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# Probing Buccal-Customized Epigenetic Clocks to Investigate Socioeconomic and Health Disparities

Qiao Wu Ph.D.
Postdoctoral Research Scientist
Max Planck Research Group Biosocial
Max Planck Institute for Human Development

# Acknowledgment

Coauthors: **Marta Bosanac** and **Laurel Raffington**

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We do not have financial interests related to the work

We thank **Maxim N. Shokhirev** and colleagues for the computation of CheekAge

# Background

## “Clock”s

DNA-Methylation-Based Measures of Biological Aging

**1st Generation**
- Trained on Chronological Age

**2nd Generation**
- Trained on Aging-Related Biomarkers/Outcomes

**3rd Generation**
- Trained on Longitudinal Change of Aging Biomarkers

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(Many of these clocks are developed in blood)

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How to Collect a Saliva Sample
By The Hospital for Sick Children (SickKids)
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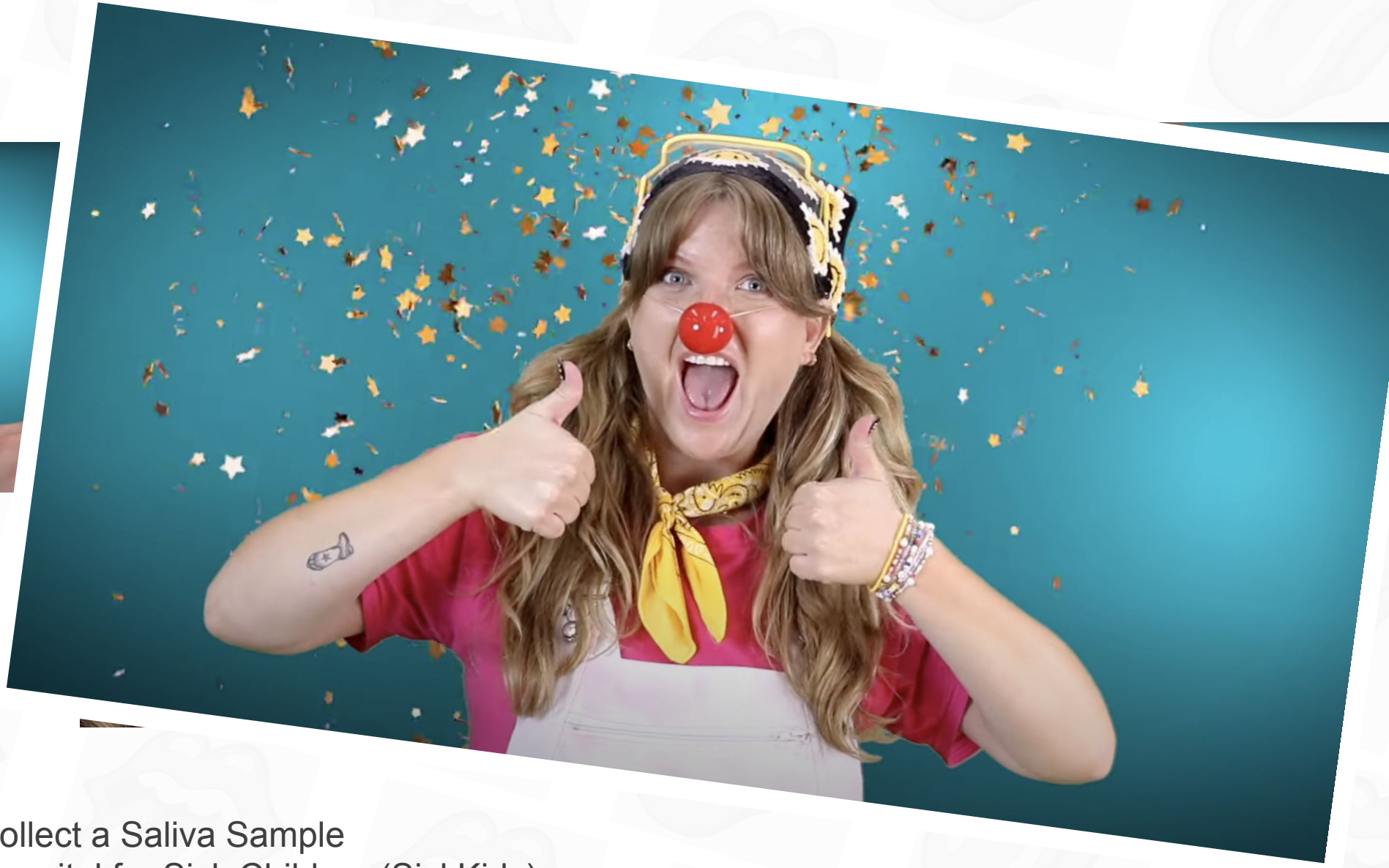

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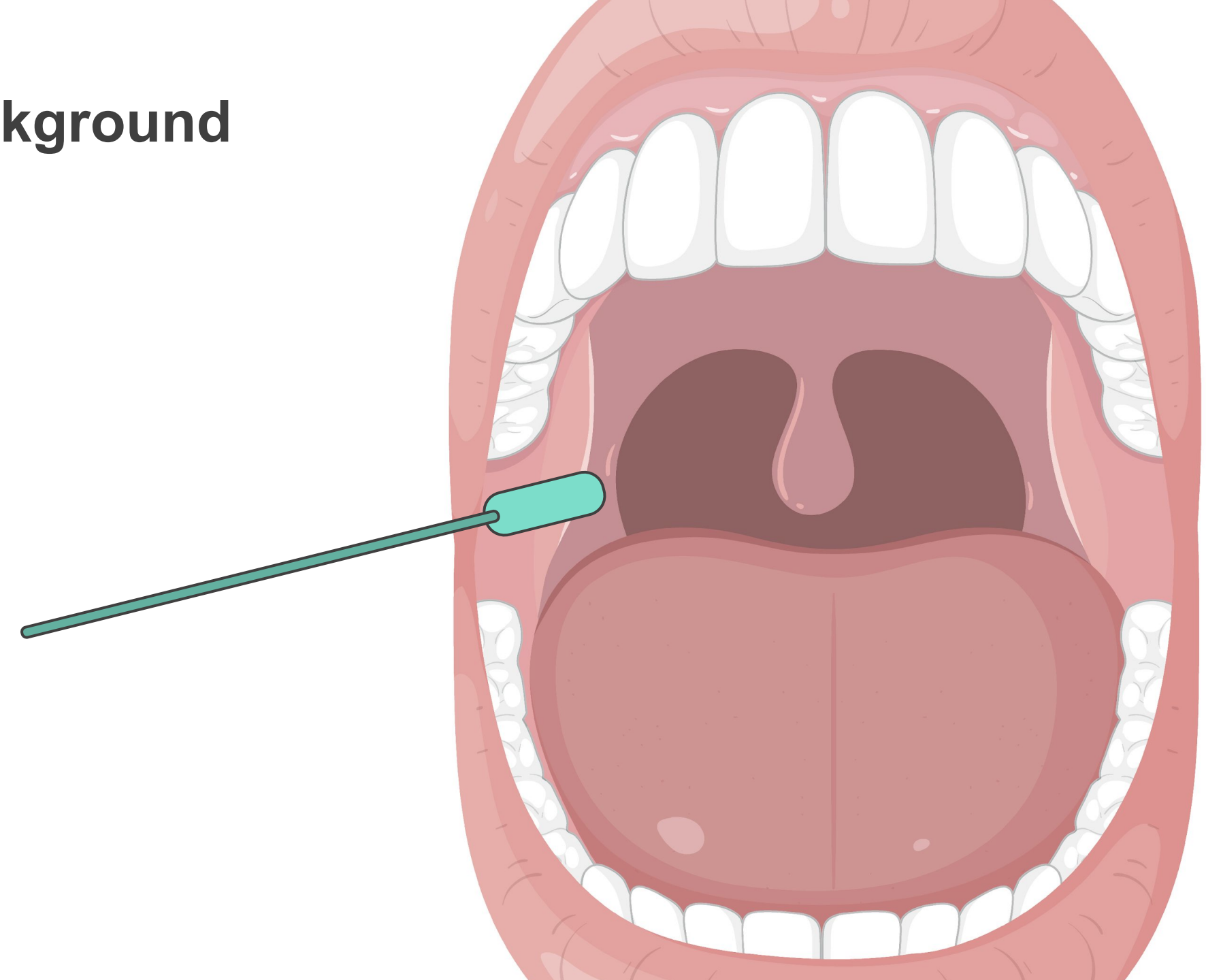

