

Effect of Air Pollution on Asthmatic Patients in China: Role for DNA Methylation Changes

Study PIs:

Yahong Chen, MD PhD

Steven Huang, MD



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Asthma and Pollution:

- Asthma affects all people: both genders, all races and ethnicities, and all ages (children and adults)
- The incidence of asthma in China is **rising**
 - Prevalence in some cities (e.g. Hong Kong) is 11%
- The increased incidence correlates with the increase in industrialization
 - Mirrors that in Europe, United States, and other Asian countries
- Air pollution is one risk factor for asthma that is associated with:
 - Increased development of asthma (esp among children)
 - Increased disease severity
 - Increased hospitalizations and rates of exacerbations

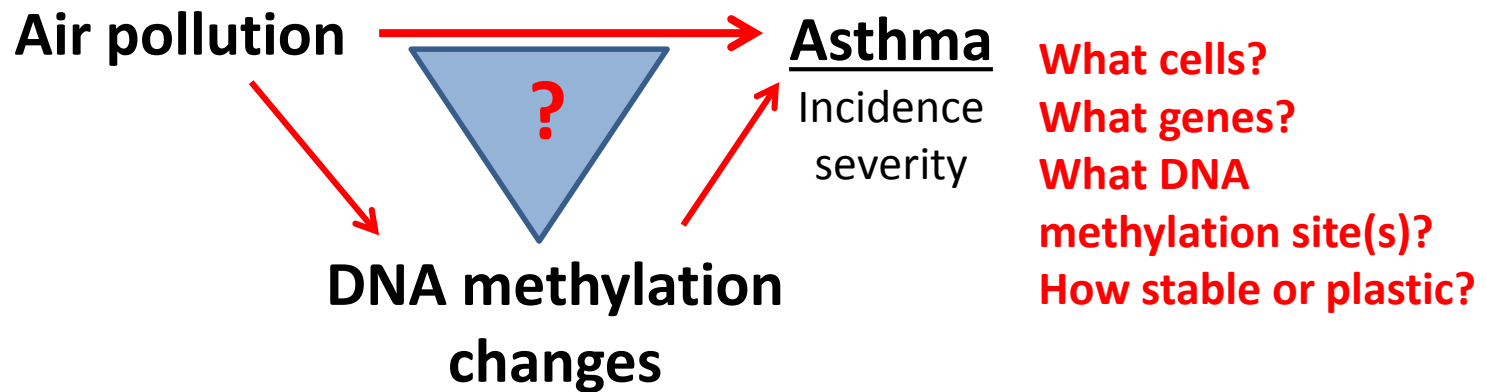


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- Air pollution has been associated with DNA methylation changes detected in certain tissues and cells
 - Whole blood
 - Peripheral blood mononuclear cells
 - Nasal epithelium
- Certain DNA methylation changes have been identified in higher proportion of patients who develop asthma



Hypothesis: Air pollution contributes increased adult asthma severity via changes in DNA methylation of key genes found in T cells and bronchial epithelial cells

Specific Aims

- **Aim 1:** Examine how variations in pollution levels correlate with the symptoms, severity, and DNA methylation changes among adults with asthma
 - Correlate variations in levels of pollution with clinical indices of asthma severity
 - Correlate variations in levels of pollution with levels of DNA methylation for specific genes in whole blood and isolated CD3+ T cells
- **Aim 2:** Examine the mechanisms by which particulate matter induce DNA methylation changes in bronchial epithelial cells

Patient enrollment

- **Inclusion criteria:** 18-60 yo non-smokers or ex-smokers diagnosed with asthma based on Global Initiative for Asthma (GINA) criteria, live in the Beijing District, exacerbation-free in the past month, symptoms not fully controlled (moderate-to-severe disease)
- **Exclusion criteria:** Current smoker, known history of COPD or alternative lung disease, inability to follow instructions
- **Goal:** enroll **50** patients and follow them **longitudinally** for 1 year, monitoring their asthma symptoms and severity. These data will be compared with variations in pollution exposure over time and DNA methylation from peripheral blood T cells.



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Study Flow Chart

	baseline	daily	monthly	At 3 months	At 6 months	At 9 months	At 12 months	At acute exacerbations
demography	√							
Asthma control test (ACT)	√		√	√	√	√	√	√
Exacerbation	√		√	√	√	√	√	√
PFT	√		√	√	√	√	√	√
Airway inflammation	√		√	√	√	√	√	√
Air pollution monitor	√	√	√	√	√	√	√	√
Blood sample	√			√	√	√	√	√



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Study Progress

Type	Total
Enrolled	37
Finished	19
At least 6 months	33
Being followed	13
Dropped out	5

- Clinical data: baseline demographics, medication usage, monthly PFTs, monthly asthma control questionnaires, FE_{NO}
- Blood was taken at baseline and every 3 months
 - DNA was isolated from whole blood
 - DNA was also isolated from purified peripheral blood CD3+ T cells selected using magnetic beads
- Daily records of PM₁₀, PM_{2.5}, CO, SO₂, NO, and O₃ levels in Beijing were obtained



