Estimating the Causal Effects of Adolescent and Adult Smoking Behavior on DNA Methylation Signatures

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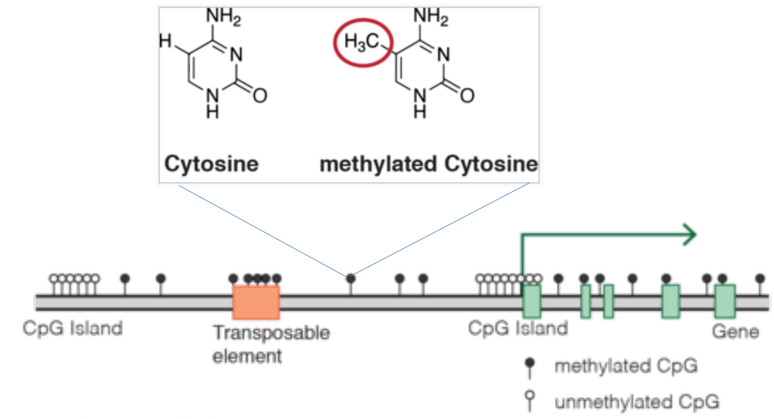
Background

- Smoking is one of the leading causes of mortality & morbidity – 7M deaths/ year globally (WHO, 2017)
- Confers long-term disease risk e.g. cancer, respiratory and CVDs
- Mechanisms behind long-term effects not well understood
- DNA methylation (DNAm) at suggested as one possible explanation
- DNAm is the addition of methyl groups to the DNA molecule at cytosine bases; usually represses gene transcription when located in a gene promoter (does not alter DNA sequence)
- DNAm is essential for normal development - associated with several processes e.g. carcinogenesis, aging, genome imprinting, etc.
- Epigenome Wide Association Studies (EWAS) have shown reproducible association between smoking & altered DNAm at thousands of CpGs (Joehanes et al., 2016; Sikdar et al., 2019)
- However, it is not clear whether differential DNAm are:
  - Caused by smoking
  - Downstream consequences of related co-morbidities
  - Caused by environmental factors highly correlated with smoking
- Research is needed to refine & validate smoking DNAm signatures
- Smoking DNAm signatures may serve as biomarkers of lifetime smoking exposures, shed light on molecular mechanisms & used as predictors of smoking-related diseases
- This study aims to estimate the causal effect of smoking in adolescence and adulthood on DNAm by exploiting state-year variation in cigarette taxes and data.

Data

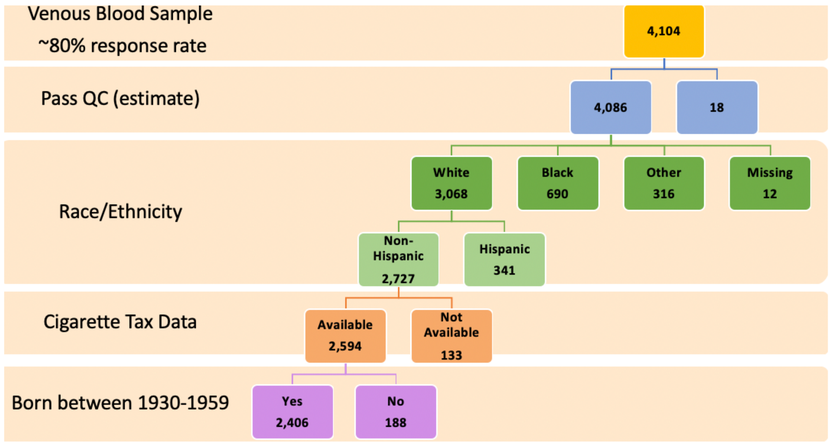
- **Health and Retirement Study (HRS):**
  - All available waves (1992-2016) ~4,000 DNAm samples (pop. representative); ~2,406 European ancestry DNAm samples
  - Smoking initiation (SI) as extensive margin and cigarettes per day (CPD) as an intensive margin measure of smoking
  - For SI, CpGs as reported by Sikdar et al., 2019
  - For CPD, CpGs reported by Joehannes et al., 2016
  - We'll exclude CpGs unique to newborns from maternal smoking & those overlapping between newborns & adults
- **Cigarette Tax Data:**
  - Annual excise taxes per cigarette pack from The Tax Burden on Tobacco (Orzechowski & Walker, 2016) adjusted for inflation
  - Cigarette tax data span all available years the HRS cohorts were in adolescence or adulthood (1940-2016)
  - Average of state and federal cigarette taxes respondents were exposed to:
    - Ages10-18 (1940-1977) to capture adolescent exposure
    - Average of HRS survey years (1992-2016) for adult exposure
- **Tobacco Control Policies Data (controls):**
  - State-level tobacco control policies extracted from *ImactTeens Project* & the Non-Smokers' Rights Foundation (ANRF) databases
  - Key variables to be used are youth access laws & smoke-free air laws