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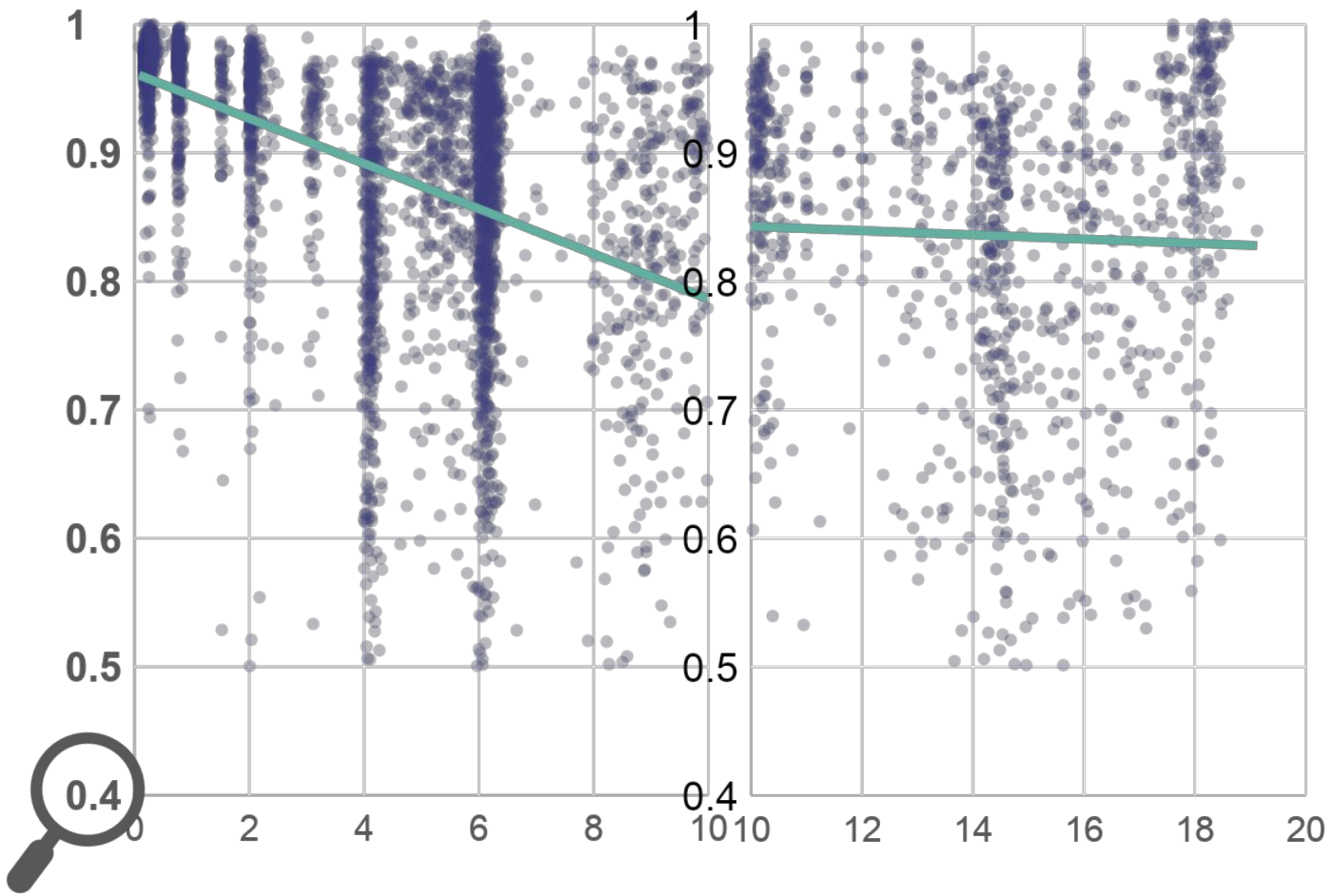



**Blood Samples:** **Predominantly Leukocytes**

**Buccal Samples:** **Primarily Epithelial Cells (>80% of the cellular content)**

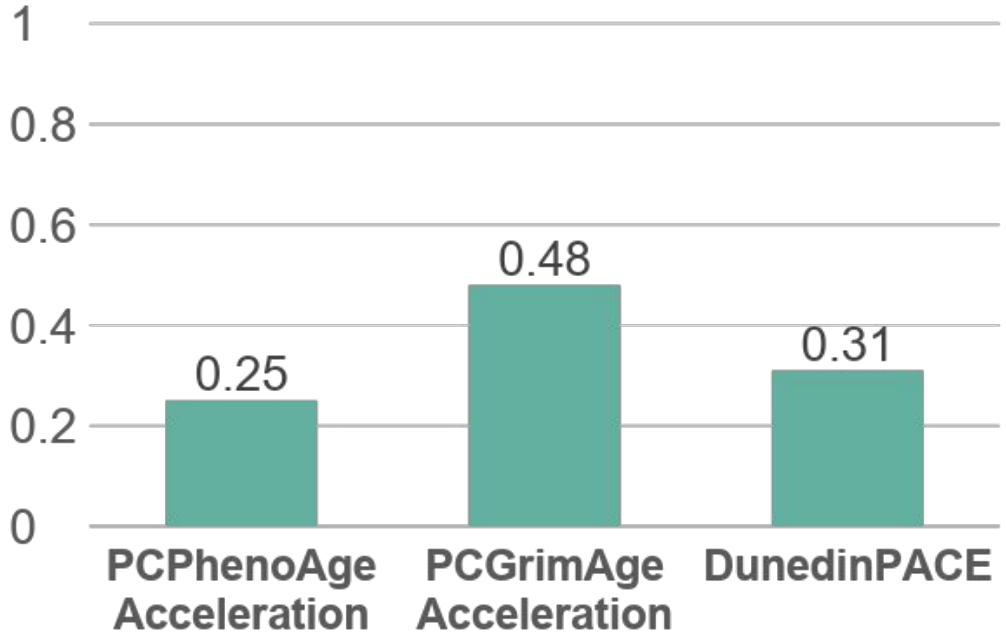
(Merrill et al. 2025, Wong et al. 2022, Theda et al. 2018)