(1) Baseline characteristics

Variable	Value
Sex (male/female, n)	16/12
Age (year)	44.4±12.0
BMI (kg/m ²)	25.6±3.9
smoking status [n, (%)]	
Current smoker	3(10.7)
Ex-smoker	5(17.9)
Never smoke	20(71.4)
Smoking amount (pack years)	6.5 (1.4, 32.0)
Passive smoking [n, (%)]	8(28.6)
Occupational history [n, (%)]	2(7.1)
Allergic history [n, (%)]	14(50.0)
Family history [n, (%)]	
Chronic bronchitis	3(10.7)
COPD	1(3.6)
Lung cancer	1(3.6)
Combination [n, (%)]	
Allergic rhinitis	19(67.9)
Hypertension	5(17.9)
Hyperlipidemia	3(10.7)
hyperuricemia	2(7.1)
Peptic ulcer	2(7.1)
Sleep apnea	1(3.6)
Angina	1(3.6)

Variable	Value
Onset age (year)	37.1±11.3
Onset month	Nov
Exacerbation [n, M (min, max)]	0(0, 2)
Severity [n, (%)]	
Intermittent condition	4(14.3)
Mild persistent	8(28.6)
Moderate persistent	11(39.3)
Severe persistent	5(17.9)
Control level [n, (%)]	
Well controlled	2(7.1)
Partly controlled	16(57.1)
Uncontrolled	10(35.7)
ACT score	18.1±4.6
FEV1% pred (%)	78.5±23.3
FEV1/FVC (%)	70.2±14.3
FEV1 change (%)	6.0±7.4
FENO (ppb)	51.4±37.5
Inhaled drugs [n, (%)]	
Budesonide/formoterol powder inhaler	20(71.4)
Salmeterol/fluticasone powder inhlaer	5(17.9)
Albuterol aerosol	7(25.0)
Tiotropium inhaler	1(3.6)
Budesonide powder inhaler	1(3.6)
Budesonide aerosol	1(3.6)
Oral drugs [n, (%)]	
Montelukast	19(67.9)
Expectorant	3(10.7)
Antiallergic Drugs	2(7.1)
Theophylline	2(7.1)
Antibiotic	1(3.6)



(2) Distribution of particulate matter exposure levels

Daily ambient air pollution data :
from nearby central air-monitoring stations.

Average exposure concentrations:
during 1 day to 7 days before follow up

Pollutants	Mean \pm S D	Percentiles					IQR
		Minimum	25th	50th	75th	Maximum	
PM 2.5,ug/m ³	75.0 \pm 43.8	17.3	45.0	63.9	93.9	255.1	48.9
PM 10,ug/m ³	73.0 \pm 46.2	2.4	37.3	66.8	102.4	214.7	65.1
SO ₂ ,ug/m ³	10.5 \pm 8.1	1.4	3.7	8.0	14.4	33.7	10.7
NO ₂ ,ug/m ³	56.6 \pm 20.2	15.9	41.4	56.3	68.7	124.9	27.3
CO,ug/m ³	1.3 \pm 0.8	0.3	0.7	1.0	1.6	4.8	0.9
O ₃ ,ug/m ³	52.9 \pm 34.4	6.6	18.6	50.7	84.2	122.1	65.6

