Clinical Implication of Smoking-Related Aryl-Hydrocarbon Receptor Repressor (*AHRR*) Hypomethylation in Japanese Adults

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COI Disclosure

Takeuchi F, Takano K, Yamamoto M, Isono M,
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Biomarker for smoking status/history

- Tobacco smoking is a leading preventable cause of morbidity and mortality.
  - Increases the risk for cardiovascular and respiratory disease, and cancer.

- Biomarker for long-term smoking behavior is needed.
  - Self-reporting is inaccurate.
  - Among current smokers, cotinine is a reliable measure of nicotine exposure, but its half-life is 15–24 hours.

➔ Use DNA methylation of *AHRR* gene as a biomarker

DNA methylation

- DNA methylation controls gene transcription.
  - CpG in promoter is unmethylated
    ➔ gene is activated
  - CpG in promoter is methylated
    ➔ gene is repressed
- DNA methylation is involved in many biological processes.
  - Cell differentiation, aging, cancer, other diseases …

https://github.com/kdkorthauer/fungeno2019
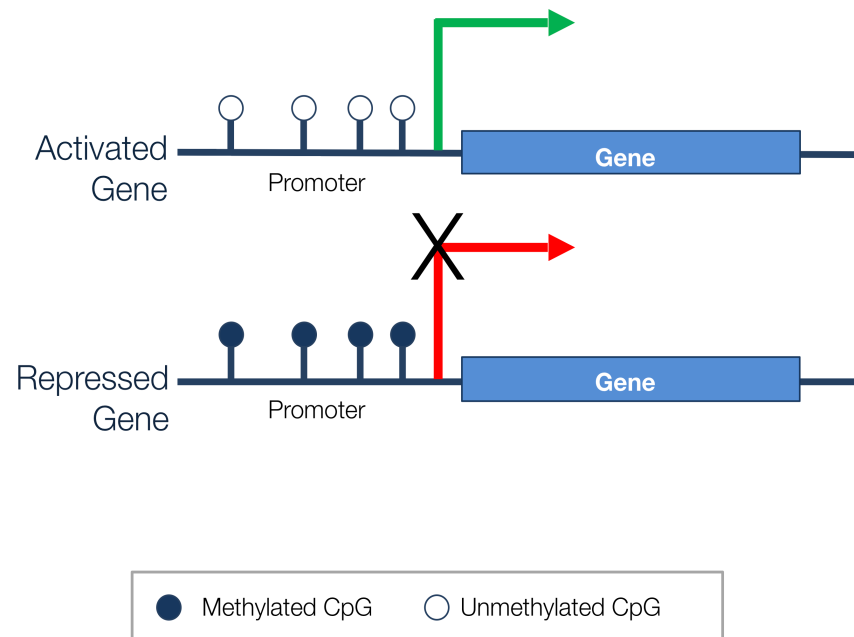
DNA methylation of *AHRR* and smoking

- *AHR, Aryl hydrocarbon receptor*
  - Receptor of xenobiotics in tobacco smoke
  - Induces cytochrome P450-mediated catabolism of the xenobiotics

- *AHR**R**, Aryl hydrocarbon receptor repressor*
  - Inhibits *AHR*
  - In smokers
    - DNA methylation is lower
    - mRNA expression is higher
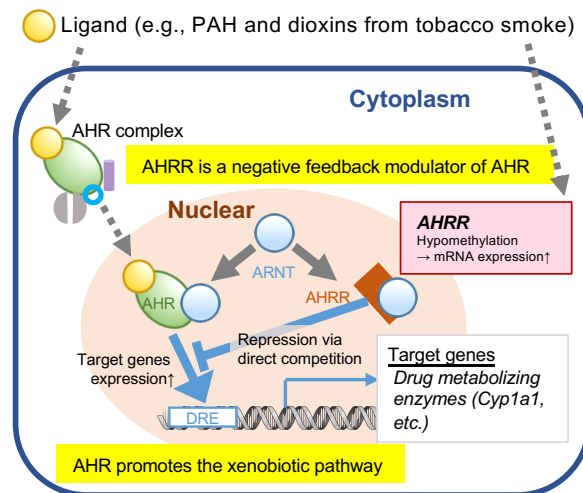
- Previous studies were in epidemiology.

- ➔ We assess the feasibility as a biomarker in clinical practice.