**Estimated Buccal Epithelial Cell Proportion with Chronological Age**

Estimated by EpiDISH-RPC method
Figures Remade from Merrill et al. Source Data
https://doi.org/10.1038/s41467-025-55909-8

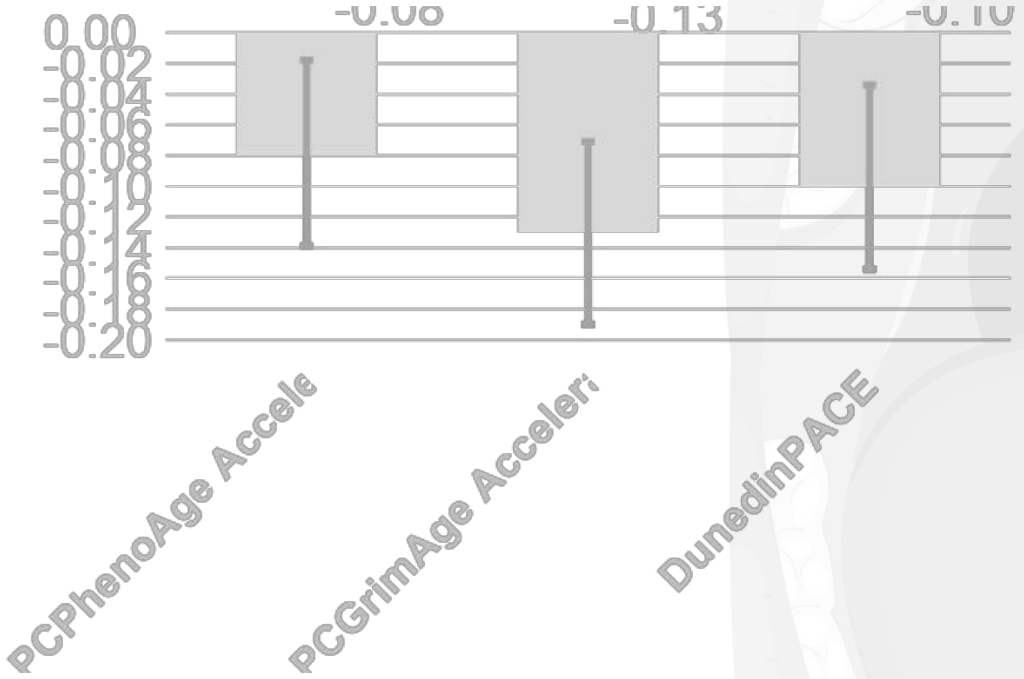
# Background

## Low-to-Moderate Blood-Buccal Cross-Tissue Correlation for Some Blood-Originated Clocks




N=21, Illumina EPIC array dataset in Gene Expression Omnibus accession GSE11116
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111165

## Significant Associations with Household SES when Directly Calculated in Buccal Samples




N=1128, Standardized Coefficients Reported
Raffington et al. 2023
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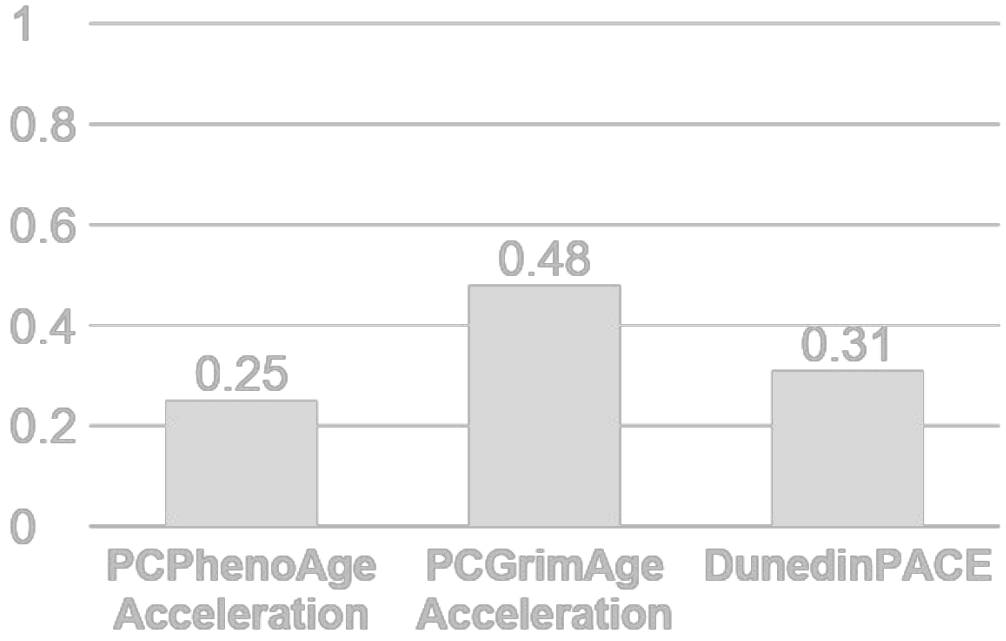
Low-to-Moderate Blood-Buccal Cross-Tissue Correlation for Some Blood-Originated Clocks