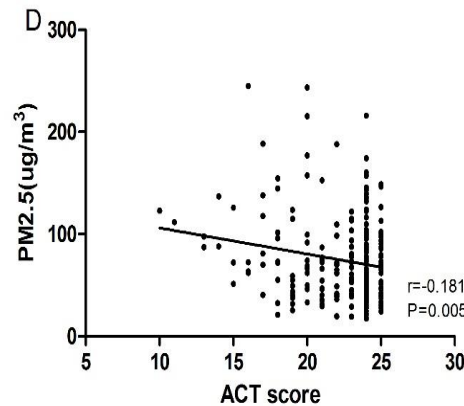
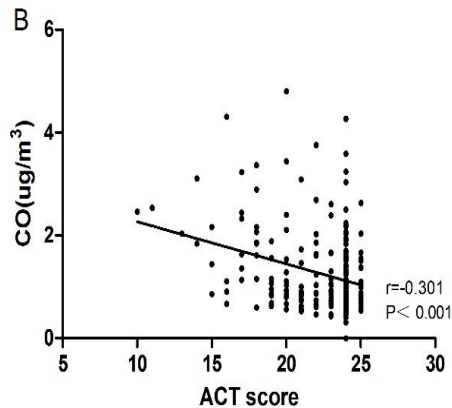
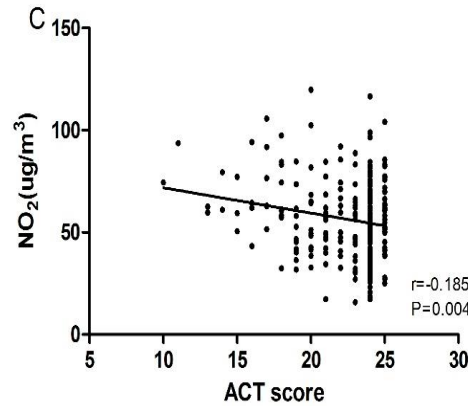
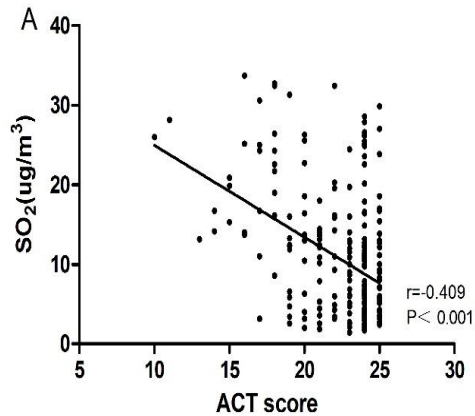
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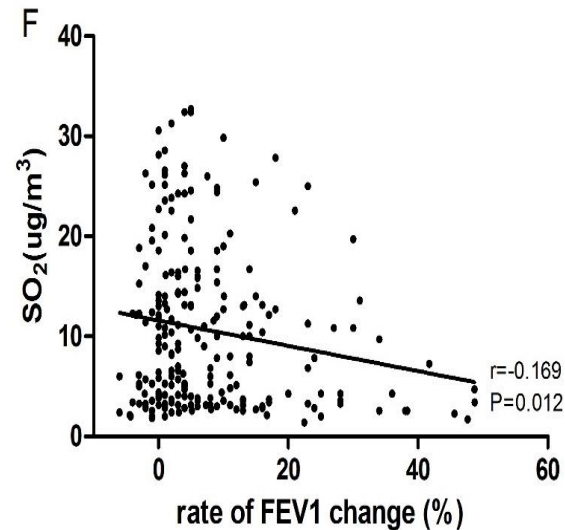
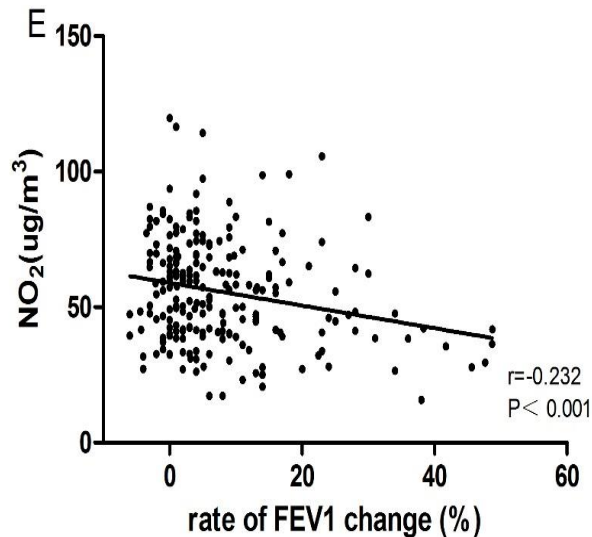
(3) Increased pollution is associated with worse asthma symptom control



SO₂ ($r = -0.409$),
CO ($r = -0.301$),
NO₂ ($r = -0.184$),
PM_{2.5} ($r = -0.181$)
were negatively
correlated with
asthma control test
(ACT) scores



Certain pollutants were negatively associated with lung function



NO₂ ($r^2 = -0.23$) and SO₂ ($r^2 = -0.168$) and were negatively correlated with rate of FEV1 change



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(4) Linear mixed model

Take ACT and SO₂ for example:

Covariates:

Age

Gender

BMI

smoking status

severity of asthma

asthma control level

allergic history

Index	Estimate (95% confidence interval)	t	-2 Restrcted Log Likelihood
Intercept	27.09 (23.23, 30.94)	<0.001	1100.62
SO ₂	-0.14 (-0.18, -0.10)	<0.001	
Gender	-1.63 (-2.60, -0.67)	<0.01	
BMI	-0.12 (-0.22, -0.03)	<0.05	
Somke status	1.21 (0.08, 2.33)	<0.05	
Asthma control level	-0.46 (-1.07, 0.15)	0.14	
Age	0.03(-0.01, 0.06)	0.16	
Allergy history	0.41 (-0.27, 1.09)	0.24	
Severity of asthma	-0.20(-0.65, -0.26)	0.40	

Estimated changes with 95% CI in ACT associated with one unit change in clinical data in SO₂-pollutant model



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Significance in association between certain pollutant and ACT or lung function in single-pollutant and all-pollutant models

