6)
≥50,000	182(23.4)	1004(33.5)
Highest Parental Education Level†		
Less than High School	145(18.7)	444(14.8)
College	533(68.5)	2077(69.2)
Graduate	66(8.5)	358(11.9)

* The difference is statistically significant (p-value<0.05).

†The numbers do not add up to the total due to missing data.

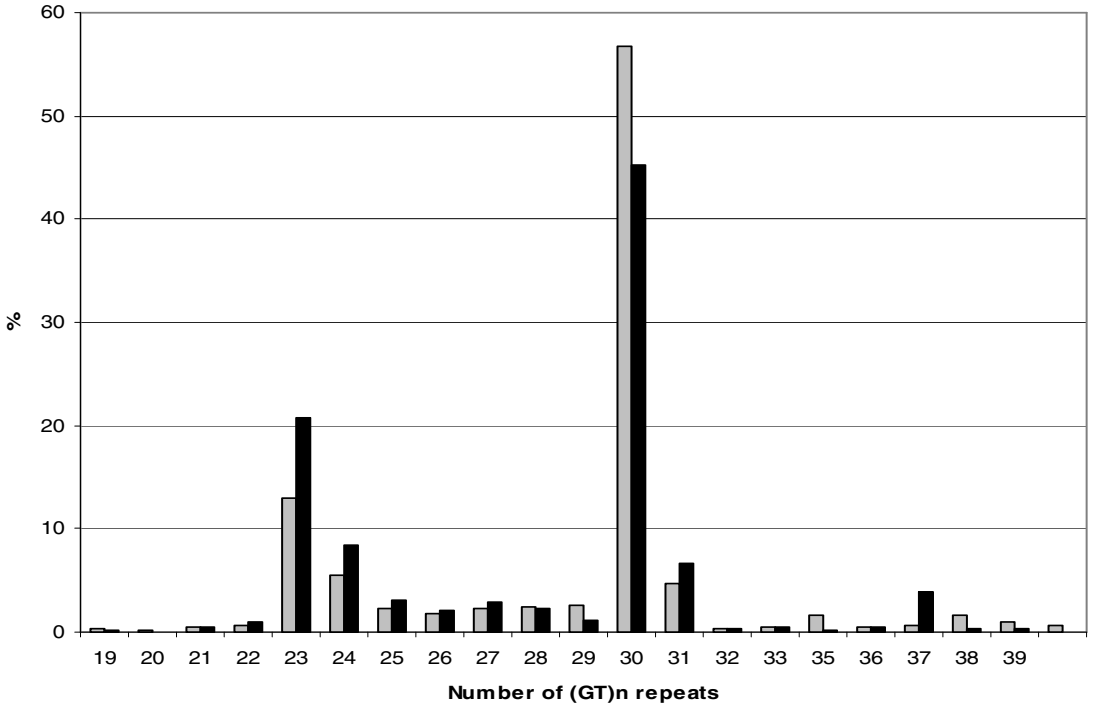

Figure E1

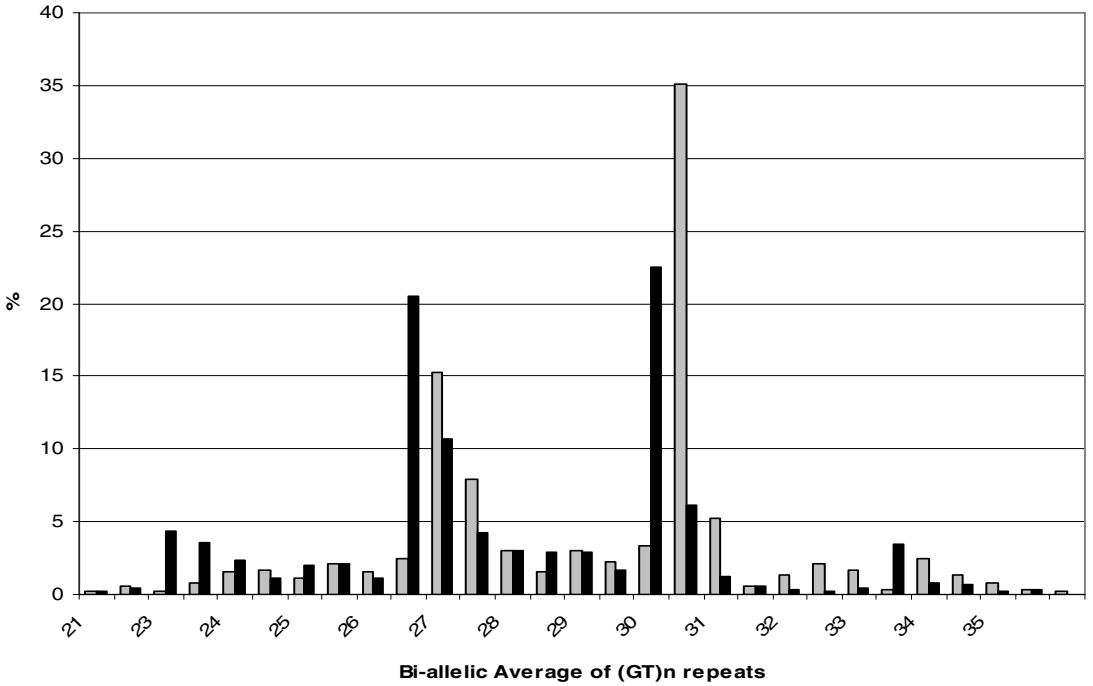