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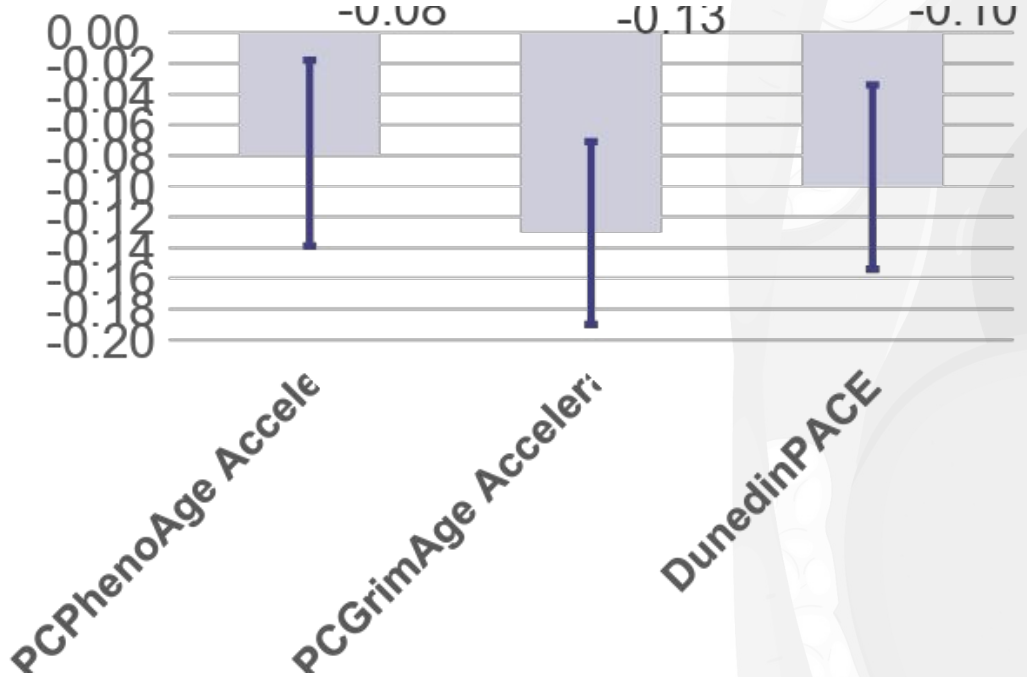


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# Research Questions

*Buccal-Originated Clocks*

*Blood-Originated Clocks*

*Buccal DNAm Sample* ?

Are buccal-derived clocks more strongly related to **SES** and **health** compared to blood-originated clocks applied to a buccal DNAm sample?

# Data

SOEP German Socio-Economic Panel
Nationally representative longitudinal sample of people living in private households

**SOEP-G** SOEP Innovative Sample of Genetic Data (N=2598)
First German genotyped dataset based on a representative population sample

**Buccal DNAm Subsample of SOEP-G**

N=1058, Ages 0-72 (*Mean*=42.4, *SD*=21.2), 57% Female

# Epigenetic Clocks

## Blood-Originated Clocks

- Second-Generation: **PC GrimAge and PC PhenoAge**
- Third-Generation: **DunedinPACE**

## Buccal-Originated Clocks

- First-Generation: **PedBE**
- Second-Generation: **CheekAge**

# Epigenetic Clocks

## Blood-Originated Clocks

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Third-Generation: **DunedinPACE**

## Buccal-Originated Clocks

First-Generation: **PedBE**

Second-Generation: **CheekAge**

**PedBE (Pediatric-Buccal-Epigenetic Score)**
(Developed in 0–20-yr-olds, trained on chronological age)
(McEwen et al. 2020)

**CheekAge**
(Developed in adults, trained on age, lifestyle, and health factors)
(Shokhirev et al. 2024)

# Socioeconomic Status (SES)

The **Average** of

Z-Scored **Household Income**

and

Z-Scored **Educational Attainment**

(Raffington et al. 2023)

**Household Income**

*Monthly household net income in Euros from all sources reported by the head of the household*

*Divided by the number of people in the household and sqrt transformed to correct for skew*

**Education Attainment**

*Highest degree obtained by any individual in the household*

*Converted to the years of education*

# Health Outcomes

## Multimorbidity (three measures, all binary)

(1) Diabetes, Cardiopathy, Stroke, and High Blood Pressure

(2) (1) + Cancer

(3) (2) + Sleep Disturbance, Migraine, Asthma, Depressive Disorder, Dementia, Joint Disorder, Chronic Back Pain, and Other Illnesses

## Limitation of Daily Activities (binary)

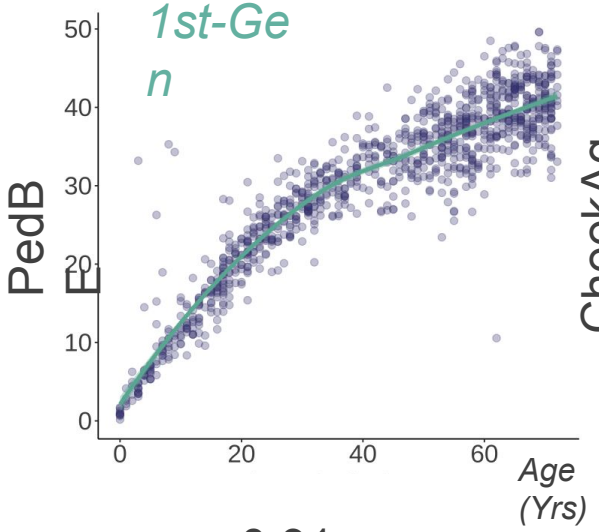
"How severely your health restricts you from ascending stairs, makes tasks tiring, and limits their normal daily activities" – Coded as 1 when strongly or somewhat limited

## Self-Reported Unhealthy Level (continuous)

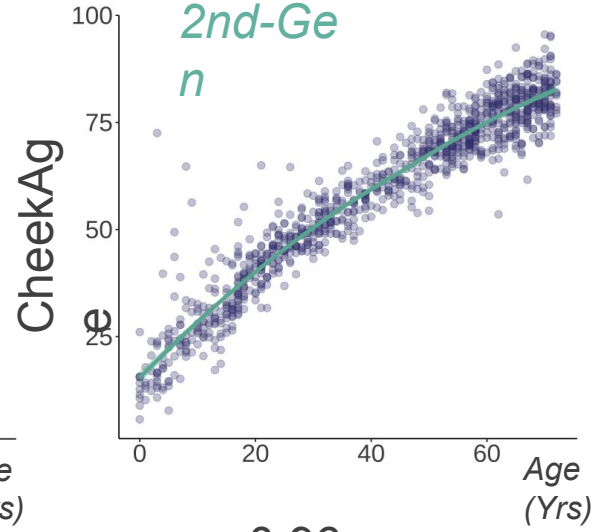
Self-rated state of health, with a higher score indicating a worse health state (1=very good, 2=good, 3=satisfactory, 4=poor, 5=bad).