Index	Main pollutants	Estimate (95% confidence interval)	t	-2 Restricted Log Likelihood
Single-pollutant model				
ACT score	PM2.5	-0.0095 (-0.0183, -0.0007)	<0.05	1159.239
	SO ₂	-0.1405 (-0.1818, -0.0991)	<0.001	1100.622
	NO ₂	-0.0217 (-0.0413, -0.0021)	<0.05	1157.409
	CO	-0.9717 (-1.4300, -0.5135)	<0.001	1124.795
Rate of FEV1 Change	SO ₂	-0.2342(-4.0001,-0.0684)	<0.01	1592.020
	NO ₂	-0.1163(-0.1872, -0.0454)	<0.01	1604.237
All-pollutant model				
ACT score	SO ₂	-0.1477 (-0.2055, -0.0899)	<0.001	1102.831
Rate of FEV1 Change	SO ₂	-0.2404 (-0.4682, -0.0127)	<0.005	1561.503
	NO ₂	-0.1873 (-0.3084, -0.0662)	<0.01	1561.503
	CO	5.5887 (1.5178, 9.6600)	<0.01	1561.503

Table 2. Estimated changes with 95% CI in ACT or lung function associated with one unit change in particular matter at key exposure metrics in single-pollutant and all-pollutant models.

DNA

Type of sample	Sample #	Volume/tube
DNA of whole blood	120	200ul/tube
DNA of CD3+ lymphocytes	120	100ul/tube

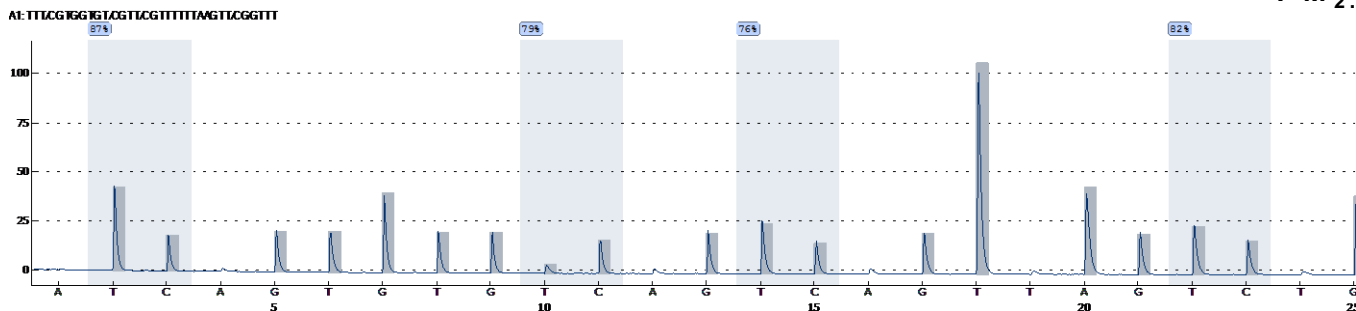
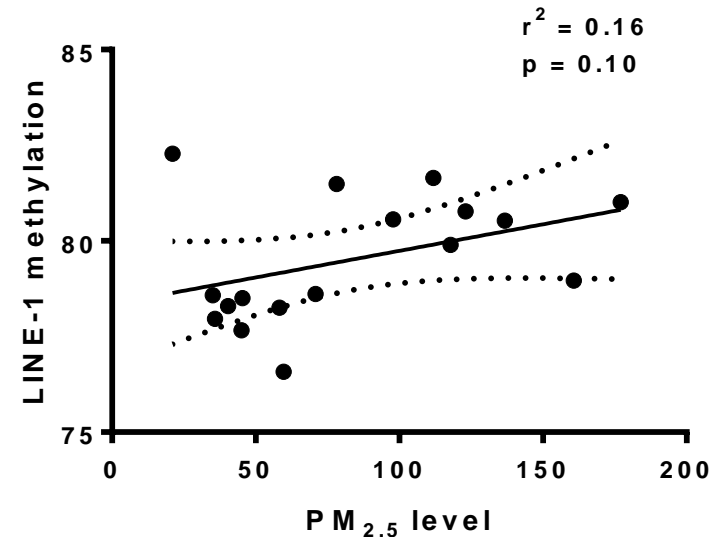
- 99 tubes of each have been successfully transported to the University of Michigan for DNA methylation analysis!