Figure E2

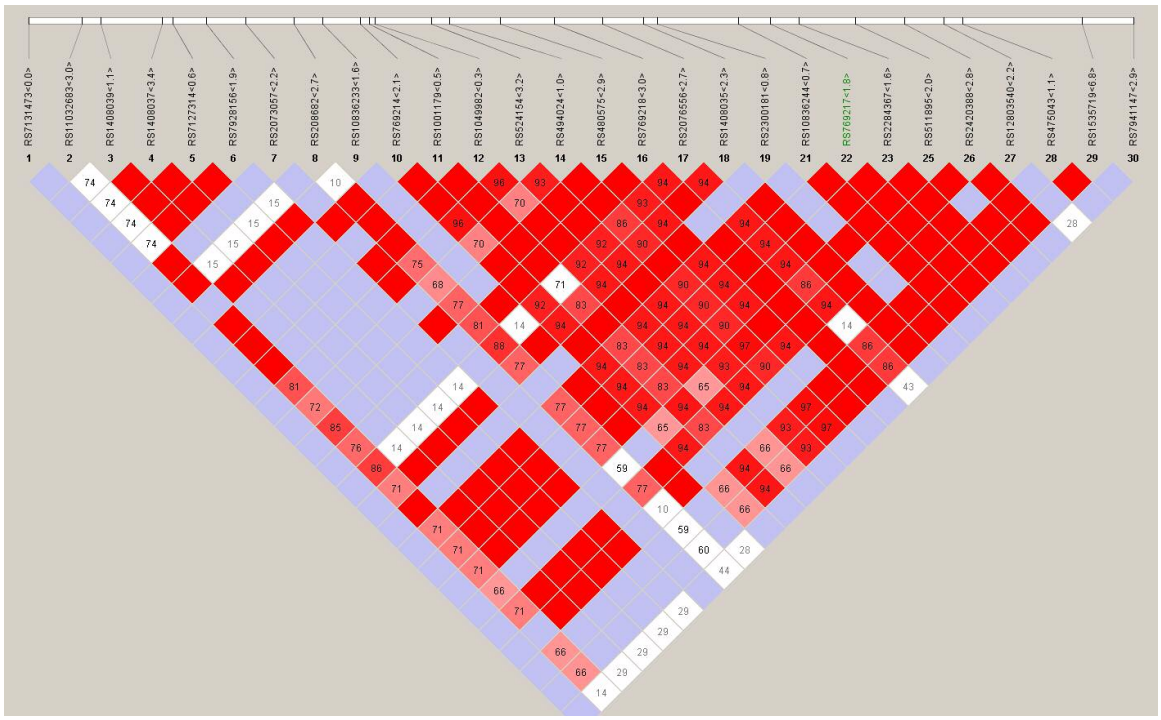

Figure E3

Figure E4

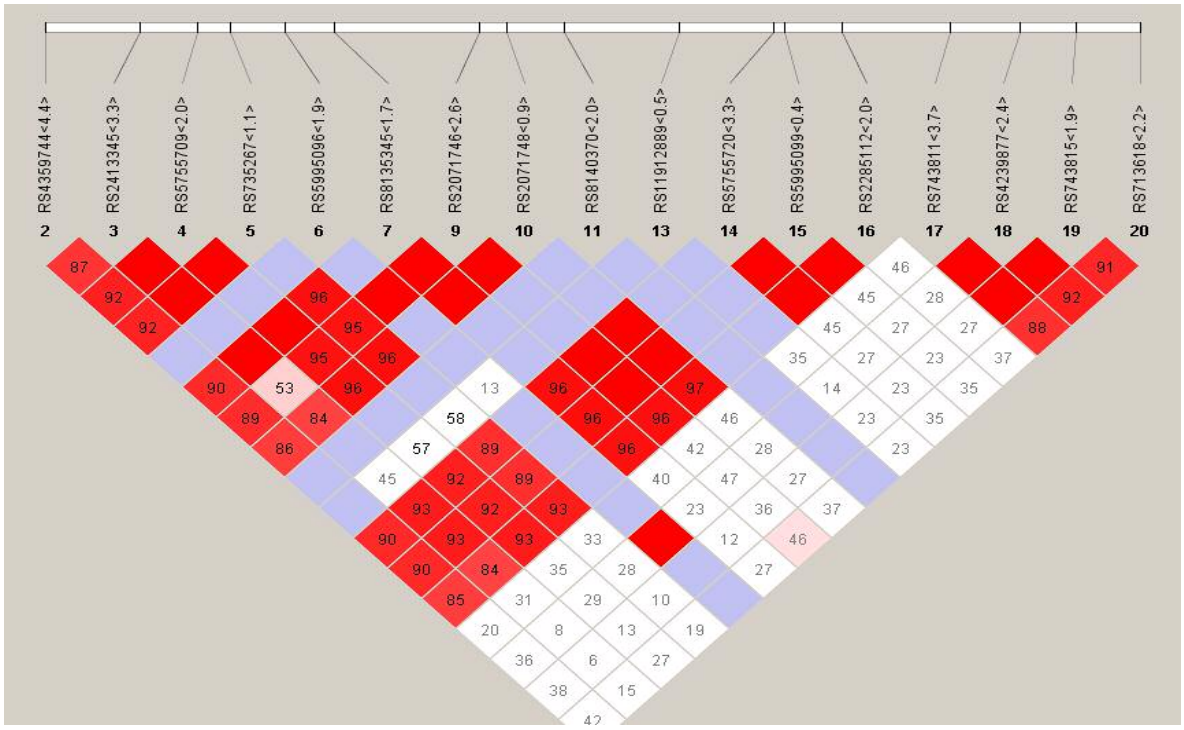

Figure E5

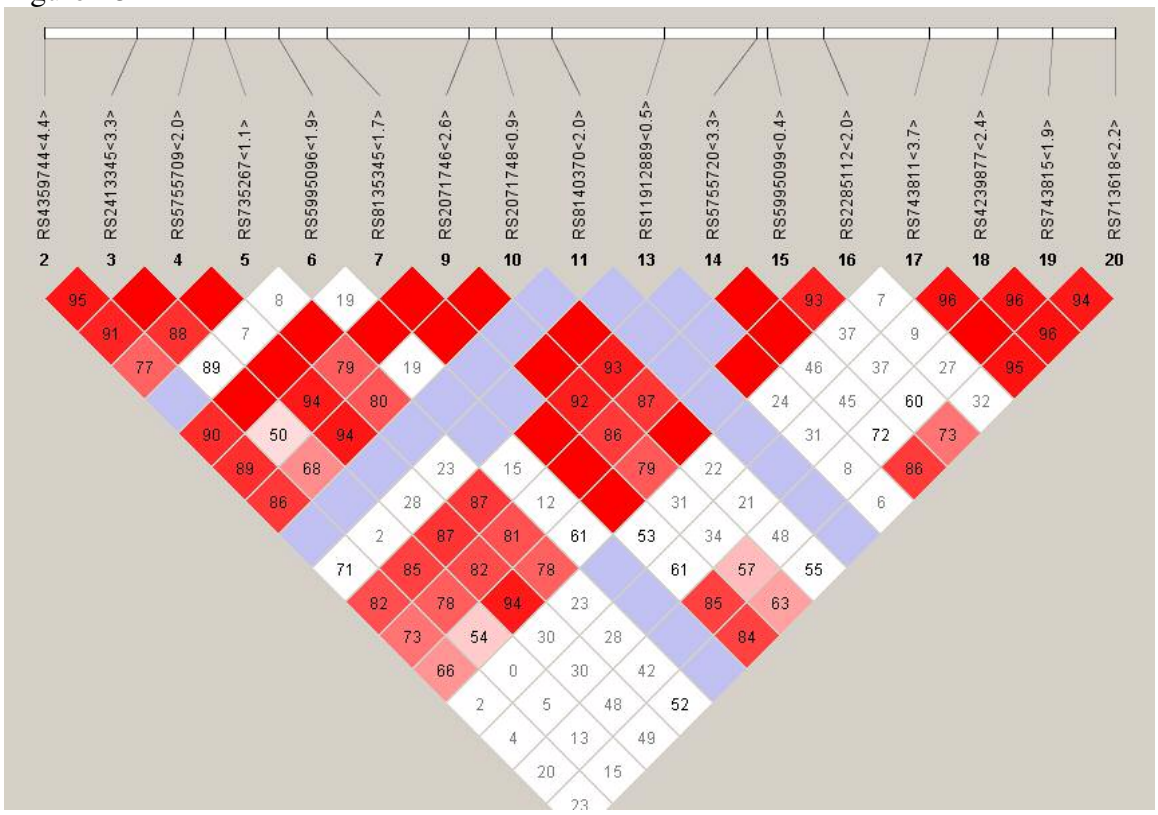

Figure E6