teratoma formation. In addition, lung induced pluripotent stem cells exhibited no chromosomal aberrations, complete silencing of reprogramming transgenes, genomic hypermethylation, upregulation of genes encoding components of polycomb repressive complex 2, hypermethylation of stem cell polycomb targets, and modulation of more than 15,000 other genes relative to parental small airway epithelial cells. Additional sex combs like-3 (ASXL3), encoding a polycomb repressive complex 2-associated protein not previously described in reprogrammed cells, was markedly upregulated in lung induced pluripotent stem cell as well as human small cell lung cancer lines and specimens. Overexpression of the additional sex combs like-3 gene correlated with increased genomic copy number in small cell lung cancer lines. Knock-down of the additional sex combs like-3 gene inhibited proliferation, clonogenicity, and teratoma formation by lung induced pluripotent stem cells and significantly diminished *in vitro* clonogenicity and growth of small cell lung cancer cells *in vivo*.

Conclusions: Collectively, these studies highlight the potential utility of this lung induced pluripotent stem cell model for elucidating epigenetic mechanisms contributing to pulmonary carcinogenesis and suggest that additional sex combs like-3 is a novel target for small cell lung cancer therapy.

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Low-to-Moderate Arsenic Exposure and Respiratory Health in American Indian Communities

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Rationale: Exposure to inorganic arsenic, through drinking naturally-contaminated water, is an established cause of lung cancer. Evidence on the impact of arsenic exposure on lung function, however, is less conclusive. The evidence available, mostly from populations exposed to water arsenic levels $>100 \mu\text{g/L}$, suggests that arsenic exposure is associated with lower lung function. Prospective studies and studies examining low-to-moderate levels of water arsenic exposure ($<50 \mu\text{g/L}$) the level relevant for U.S. populations, are very limited.

Objectives: We evaluated the association between chronic low-to-moderate arsenic exposure with lung function and disease in an American Indian population.

Methods: The Strong Heart Study is a multicenter prospective study of cardiovascular disease and its risk factors among American Indian adults. The present analysis, in 2,166 adults, used urinary arsenic measurements at baseline (1989–1991) and lung symptoms and function assessment by standardized spirometry at the second examination (1993–1995). We evaluated associations between arsenic exposure and airflow obstruction, defined as ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) of less than 0.70, and restrictive pattern, defined as FEV_1/FVC ratio greater than 0.70 and FVC less than 80% predicted; respiratory symptoms; and self-reported physician diagnosis of nonmalignant respiratory disease.

Results: The prevalence of airflow obstruction between 1993 and 1995 was 21.4% (463/2,166); restrictive pattern was 14.5% (314/2,166). Median urinary arsenic concentrations were higher in participants with airflow obstruction ($11.0 \mu\text{g/g}$ creatinine) compared to those without obstruction ($9.8 \mu\text{g/g}$ creatinine), and higher in those with restrictive pattern ($12.0 \mu\text{g/g}$) compared to those without restrictive pattern ($9.4 \mu\text{g/g}$). The odds ratio (95% confidence interval) for obstructive and restrictive patterns comparing the 75th to 25th percentile of arsenic was 1.13 (0.96–1.32) and 1.27 (1.01–1.60), respectively, after adjustment for age, sex, education, study site, smoking status, smoking pack-year, estimated glomerular filtration rate, tuberculosis, and body mass index. Emphysema, cough 4–6 times a day, phlegm, and stopping for breath were also positively associated with arsenic.

Conclusions: In this American Indian population, exposure to low-to-moderate levels of inorganic arsenic, as measured in urine, was positively associated with restrictive pattern as measured by spirometry, self-reported emphysema diagnosis, self-reported shortness of breath, and more frequent cough and phlegm among those with cough, independent of smoking status. These findings suggest that low-to-moderate arsenic exposure can contribute to nonmalignant lung disease, and may be associated with restrictive lung disease.

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Cysteinyl Leukotriene Receptor 1 and Health Effects of Particulate Exposure in Asthma

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Rationale: Acute exposure to ambient particle matter is associated with increased levels of the cysteinyl leukotriene (CysLT) biomarker, urinary leukotriene E4 (uLTE₄), in subjects with asthma.