- **All First- and second-gen clocks correlate with chronological age well (*r* ≥ 0.89)**
- **DunedinPACE moderately correlated with chronological age as expected**

**Buccal-Originated Clocks**

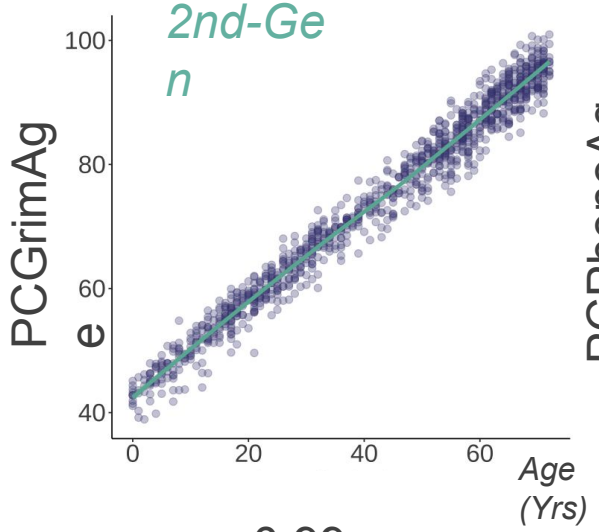



*r* = 0.91, *p* < 0.001

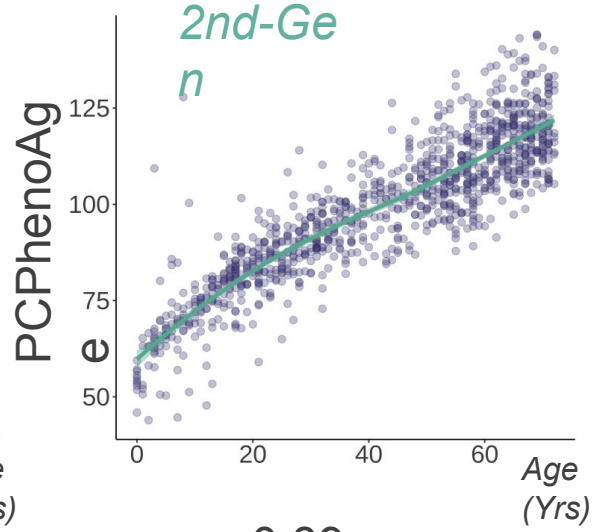



*r* = 0.96, *p* < 0.001

**Blood-Originated Clocks**

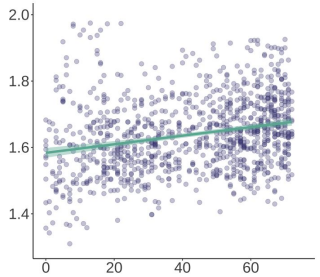



*r* = 0.99, *p* < 0.001

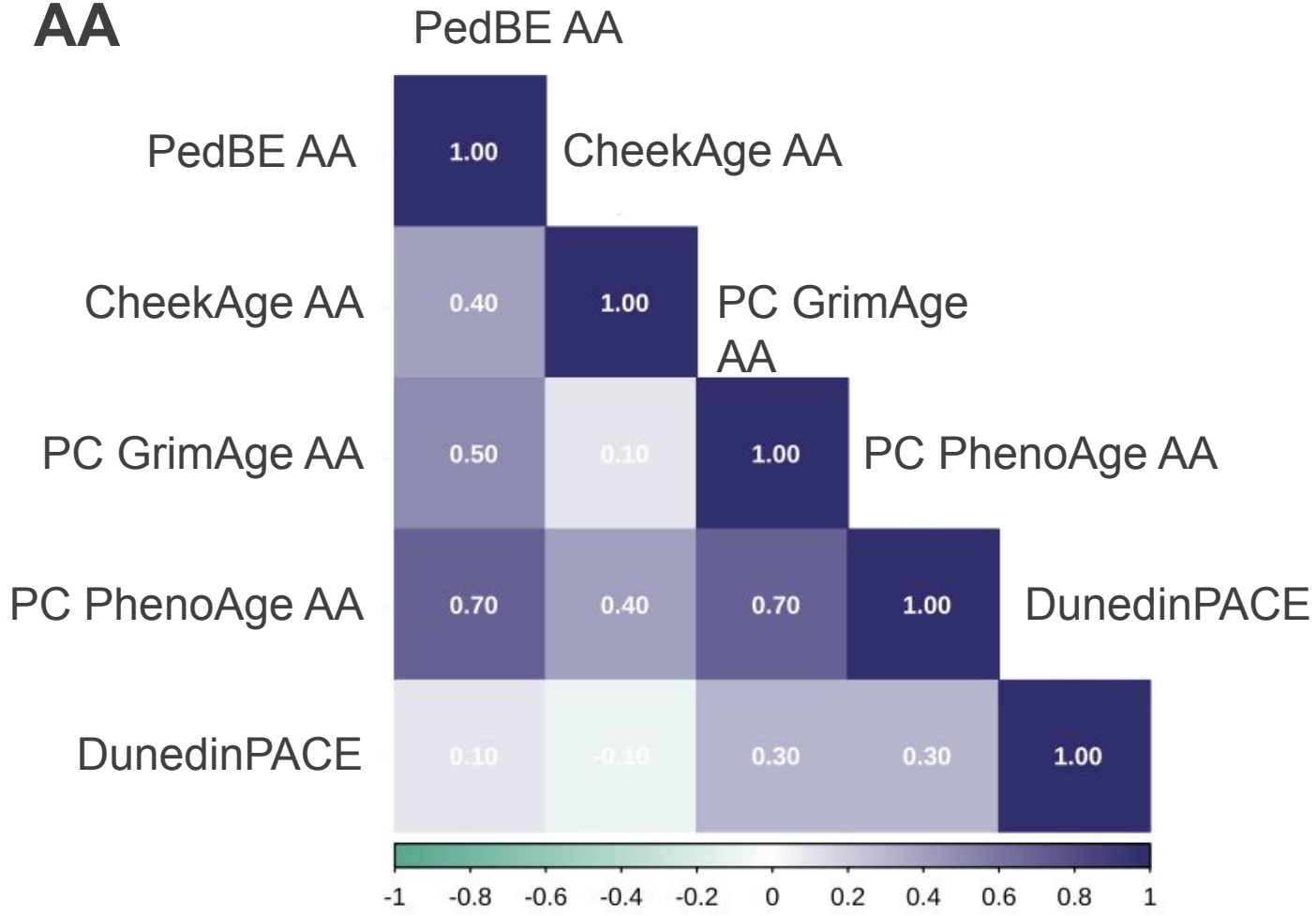



*r* = 0.89, *p* < 0.001

**DunedinPACE:** *r* = 0.24, *p* < 0.001

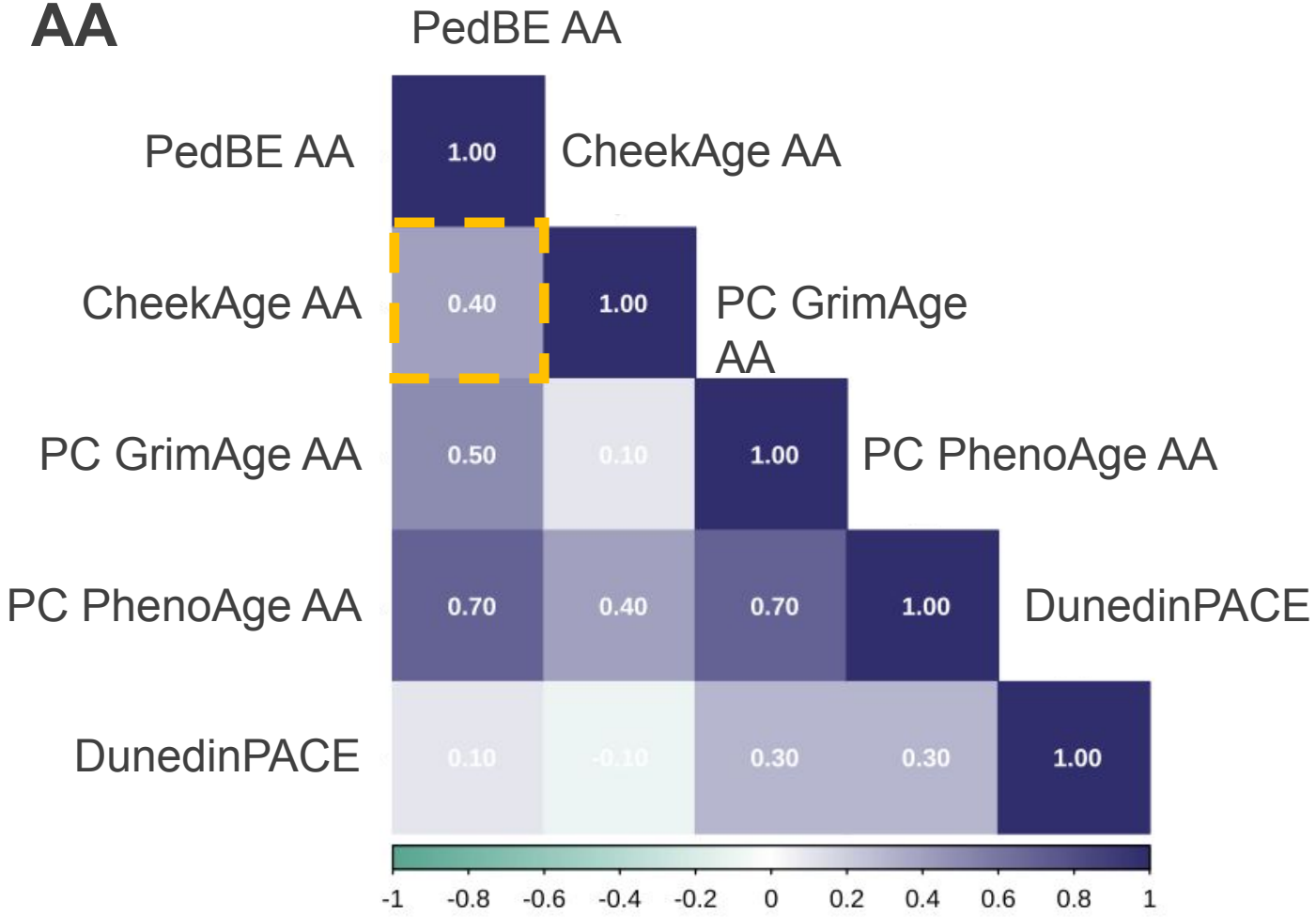


- **Epigenetic age acceleration and pace of aging are correlated**