	V0		V3		V6		V9		V12		Total
	concentration	OD260/280	concentration	OD260/280	concentration	OD260/280	concentration	OD260/280	concentration	OD260/280	
001	60				8.8	1.93	18.2	2.49	70.2	1.51	4
002	58		36.9	2.4	30.3	1.78	32.4	1.73	72.4	1.75	5
003	73.9	2.13									1
004	63.21		32.5	2.6	6.3	1.52	29.1	1.47			4
005	47	2.5	36.4	2.3	10.3	1.64					3
006	61.3	2.3	38.2	2.4	18.4	1.79	17.5	2.51	21.5	1.49	5
007	46.7	2.3	28.8		26.7	1.23					3
008	28.9	2.2	34.1	2.7	19	1.7	27.6	1.87	29.4	1.86	5
009	39	2.42	42.7	2.3							2
010	46	2.66	38.6	2.24			43	1.41	24.2	1.77	4
011	44.4	2.21	35.1	2.3			14.8	1.7			3
012	93.6	2.15	26.5	1.56	7.1	1.45			99.8	1.85	4
013	40	2.5	54.1	2.3	6.4	1.59	37.2	1.86			4
014	46.3	2.3									1
015	71.4	2.15	40	1.86	12.2	1.5	21.5	1.87			4
016	76.5	2.15	80.5	2.12	8.5	1.61	21.3	1.88	53.4	1.82	5
017	59.7	2.14	24.9	1.75	10.5	1.7	19.2	1.78			4
018	30.5	2.25			41.6	2.05					2
019	32.2	2.23	27.1	1.9	39.1	2.37	31.6	1.76			4
020	63.6	2.18	1.4	1.95							2
021	75.9	2.12	10.5	1.5	20.6	1.82					3
022	36.4	1.75	42.9	2.28	52.6	1.76					3
023	16.5	1.71	21.4	2.64	39	2.5					3
024	28	1.71	30	1.8	94.9						3
025	22.9	1.74	35.1	1.65	46.9	1.75					3
026	16.9	1.78	18.5	2.11	47.8	1.78					3
027	26.5	1.8	37.9	2.16	37.6	1.77					3
028	25.8	1.82	45.5	2.25	29.4	1.86					3
029	37.3	2.35	52.5	1.8							2
030	21.3	1.88									1
031	46.4	1.73									1
032	6.8	1.85									1
033	33.3	1.76									1
Total											99

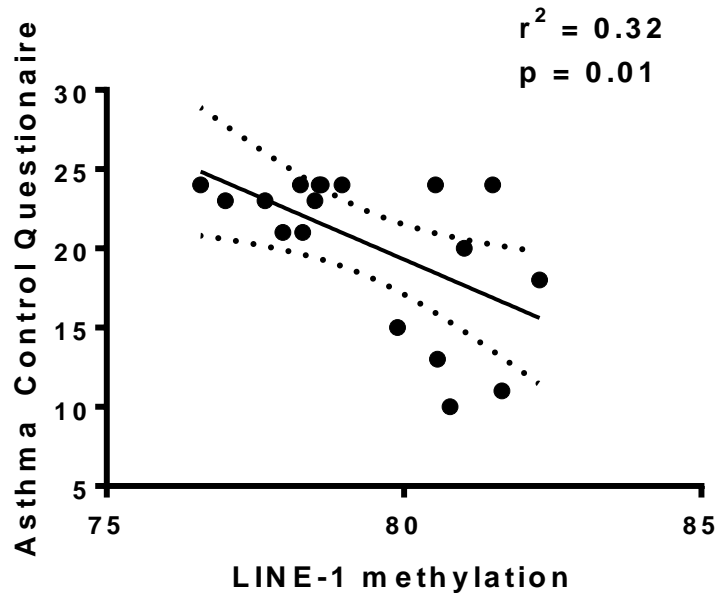


LINE-1 DNA methylation correlates with increased PM_{2.5} levels

- LINE-1 is a repeat element whose level of methylation is a surrogate for global DNA methylation
- Levels of LINE-1 DNA methylation can be analyzed via pyrosequencing



Increased LINE-1 methylation is associated with worse asthma control



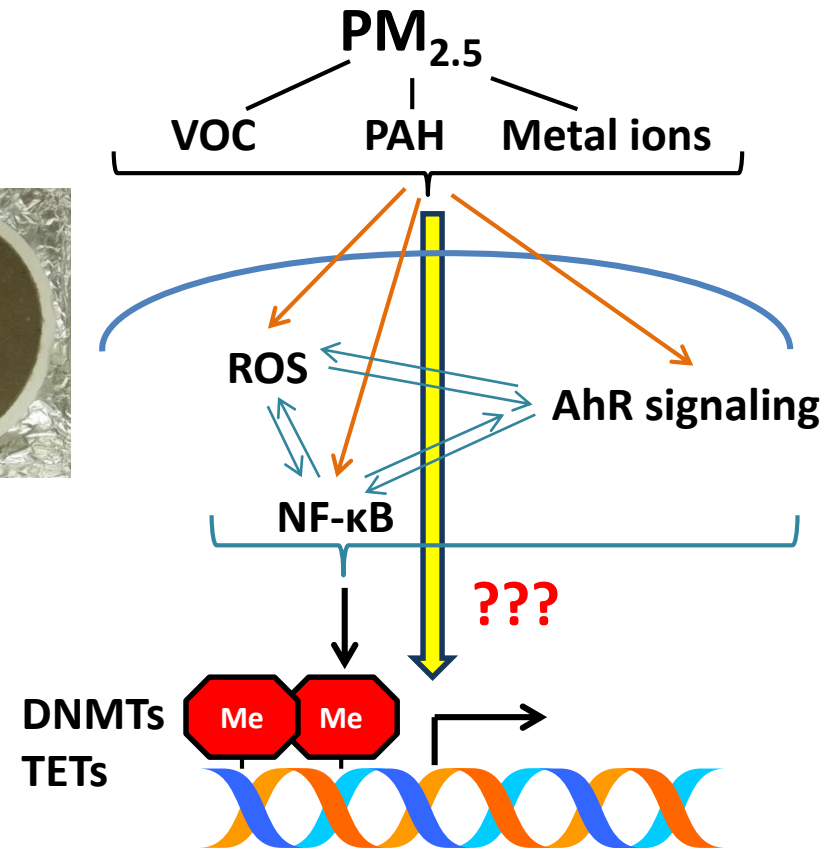
Future plans:

