Accelerated Epigenetic Aging in Bladder Cancer Patients

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Abstract

Aging represents the most important risk factor for many chronic diseases including cardiovascular diseases, diabetes, and cancer, therefore understanding the mechanisms of aging is a fundamental step for designing new treatments for chronic diseases. DNA methylation is the most reliable and accurate molecular marker for aging quantification, however, genome-wide DNA methylation profiling techniques, such as reduced representative bisulfite sequencing and illumina Bead Array that are widely used in aging research are prohibitively expensive and have poor data quality at low-read coverage sites. Here we report a robust targeted bisulfite sequencing approach, called SWARM® (Simplified Whole-panel Amplification Reaction Method), for the accurate biological age determination. SWARM® is flexible and low cost, requires relatively low DNA starting material, allows the simultaneous amplification and sequencing of hundreds of loci, and has shown to increase sample throughput. Using the SWARM® approach, we were able to analyze the methylation level of several hundreds of age-associated loci including the published Horvath Clock sites. Gender-specific age-predictive models were built using the elastic net regression of DNA methylation levels of the loci and chronological age of urine DNA samples of over 300 healthy subjects of 18 to 88 years old. Urine samples from bladder cancer patients exhibit significant age acceleration, with an average of >10 years. In brief, our gender-specific urine DNAge® analysis is a tool for the precise biological aging quantification and can be used to address questions in aging and urinary tract health.

Keywords: DNA methylation, targeted bisulfite sequencing, epigenetic age clock, DNAge®, elastic net

The DNAge® Test

The DNAge® test is built based on the principles originally described in the Horvath Clock method[1], that utilizes DNA methylation levels of 353 human epigenetic markers for biological age determination. This test utilizes SWARM® (Simplified Whole-panel Amplification Reaction Method) to analyze DNA methylation patterns >1,000 loci including the Horvath's markers, providing epigenetic age predictions in a high throughput manner.

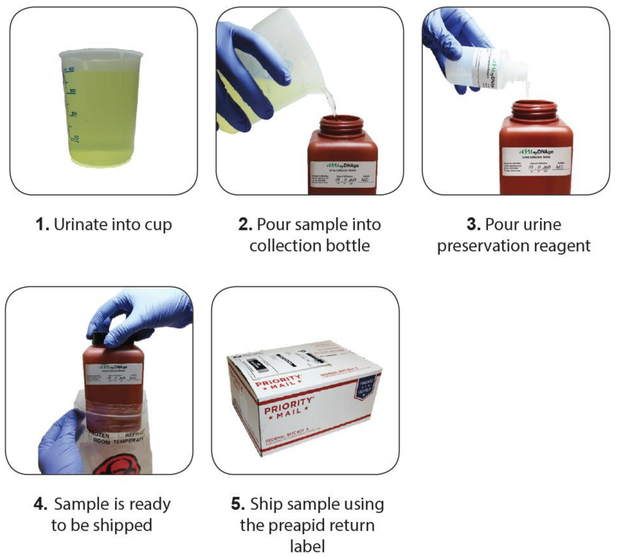
A penalized regression model's coefficients b_0, b_1, ..., b_n relate to transformed age as follow:

$$F(\text{chronological age}) = b_0 + b_1CpG_1 + \cdots + b_nCpG_n + \text{error}$$

DNAge™ is estimated as follow:

$$DNAge® = inverse.F(b_0 + b_1CpG_1 + \cdots + b_nCpG_n)$$

At-Home Sample Collection



DNAge® Test

SWARM® Performs Better than Array

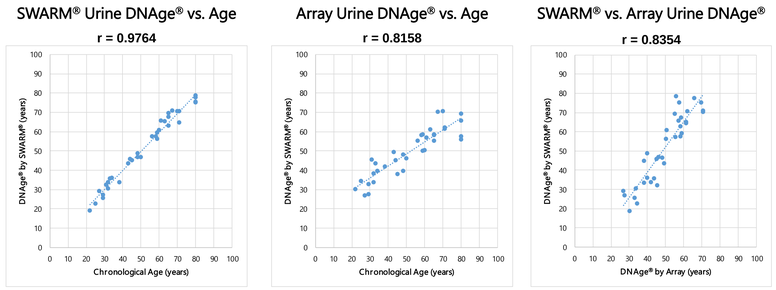
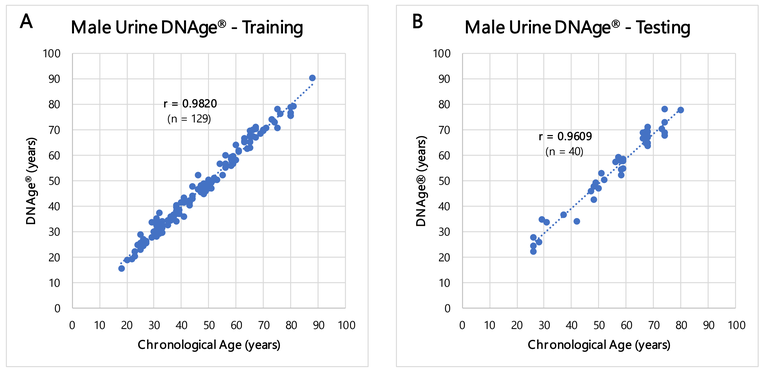


Figure 1. Performance comparison of DNA methylation analysis platforms for urine DNAge® prediction. DNAge® prediction of the same urine samples (n=35) which are analyzed using (A) SWARM®, (B) Illumina 450K or EPIC BeadChip. (C) The correlation between samples from 35 individuals analyzed by SWARM® platform or array-based platforms.