Methods

- Study cohorts

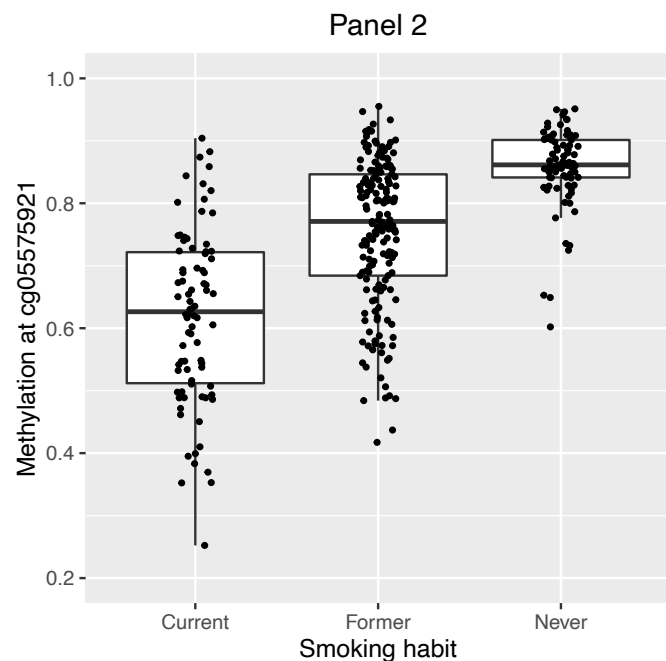|	Panel 1	Panel 2	Panel 3
No. of individuals (F/M)	94 (29/65)	339 (47/292)	300 (155/145)
Age, yr	60.3 ± 1.2	67.8 ± 0.6	64.3 ± 0.7
Smoking habit			
Never, n (%)	39 (41%)	88 (26%)	145 (48%)
Former, n (%)	31 (33%)	169 (50%)	115 (38%)
Current, n (%)	24 (26%)	82 (24%)	40 (13%)
Complication			
Hypertension, n (%)	51 (54%)	259 (76%)	155 (52%)
Diabetes, n (%)	23 (24%)	131 (39%)	57 (19%)
Hypercholesterolemia, n (%)	45 (48%)	239 (71%)	300 (100%)
CAD, n (%)	46 (49%)	295 (87%)	9 (3%)

- Multi-omics measurements
  - DNA methylation, epigenome-wide
    - Buffy coat
    - EPIC array in Panel 1
  - DNA methylation at *AHRR* cg05575921
    - ddPCR in Panels 2&3
  - mRNA expression of *AHRR*
    - Whole blood
    - qPCR in Panel 2
  - SNP genotyping, genome-wide
    - OmniExpress in Panels 2&3

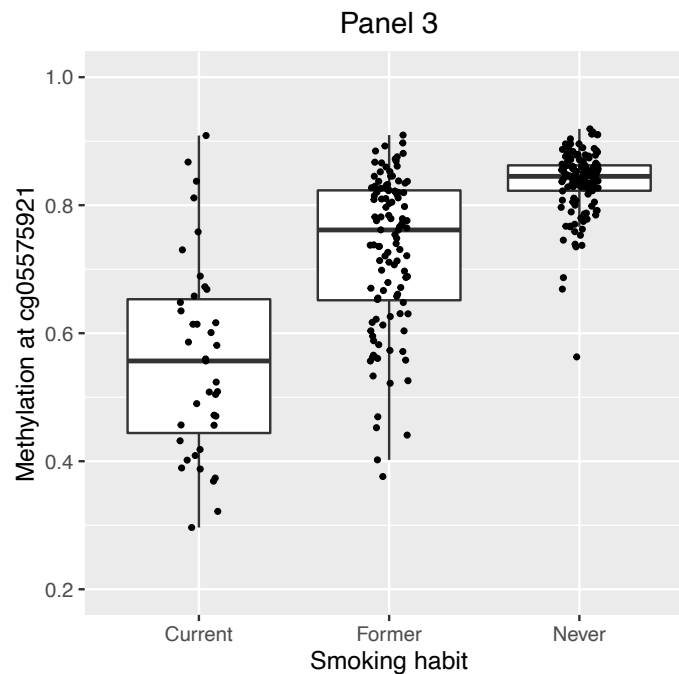# Epigenome-wide testing for association of DNA methylation with smoking status