- **PedBE AA and CheekAge AA have a moderate correlation of 0.40**
- **PedE n blood clocks than CheekAge AA**




*PC: Principal Component Version*
*AA: Age Acceleration*

- **Epigenetic age acceleration and pace of aging are correlated**
- **PedBE AA and CheekAge AA have a moderate correlation of 0.40**
- **PedBE AA is more strongly correlated with blood clocks than CheekAge AA**

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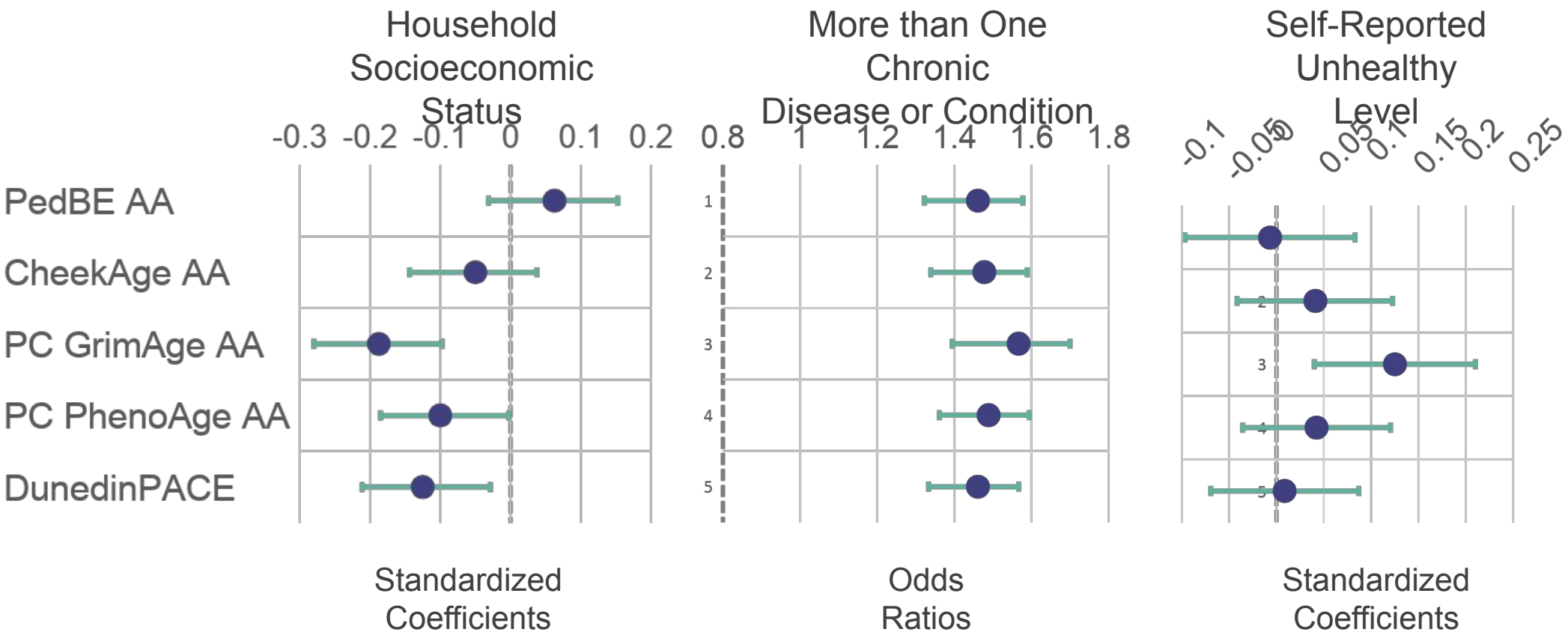
- **Buccal-originated clocks are not sensitive to SES**
- **CheekAge AA performs similarly to blood-originated clocks predicting health, while PedBE does not**