Typical Mammalian DNAm Landscape

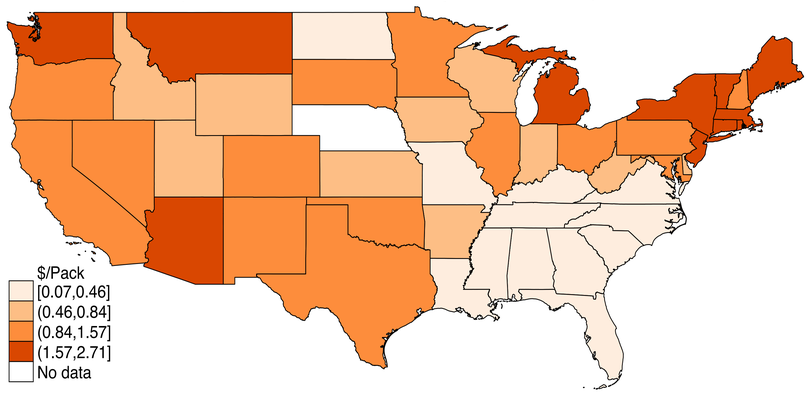


Source: https://en.wikipedia.org/wiki/DNA_methylation

HRS DNAm Sample (approx., data will be released soon)



Spatial Variation in the Inflation Adj. State Excise Tax, 2007



Empirical Strategy

- Exploit state-year variation in cigarette taxes as an instrumental variable
- Use 2-Stage Least Square (2SLS) model:

First stage:

$$\boldsymbol{SmokingPhen_{isbt}} = \boldsymbol{\pi_0} + \boldsymbol{\pi_1 CigTax_{st}} + \boldsymbol{X'_{isbt}}\,\boldsymbol{\pi_2} + \boldsymbol{S_s} + \boldsymbol{B_b} + \mathrm{P}_{st} + \boldsymbol{\varepsilon_{isbt}} \quad (1)$$

- $SmokingPhen_{isbt}$ is smoking phenotypes of interest for individual *i* in state s born in year *b* & interviewed in time *t*
- $CigTax_{st}$ is the average state cigarette tax during adulthood or adolescence
- X_{isbt} is a vector of individual characteristics including sex, race & education
- S_s *and* B_b are fixed effects that control for time-invariant state & cohort characteristics
- P_{st} is a vector of state-level tobacco control policies

Second stage:

$$\boldsymbol{CpG_{isbt}} = \boldsymbol{\delta_0} + \boldsymbol{\delta_1 SmokingPhen_{it}} + \boldsymbol{X'_{isbt}\delta_2} + \boldsymbol{S_s} + \boldsymbol{B_b} + P_{st} + \boldsymbol{\varepsilon_{isbt}} \quad (2)$$

- CpG_i is a single *CpG* site or average (aggregate) methylation beta value for *CpG* sites linked with a single known gene

- Additionally, we'll examine whether mQTLs or PGSs for smoking moderate the relationship between cigarette taxes and DNAm
- Moreover, we'll validate our results by testing for reverse causality using a Mendelian Randomization approach – use mQTLs as IV for DNAm & test whether DNAm influences smoking behavior

- **Power Calculation:**
  - Approximate effect size of *CpG* site *p* from an "IV EWAS" as:

$$\boldsymbol{R^2_{p\ IV\ EWAS}} = \boldsymbol{R^2_{p\ 1st\ STAGE}} \times \boldsymbol{R^2_{p\ EWAS}} \quad (3)$$

  - $R^2_{p\ EWAS}$ taken from most recent smoking EWAS (Sikdar, Joehanes, Joubert, Xu, Vives-Usano, et al., 2019)
  - $R^2_{p\ 1st\ STAGE}$ estimated for smoking outcomes using incremental R^2 of the $\pi_1 CigTax_{st}$ term in equation (1)

$$\boldsymbol{\Phi}\left(\boldsymbol{\Phi}^{-1}\left(\frac{\boldsymbol{\alpha}}{\boldsymbol{k}}\right) - \sqrt{\boldsymbol{N R^2_{p\ IV\ EWAS}}}\right) + \mathbf{1} - \boldsymbol{\Phi}\left(\boldsymbol{\Phi}^{-1}\left(\mathbf{1} - \frac{\boldsymbol{\alpha}}{\boldsymbol{k}}\right) - \sqrt{\boldsymbol{N R^2_{p\ IV\ EWAS}}}\right) \quad (4)$$

  - Φ is *CDF* of the standard normal distribution and Φ^{-1} denotes its inverse function
  - 80% power to detect effects in the European ancestry sample (N=2,406) when $R^2_{p\ IV\ EWAS} > 0.003$
  - Powered to detect effects at 386 differentially methylated *CpGs* b/n never & current smokers (Sikdar et al., 2019).
  - For CPD, well powered to detect effects at 70 differentially methylated *CpGs* (Joehanes et al., 2016)

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