Objectives: The role of CysLTs in mediating asthma worsening after particulate matter exposures was explored.

Methods: Daily concentrations of particulate matter of 2.5 µm and smaller diameter (PM_{2.5}) and repeated measurements of albuterol use over a 5-month period were collected in 44 urban school children with persistent asthma. DNA was analyzed for gene polymorphisms on genes involved in the CysLT pathway to identify gene–environment interactions. An experimental challenge study in 16 adults with mild, nonpersistent asthma was performed to define biological pathways explaining these gene–environment interactions. Subjects in the challenge study were exposed on two different days to filtered air or diesel exhaust (300 µg PM_{2.5}/m³). FEV₁ and CysLT-related gene DNA methylation and messenger RNA expression changes were measured before and 6 hours after exposure challenges.

Results: In school children with asthma, associations between PM_{2.5} and school-time albuterol usage were significantly greater in those with the variant C allele in the CysLT receptor 1 (CysLTR₁) rs320995 locus (5.4% increase per interquartile range PM_{2.5} increase) compared with those homozygous for the wild-type T allele (1.6% decrease; $P = 0.005$ for allele $\times$ PM_{2.5} interaction). In the challenge study, declines in forced expiratory volume in 1 second after diesel exhaust exposure were associated with lower CysLTR₁ expression ($r^2 = 0.52$; $P = 0.01$), which, in turn, was associated with decreased leukotriene C4 synthase cg1631890 ($P = 0.02$) and increased CysLTR₁ cg26848126 ($P = 0.01$) methylation, as assessed in a multivariable model ($r^2 = 0.69$).

Conclusions: Health effects of acute particulate exposure on asthma are associated with changes in CysLTR₁ expression and methylation of CpG sites on CysLTR₁ and leukotriene C4 synthase genes.

Keywords: asthma; cysteinyl leukotriene receptor 1; methylation; polymorphisms; particulate matter

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Signal Transducer and Activator of Transcription 1 Regulates Multiwalled Carbon Nanotube–induced Pulmonary Fibrosis in Mice via Suppression of Transforming Growth Factor-β1 Production and Signaling

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Rationale: Multiwalled carbon nanotubes (MWCNTs) are a potential risk for pulmonary fibrosis because of their fiber-like shape; other physicochemical features, such as rigidity, could also confer fibrogenicity. The signal transducer and activator of transcription 1 (STAT1) is an important antifibrogenic transcription factor that promotes fibroblast growth arrest. STAT1-deficient (*Stat1*^{−/−}) mice are susceptible to pulmonary fibrosis.

Objectives: In this study, we hypothesized that *Stat1*^{−/−} mice exhibit a differential fibrogenic response to tangled MWCNTs versus rigid MWCNTs above that seen with wild-type (*Stat1*^{+/+}) mice.

Methods: *Stat1*^{+/+} and *Stat1*^{−/−} mice were exposed to tangled MWCNTs or rigid MWCNTs (4 mg/kg) via oropharyngeal aspiration, and lung tissues were collected after 1 and 21 days to measure messenger RNA and protein levels of fibrogenic mediators.

Results: Compared with tangled MWCNTs, rigid MWCNTs caused mucous cell metaplasia, epithelial cell proliferation, increased fibrosis, and larger granulomas in the lungs of mice. Both MWCNT types induced acute neutrophilia; however, only rigid MWCNTs induced chronic neutrophilia. *Stat1*^{−/−} mice exhibited higher serum levels of immunoglobulin E and even higher levels when treated with rigid MWCNTs. Twenty-one days after treatment with rigid MWCNTs, compared with *Stat1*^{+/+} mice, *Stat1*^{−/−} mice had higher levels of transforming growth factor-β1 (TGF-β1) protein in bronchoalveolar lavage fluid, increased transforming growth factor-β1 signaling activation, and higher airway collagen deposition. Some of the results of this study were previously reported in the form of an abstract and a paper (1, 2).

Conclusions: Our data emphasize that rigid MWCNTs produce much stronger immune and fibrogenic responses than tangled MWCNTs and that these effects are exaggerated by