AHRR
cg05575921
cg23576855
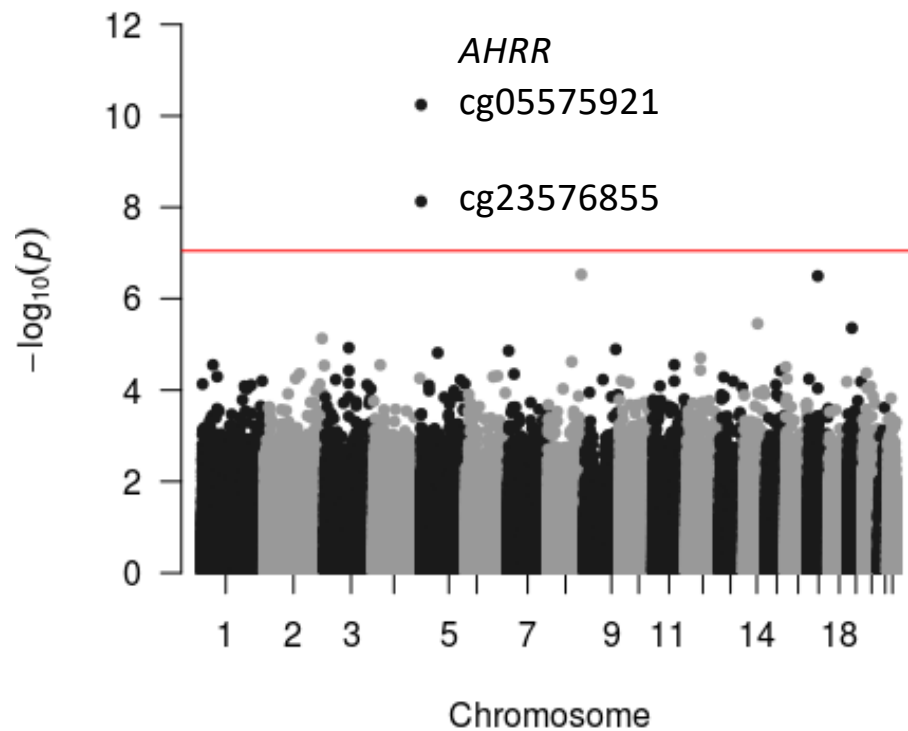
- Genome-wide significant association at two CpG sites in *AHRR* gene.

Panel 2

Panel 3

Comparison of *AHRR* methylation between smoking statuses

Panel 2

Methylation at cg05575921

1.0
0.8
0.6
0.4
0.2

Current   Former   Never
Smoking habit

Current↓↓   Former ↓   Never

$P = 2.5 \times 10^{-28}$

Panel 3

Methylation at cg05575921

1.0
0.8
0.6
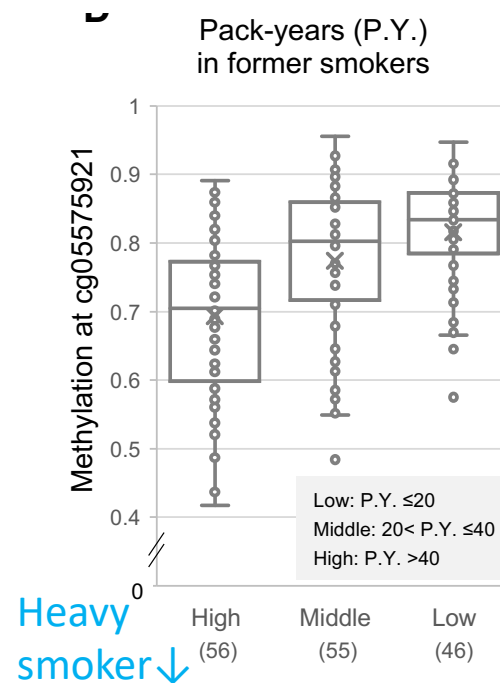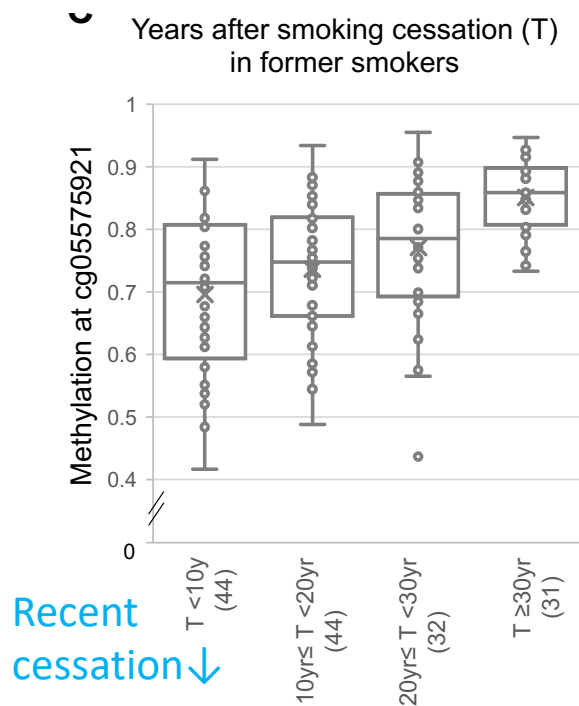0.4
0.2

