# Latent Tuberculosis Infection is Strongly Associated with Increased Epigenetic Aging

764

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## Study Aim

Estimate the association between positive QuantiFERON (QFT) results and epigenetic age acceleration among close contacts of index TB patients in Tbilisi, Georgia.

## Background

- Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is the leading infectious cause of death worldwide and accounts for 30% of mortality among people with HIV.
- Latent TB infection (LTBI) is characterized by granulomas encasing *Mtb*, maintained by a balanced host T-cell response.
- While advanced age traditionally increases the risk of LTBI reactivation through the process of immunosenescence, emerging evidence suggests a bidirectional relationship where chronic immune stimulation from the infection drives biological aging, a process referred to as "inflammaging."
- DNA methylation (DNAm) is used in epigenetic clocks to estimate epigenetic age, a proxy for biological age; contrasting these estimates with chronological age defines epigenetic age acceleration (EAA).
- Despite the high prevalence of LTBI globally, whether it impacts accelerated aging remains largely unexplored.

## Methods

**Study Design:** Cross-sectional study of household contacts (N = 75) of index active pulmonary TB cases at NCTLD (Tbilisi, Georgia). Participants were aged 18 years or older, had documented exposure to household index TB patients, and screened sputum/X-ray negative for active pulmonary TB. We enrolled 37 QFT positive (QFT+) and 38 QFT negative (QFT-) individuals **(Table 1)**.

**DNA methylation profiling**: We used Illumina Infinium MethylationEPIC v2 array (> 935,000 CpG sites). Quality control, including background correction, probe filtering, and quantile normalization were performed using the R package *minfi*.

**Epigenetic Clocks and Statistical Analyses**: EAA was calculated for the Horvath clock (HorvathAA), Hannum clock (HannumAA), Pheno clock (PhenoAA), and Grim clock (GrimAA). Multiple linear regression models evaluated associations between EAA (dependent variable) and predictors: QFT status (binary) and log10-transformed TB1-Nil and TB2-Nil antigen responses. All models were adjusted for sex.

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QFT+ individuals exhibit up to 4.2 years of Epigenetic Age Acceleration compared to QFT- individuals across three epigenetic clocks (HorvathAA, HannumAA, and PhenoAA).

TB 1 antigen response demonstrated a more robust and higher-magnitude association with Epigenetic Age Acceleration ($\beta$ = 3.56 – 4.61 years across three clocks) than the TB2 antigen response ($\beta$ = 3.04 – 3.11 years across two clocks).

## Results

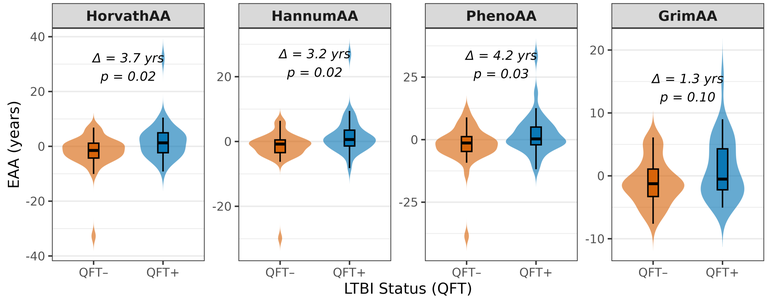



**Figure 1. QFT+ individuals exhibit significantly higher EAA.** Sex-adjusted linear regression revealed significant EAA increases (Δ) in QFT+ group (n = 37, blue) compared to QFT- group (n = 38, orange) for HorvathAA, HannumAA, and PhenoAA. Violin plots and embedded boxplots illustrate the EAA distributions across all DNAm clocks.

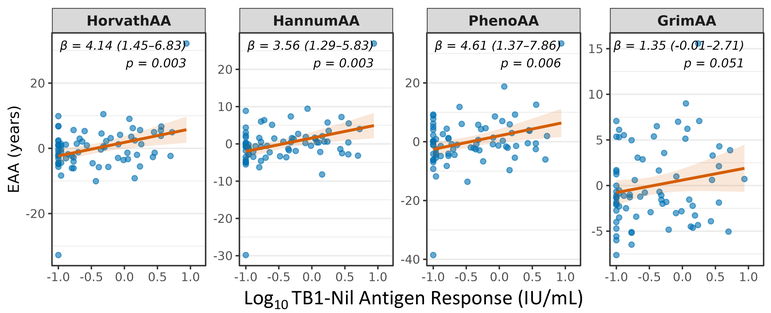



**Figure 2. EAA significantly increases with higher log-transformed TB1 antigen response.** Sex-adjusted linear regression revealed significant positive correlations between $\log_{10}$ TB1 antigen levels (IU/mL) and EAA for HorvathAA, HannumAA, and PhenoAA ($p < 0.05$). Scatter plots (N = 75) include regression fits (orange lines) and 95% CIs (shaded ribbons).