- We only analyzed DNA methylation in a small fraction of samples
- Examine DNA methylation for multiple genes (*FOXP3*, *NOX2*)
 - Perform whole-genome methylation
- Compare DNA methylation levels in whole blood with CD3+ T cells
- Compare DNA methylation changes with asthma severity and lung function
- Analyze how changes in pollution level over time (within each patient) affects DNA methylation levels

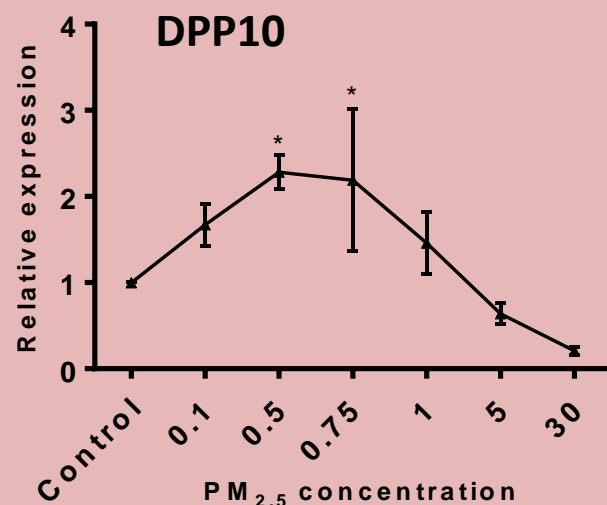
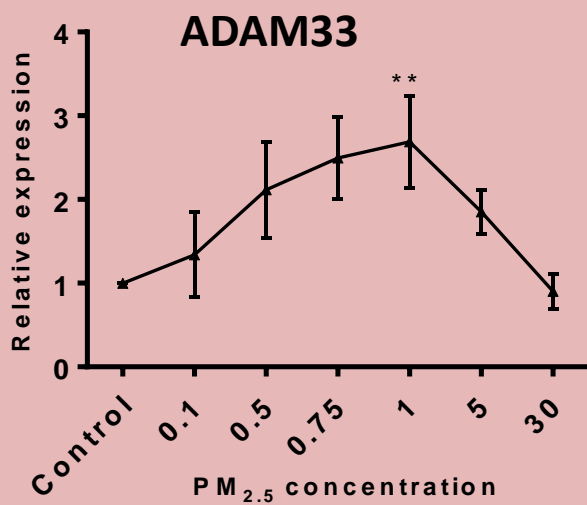
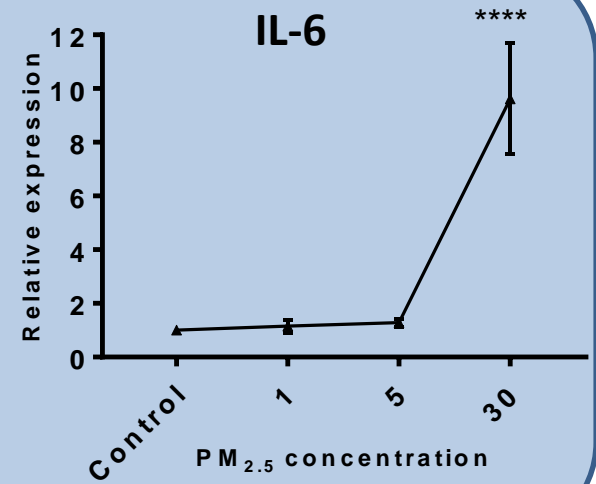
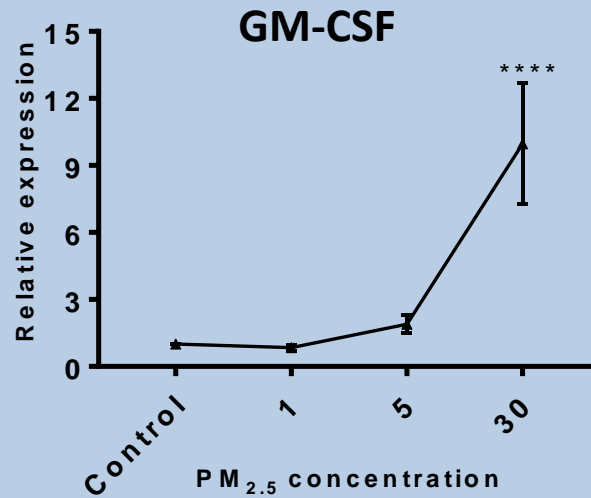
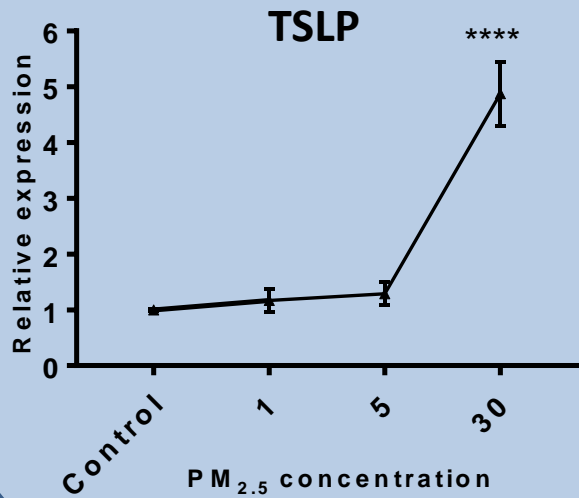