Current   Former   Never
Smoking habit

Current↓↓   Former ↓   Never

$P = 5.1 \times 10^{-33}$

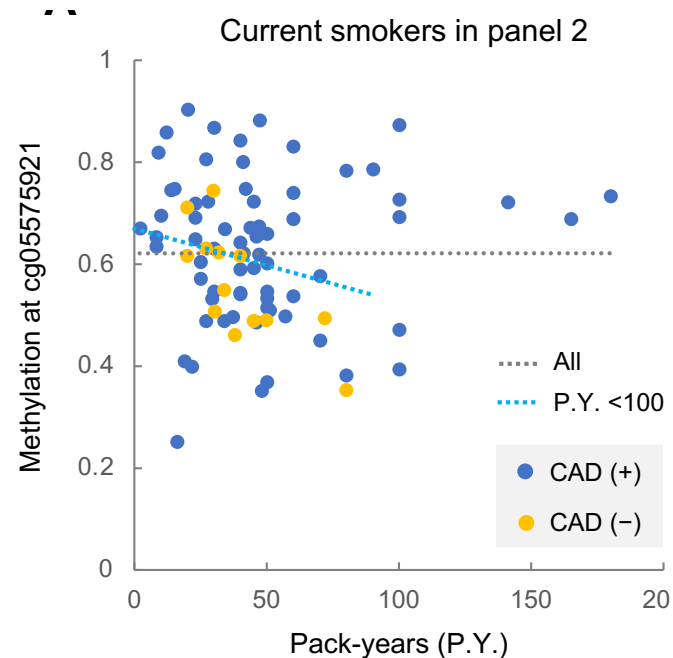- Significant differences in cg05575921 methylation between smoking statuses.

DNA methylation of *AHRR* in former smokers

Years after smoking cessation (T)
in former smokers

Pack-years (P.Y.)
in former smokers

Recent cessation↓

Heavy smoker↓

Low: P.Y. ≤20
Middle: 20< P.Y. ≤40
High: P.Y. >40

Panel 2

- cg05575921 hypomethylation gradually reverts with time since cessation.

- Inverse association between cumulative cigarette consumption and cg05575921 methylation.

DNA methylation of *AHRR* in current smokers

Current smokers in panel 2

Methylation at cg05575921

Pack-years (P.Y.)

- All ......
- P.Y. <100 ......
- CAD (+)
- CAD (−)

Current smokers in panel 3

Methylation at cg05575921

Pack-years (P.Y.)

- No apparent relationship between cumulative cigarette consumption and cg05575921 methylation (P = 0.998).
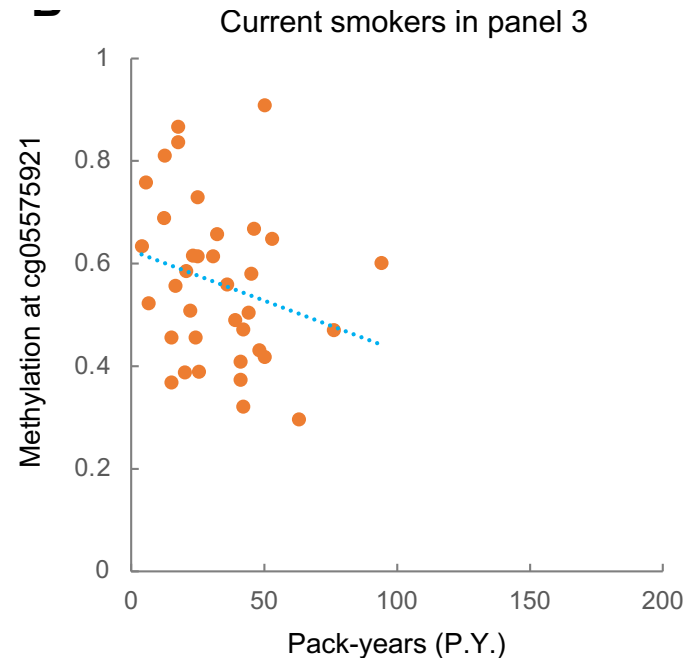
- Weak tendency of inverse association (P = 0.144).

Conclusion

- Association of DNA methylation with smoking status was most significant at *AHRR* cg05575921.

- cg05575921 hypomethylation remained for a long period of time, >20 years, after smoking cessation.

  ➔ Reversion of hypomethylation is a biomarker for smoking cessation.

- cg05575921 hypomethylation became evident from an early stage of smoking, <10 pack-years, among current smokers.

- DNA methylation is a new tool that could supplement self-report or existing biomarkers for smoking.

- Our data not only contribute to basic science but also help improve the applications of DNA methylation signature to medical care of real-world patients.