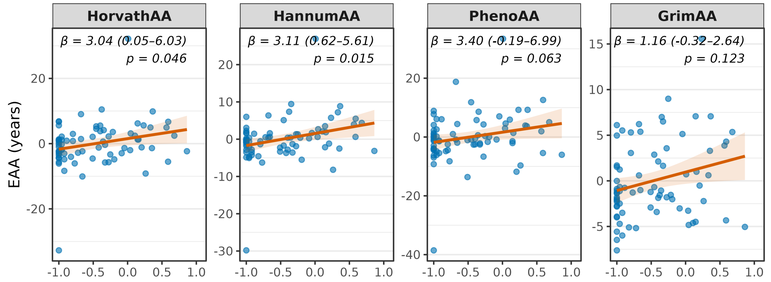



**Figure 3. EAA significantly increases with higher log-transformed TB2 antigen response.** Sex-adjusted linear regression identified significant positive correlations between $\log_{10}$ TB1 antigen levels (IU/mL) and EAA for HorvathAA and HannumAA ($p < 0.05$). Scatter plots (N = 75) include regression fits (orange lines) and 95% CIs (shaded ribbons).

## Results

| Table 1. | QFT- (n=38) | QFT+ (n=37) |
|---|---|---|
| Male (%) | 11 (28.9) | 16 (43.2) |
| Age, year (mean (SD)) | 43.16 (15.02) | 43.11 (14.61) |
| TB1 Antigen, IU/ml (mean (SD))* | 0.12 (0.19) | 1.75 (1.88) |
| TB2 Antigen, IU/ml (mean (SD))* | 0.10 (0.15) | 1.51 (1.80) |
| Body mass index, kg/m² (mean (SD)) | 26.72 (4.89) | 27.09 (5.28) |
| Smoking status (%) | -- | -- |
| Current smoker | 12 (31.6) | 14 (37.8) |
| Former smoker | 3 (7.9) | 2 (5.4) |
| Never smoker | 23 (60.5) | 21 (56.8) |
| Mitogen, IU/ml (mean (SD))* | 4.88 (1.90) | 5.79 (1.61) |
| Hypertension (%) | 6 (15.8) | 4 (10.8) |
| Systolic blood pressure, mmHg (mean (SD)) | 117.37 (6.34) | 115.68 (9.22) |
| Diastolic blood pressure, mmHg (mean (SD)) | 72.63 (4.61) | 71.62 (6.02) |

**Sensitivity Analyses**

**Adjusting for BMI and Smoking Status:** We conducted a sensitivity analysis including BMI (continuous) and smoking status (ordinal). Results remained unchanged likely due to the similar distribution across QFT+ and QFT- individuals.

**Removal of Extreme EAA Values:** We excluded individuals with EAA values corresponding to ≥ 4 SD. All associations maintained the same direction of effect, although the magnitude of the effect decreased (**Table 2**).

| Table 2. | HorvathAA (95% CI) | HannumAA (95% CI) | PhenoAA (95% CI) | GrimAA (95% CI) |
|---|---|---|---|---|
| QFT status | 2.02 (-0.03, 4.07) | 1.71 (0.08, 3.35) | 2.35 (-0.37, 5.06) | 1.29 (-0.26, 2.85) |
| TB1 Antigen | 1.62 (-0.27, 3.50) | 1.40 (-0.10, 2.89) | 1.92 (-0.57, 4.41) | 1.35 (-0.01, 2.71) |
| TB2 Antigen | 1.47 (-0.49, 3.44) | 1.76 (0.22, 3.30) | 1.67 (-0.92, 4.26) | 1.16 (-0.32, 2.64) |

## Conclusions

- LTBI is strongly associated with significant EAA, with QFT+ status predicting between a 3.2-to-4.2-year increase in EAA across three different clocks.
- Greater IFN-$\gamma$ T-cell response magnitude was associated with EAA, supporting the inflammaging hypothesis that chronic adaptive immune stimulation induces systemic epigenetic shifts.
- The consistency of EAA across Horvath, Hannum, and Pheno clocks strongly suggests that LTBI impacts accelerated biological aging.
- LTBI may contribute to the global burden of age-related comorbidities by independently accelerating molecular aging processes.
- Future larger, longitudinal research is required to establish causality, assess EAA reversibility post-treatment, and determine if EAA predicts progression to active TB.

## Funding

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