Male Urine DNAge® Samples



Female Urine DNAge® Samples

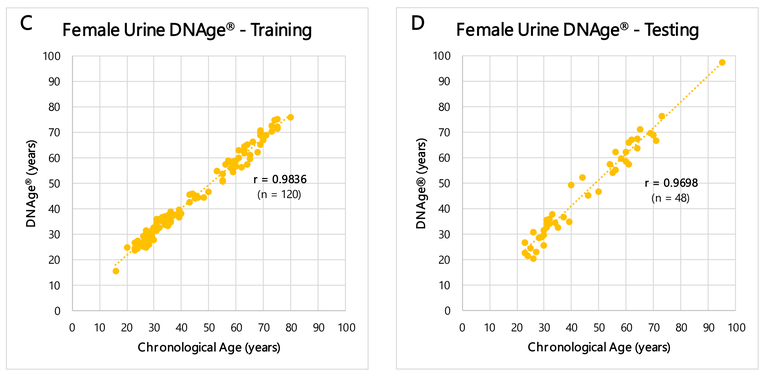
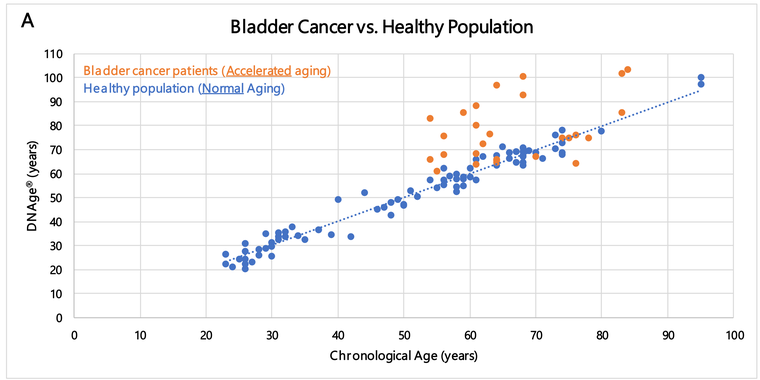


Figure 2. Gender-specific urinf DNAge® test optimized for urine samples. (A) Male urine DNAge® training data (r = 0.982, error = 0.6); (B) Male urine DNAge® testing data (r = 0.9609, error = 1.2); (C) Female urine DNAge® training data (r = 0.9836, error = 0.3); (D) Female urine DNAge® testing data (r = 0.9698, error = 1.1).

Nature vs. Nurture

Bladder Cancer Patients Age Faster Than Healthy Population



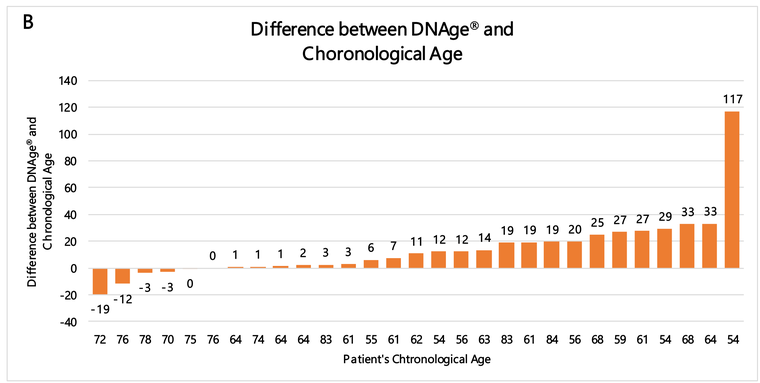


Figure 3. Age acceleration in bladder cancer patients. (A) Different populations show different aging rates, with accelerated aging in bladder cancer patients and normal aging in general populations. (B) Urine samples from bladder cancer patients exhibit significant age acceleration, with an average of 13.9 years.

Figure 4. Effect of transurethral resection of bladder tumors (TURBT) on urine DNAge®. Urine DNAge® significantly decrease after tumors removal and this decrease is correlated with Bladder CARE™ index. (See Paolo Piatti's poster for more information).

Conclusion

The urine DNAge® test is a useful tool for age quantification and monitoring, as well as for studying aging and urinary track health. This test utilized our newly developed targeted bisulfite sequencing platform – SWARM®, which is a flexible, low-cost technology that requires relatively low DNA starting material. The performance and the cost for the sequencing-based technology DNAge® test is a significant improvement compared to array-based platform.

Reference:

1. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115. PMID: 24138928