

Supplemental Table 1. Distribution of *ADRB2* 5'-UTR methylation status by asthma severity status (N=182 children with asthma, CT and MA, 2006-2009)

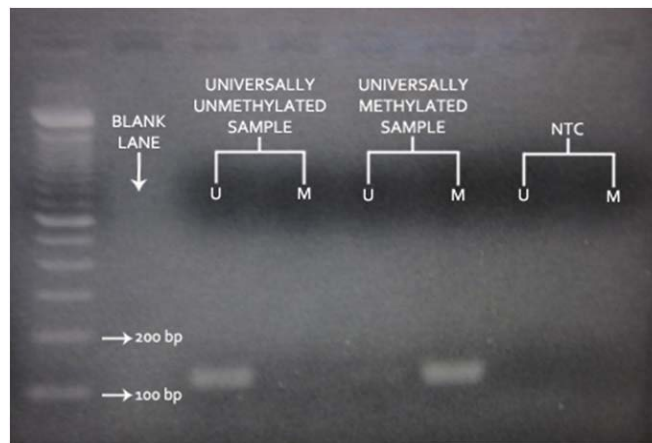
<i>ADRB2</i> methylation level	Mild cases (N = 60) N (%)	Severe cases (N = 122) N (%)	χ^2 P-value
Low	20 (33.3)	10 (8.2)	
Intermediate	20 (33.3)	39 (32.0)	
High	20 (33.3)	73 (59.8)	<0.0001

Supplemental Table 2. Distribution of NO₂ exposure levels by asthma severity status following stratification by *ADRB2* 5'-UTR methylation level (N=182 children with asthma, CT and MA, 2006-2009)

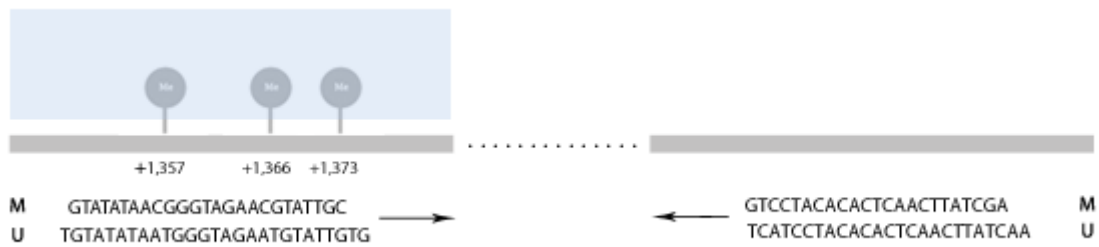
NO₂ exposure level	Low <i>ADRB2</i> methylation		<i>P</i>-value^a	High <i>ADRB2</i> methylation		<i>P</i>-value^a
	Mild cases (N = 20) N (%)	Severe cases (N = 10) N (%)		Mild cases (N = 40) N (%)	Severe cases (N = 112) N (%)	
Low	17 (85.0)	10 (100.0)	0.532	38 (95.0)	90 (80.4)	0.041
High	3 (15.0)	0 (0.0)		2 (5.0)	22 (19.6)	

^a*P*-values were calculated using Fisher's exact test.

Supplemental Figure 1. Agarose gel confirmation of amplicate length, purity, and primer specificity. A real-time PCR was performed using universally methylated and universally unmethylated DNA with methylated and unmethylated ADRB2 primers. Samples were equalized for DNA amount and the PCR was halted at the 35th cycle, or 10 cycles following the appearance of the first amplification curve. Amplificates of both methylated (M) and unmethylated (U) primers showed one specific band at 110 bp, which approximates our expected amplicon length (105 bp). The contrast in band intensities between primer-specific amplificates for both methylated and unmethylated samples suggests specificity of methylated and unmethylated primers for methylated and unmethylated sequences, respectively.



Supplemental Figure 2. Schematic representation of measured CpG loci within the *ADRB2* 5'-UTR region. The primers were designed to interrogate the methylation of 3 CpG sites, located 1,357, 1,366, and 1,373 bases downstream of the transcriptional start site within a CpG island (highlighted in light blue).



Supplemental Figure 3. Frequency distribution of *ADRB2* methylation values among children with mild and severe asthma. Median % methylation were 0.81% and 1.14% for children with mild and severe asthma, respectively. A two-sided independent Student's t-test and Wilcoxon rank-sum test were used to interrogate statistical differences in *ADRB2* methylation between the two groups. Methylation was found to be significantly higher in severe asthmatics compared to mild asthmatics ($P < 0.001$ for both tests).