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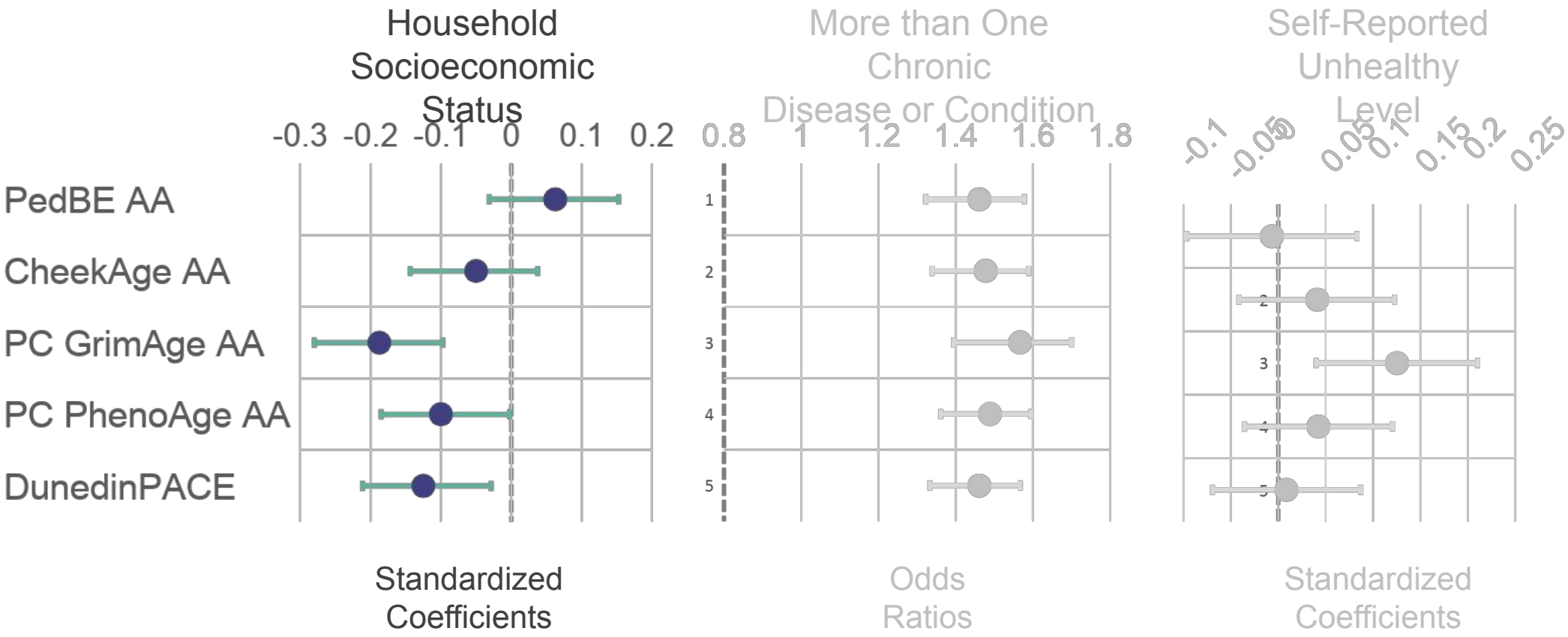

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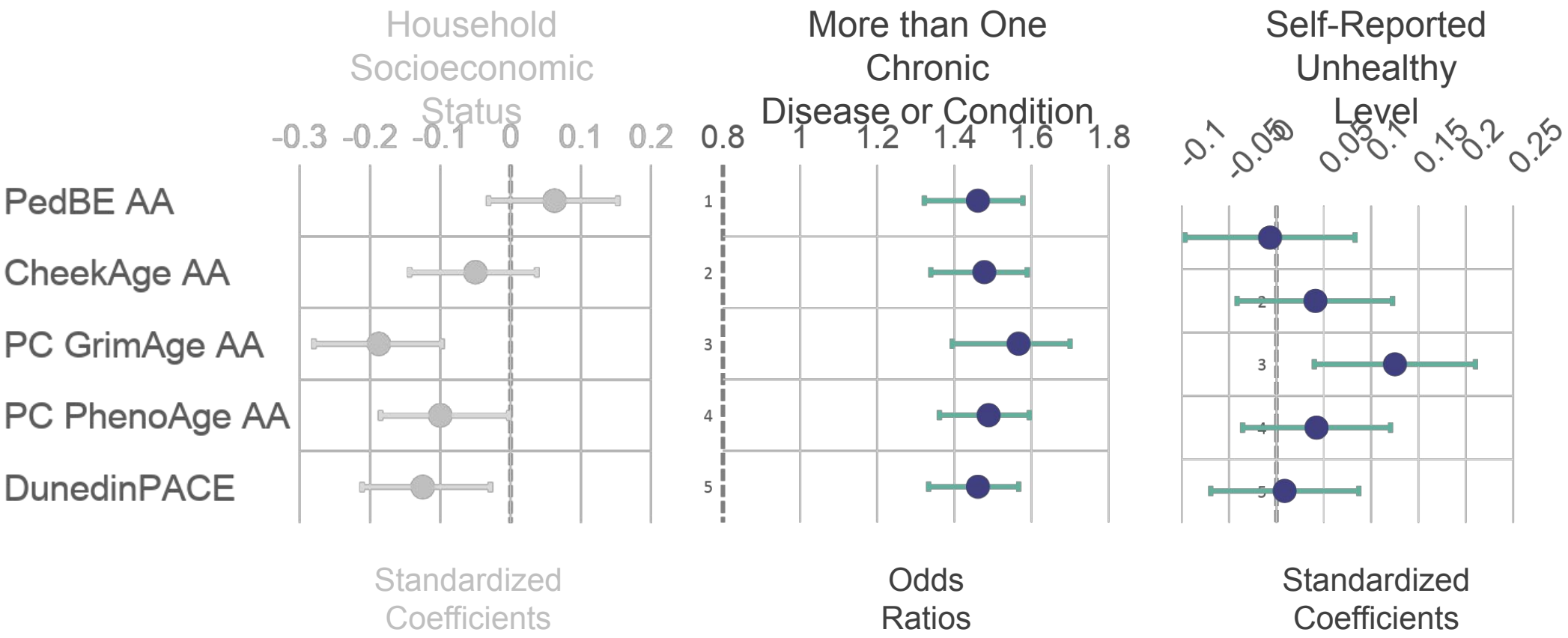