Aim 2: Examine the mechanisms by which PM_{2.5} induce DNA methylation changes in bronchial epithelial cells

- PM_{2.5} consists of combination of volatile organic compounds, polyaromatic hydrocarbons, and inorganic metals
- Although PM_{2.5} is known to have diverse cellular effects, the mechanism of its action is not completely known



Different dose-ranges of PM_{2.5} upregulate different genes associated with asthma



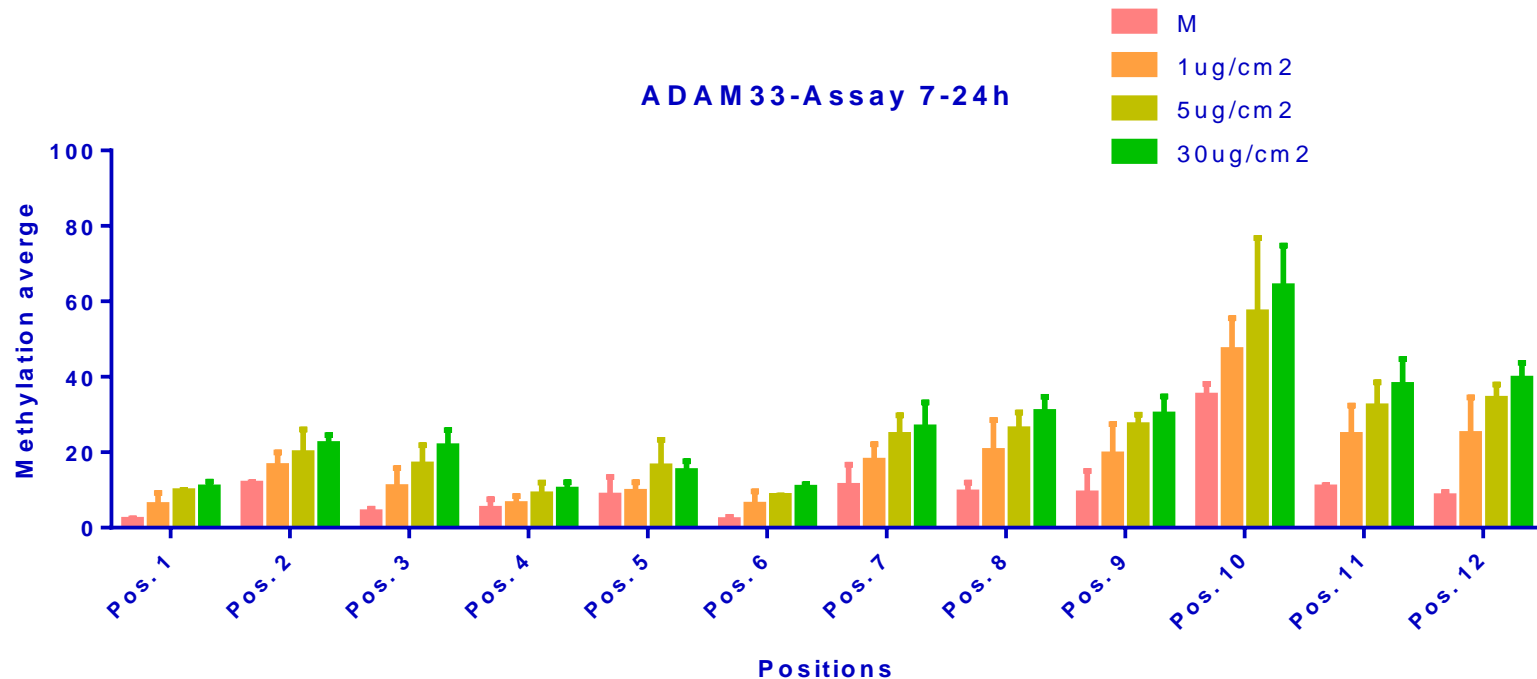
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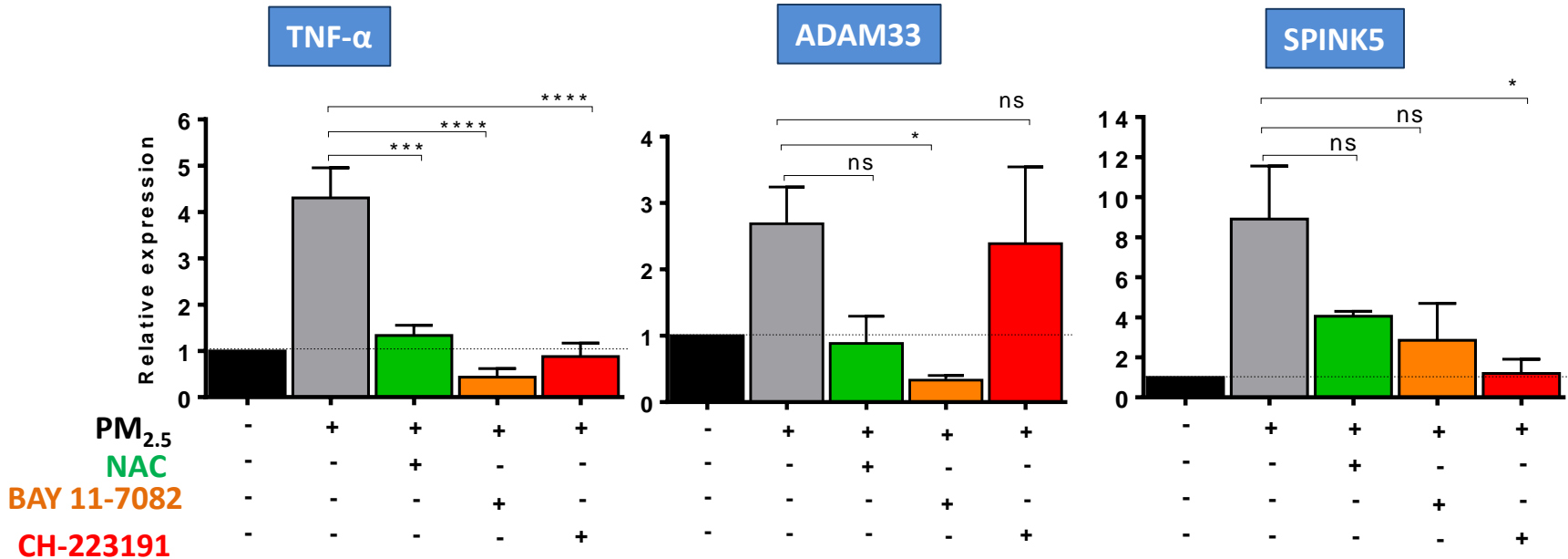
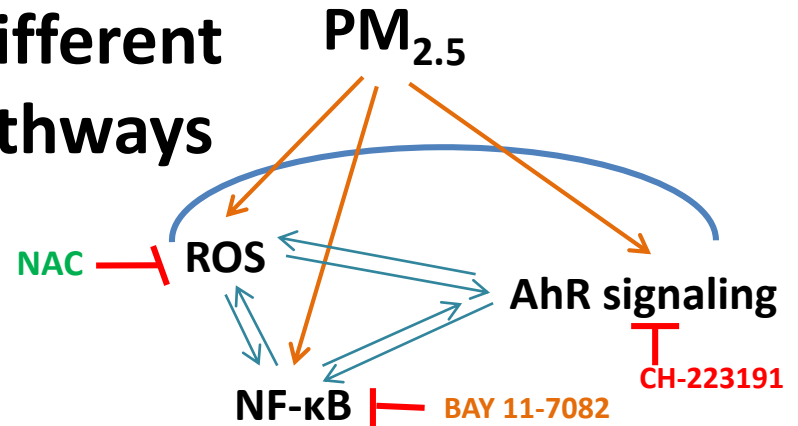


DNA methylation changes in ADAM33 associated with PM_{2.5} exposure in bronchial epithelial cells



Increased DNA methylation in gene body → Increased gene expression

PM_{2.5} alters the expression of different genes via different signaling pathways



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Conclusions

- The incidence and prevalence of asthma is increasing in China and air pollution is a major risk factor
- In our cohort, increased levels of pollution were associated with worse asthma control and worse lung function
- Global DNA methylation changes are present in the blood of asthma patients in our cohort
- *In vitro* studies suggest multiple mechanisms by which PM_{2.5} affects DNA methylation and gene expression
- In the upcoming year, we anticipate much more data focusing on the location of these DNA methylation changes in whole blood and T cells and potential mechanisms for these changes



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