- **First- and second-gen clocks have stronger associations with SES among middle-aged and older participants (Age 45+)**
- **Age-interaction for DunedinPACE is not statistically significant**

| Epigenetic Clocks | Standardized Coefficient | P Value | CI Lower | CI Upper |
|---|---|---|---|---|
| A. All Ages, N=1045 | | | | |
| PedBE AA | 0.04 | 0.273 | -0.032 | 0.115 |
| CheekAge AA | -0.05 | 0.192 | -0.122 | 0.025 |
| PCGrimAge AA | **-0.18** | **0.000** | -0.243 | -0.110 |
| PCPhenoAge AA | **-0.10** | **0.009** | -0.171 | -0.024 |
| DunedinPACE | **-0.11** | **0.002** | -0.187 | -0.040 |
| B. Age<=45 years, N=504 | | | | |
| PedBE AA | 0.05 | 0.273 | -0.042 | 0.149 |
| CheekAge AA | -0.07 | 0.292 | -0.187 | 0.056 |
| PCGrimAge AA | -0.06 | 0.197 | -0.162 | 0.033 |
| PCPhenoAge AA | -0.02 | 0.654 | -0.133 | 0.084 |
| DunedinPACE | N/A | N/A | N/A | N/A |
| C. Age>45 years, N=541 | | | | |
| PedBE AA | **-0.10** | **0.021** | -0.187 | -0.015 |
| CheekAge AA | **-0.13** | **0.002** | -0.209 | -0.045 |
| PCGrimAge AA | **-0.26** | **0.000** | -0.349 | -0.163 |
| PCPhenoAge AA | **-0.18** | **0.000** | -0.283 | -0.082 |
| DunedinPACE | N/A | N/A | N/A | N/A |

- **PedBE has the strongest association with multimorbidity without cancer among all the clocks**
- **Buccal-originated clocks have stronger associations with self-reported health among middle-aged and old adults**

| Epigenetic Clocks | Self-Reported Diseases & Conditions | Multimorbidity (Including Cancer) | Multimorbidity (without Cancer) | Limitation of Daily Activities | Self-Reported Unhealthy Level |
|---|---|---|---|---|---|
| | OR (p value) | OR (p value) | OR (p value) | OR (p value) | Std. Coef. (p value) |
| PedBE AA | 1.19 (0.089) | 1.31 (0.083) | **1.46 (0.026)** | 1.03 (0.776) | **0.08 (0.042)** |
| CheekAge AA | **1.22 (0.029)** | 1.26 (0.112) | 1.28 (0.112) | 1.16 (0.070) | **0.12 (0.001)** |
| PCGrimAge AA | **1.38 (0.001)** | 1.25 (0.155) | 1.23 (0.200) | **1.23 (0.016)** | **0.19 (0.000)** |
| PCPhenoAge AA | **1.24 (0.011)** | 1.20 (0.154) | 1.30 (0.054) | 1.14 (0.099) | **0.12 (0.000)** |
| DunedinPACE | **1.20 (0.049)** | **1.46 (0.010)** | **1.39 (0.033)** | 1.09 (0.299) | **0.09 (0.007)** |

- **PedBE has the strongest association with multimorbidity without cancer among all the clocks**
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| Epigenetic Clocks | | | | | Self-Reported Unhealthy Level<br>Std. Coef. (p value) | Among ages 45+ |
|---|---|---|---|---|---|---|
| PedBE AA | 1.19 (0.089) | 1.31 (0.083) | **1.46 (0.026)** | 1.03 (0.776) | **0.08 (0.042)** | **0.10 (0.044)** |
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# Conclusions

1. In our buccal sample, unlike the blood clocks, the buccal clocks are not significantly associated with SES.
2. All clocks, except for DunedinPACE, have stronger associations with SES among older participants.
3. All clocks are associated with health outcomes. Overall, buccal clocks do not outperform blood clocks in buccal sample.
4. Buccal clocks have stronger associations with health outcomes among older participants.

# About PedBE

- PedBE AA is associated with health even among older adults and has strongest association with multimorbidity without cancer
- Future development of aging-related biomarkers may benefit from including samples of children

| Epigenetic Clocks | Multimorbidity (without Cancer) OR (p value) | Self-Reported Unhealthy Level Std. Coef. (p value) | Among ages 45+ |
|---|---|---|---|
| PedBE AA | **1.46 (0.026)** | **0.08 (0.042)** | **0.10 (0.044)** |
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## Training of Buccal Clocks

**PedBE:** Trained on chronological age

**CheekAge:** Trained on

- Lifestyle: e.g., smoking, exercise, alcohol, plant diet, education, etc.
- Health factors: e.g., self-rated health, self-perceived aging, stress level, sleep quality, BMI, etc.

# Buccal Clocks Do Not Outperform Blood Clocks in Buccal

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## Cellular Content

Buccal Sample: % **Leukocyte** very low

Great performance of blood clocks suggests the importance of leukocytes in quantifying biological aging

Blood cells circulate throughout the body, capturing signals from multiple organs and tissues

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Great performance of blood clocks suggests the importance of leukocytes in quantifying biological aging

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***Better cross-tissue correspondence of blood clocks applied to saliva (Zarandooz & Raffington, 2025)***

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Blood clocks ~ SES: In buccal $r$ = 0.08 - 0.13 **vs** in blood $r$ = 0.10 - 0.37 (Raffington et al. 2023 )

***Measures of SES? What does SES mean across societies?***

Blood clocks ~ Health: We computed same heath measures in HRS, point estimates are very similar but all $p < 0.001$

***Sample size? Variation in the DNAm signal?***

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Future studies should compare the associations of MPSs with SES and health outcomes in **paired buccal and blood samples** collected from the **same individuals**.

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BASE II?

# Given Uncertainties, Why Still Buccal Sample?

- Promising associations
- More directly exposed to **certain environmental factors** (e.g., smoking and diet)?
- Unique **DNAm pattern**?
- Value beyond prediction precision alone: In some studies, the inclusion of specific populations might be a higher priority than maximizing prediction

| Variable | Mean (SD) / Percentage | Sample size |
|---|---|---|
| Chronological age | 42.4 (21.2) | 1058 |
| PedBE | 30.2 (10.9) | 1058 |
| PedBE AA | -0.0 (4.4) | 1058 |
| CheekAge | 59.4 (19.8) | 1058 |
| CheekAge AA | -0.0 (5.8) | 1058 |
| PC PhenoAge | 99.2 (18.8) | 1058 |
| PC PhenoAge AA | -0.0 (8.6) | 1058 |
| PCGrimAge | 74.3 (15.9) | 1058 |
| PCGrimAge AA | 0.0 (2.5) | 1058 |
| DunedinPACE | 1.6 (0.1) | 1058 |
| Age | 42.4 (21.2) | 1058 |
| Female | 57.5% | 1045 |
| Socioeconomic status (Z - score) | 0.0 (0.8) | 1045 |
| % reporting more than 1 diseases & conditions | 22.0% | 797 |
| % Dichotomous coded multimorbidity (including cancer) | 7.2% | 797 |
| % Dichotomous coded multimorbidity (without cancer) | 6.3% | 797 |
| Self-reported unhealthy level | 2.6 (1.0) | 797 |
| % Free from functional limitation | 36.6 | 797 |