Some tips on age estimation using DNA methylation in saliva samples as an index across the Japanese and Indonesian ethnicities

by Maretaningtias Dwi Ariani

Submission date: 31-Mar-2023 10:34AM (UTC+0800)
Submission ID: 2051611931
File name: 2022_Legal_Medicine.pdf (703.25K)
Word count: 3591
Character count: 19368

Legal Medicine 56 (2022) 102042

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Some tips on age estimation using DNA methylation in saliva samples as an index across the Japanese and Indonesian ethnicities

Hiroko Oka [a,*], Maretaningtias Dwi Ariani [b], Tomohiko Akazaki [c], Mutsumi Miyauchi [d], Masae Kitagawa [d,e]

[a] Center for Cause of Death Investigation Research and Education, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[b] Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Indonesia
[c] Akazaki Dental Office, Iwakuni, Japan
[d] Department of Oral and Maxillofacial Pathobiology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[e] Center of Oral Clinical Examination, Hiroshima University Hospital, Hiroshima, Japan

ARTICLE INFO

Keywords:
Age estimation
DNA methylation
Forensic science
Ethnicity

ABSTRACT

Age estimation of unidentified bodies is of marked importance in forensic medicine. In previous studies, the analysis of DNA methylation in body fluids led to the identification of several age-related CpG sites in genes such as *EDARADD* and *FHL2*. However, limited information is available on whether interethnic differences may affect the age prediction results. In the present study, we examined the effect of ethnicity on the age prediction method based on methylation scores, which were determined via methylation-sensitive high-resolution melting. We found that there was a significant difference in methylation scores between Japanese and Indonesian participants of early 20 s group, and that the nationality coefficient was significant for age estimation when applying the existing method for the analysis of the methylation status of *EDARADD* and *FHL2*. This suggests that when using certain biochemical indicators as a predictor of age, the effects of ethnicity on DNA methylation should be considered to improve the accuracy of the estimation.

1. Introduction

Age estimation of unidentified remains is important for personal identification in crime scenes and forensic medicine in general. The age estimation methods used in the forensic field are roughly classified into morphological and biochemical. It has been reported that biochemical methods are more precise, since morphological methods are highly affected by error due to individual differences. Moreover, biological fluids, such as saliva and blood, which are commonly found in crime scenes, cannot be analysed with morphological techniques. In addition to biochemical age estimation methods, gene-based methods, such as those employing signal-joint T-cell receptors [1], telomere length [2,3], and somatic gene arrangement [4], are increasingly being used for the analysis of biological fluids and small specimens frequently found in disaster and crime scenes. However, while morphological similarities and differences among various ethnicities may be known, there is limited evidence of these among biochemical markers.

In recent years, the use of epigenetics in age estimation has been reported, where the amount of methylated cytosines in CpG sites has become known as a tissue-specific marker of age [5–11]. In fact, studies have reported age estimation of saliva samples based on DNA methylation of CpG sites located in *ELOVL2*, *EDARADD*, and *FHL2* [9,12,13]. Due to the cost and the complexity of DNA methylation measurement, there is an increasing expectation for using real-time PCR based techniques such as methylation-sensitive high-resolution melting (MS-HRM) [12,14] and methylation-specific PCR (MSP) [15]. However, limited information is available on whether these methods are accurate across different populations.

In our study, we took into consideration the nationality of the saliva sample donors (Japanese or Indonesian) and reported some results for age estimation via MS-HRM quantification of methylation in *ELOVL2*, *EDARADD*, and *FHL2*.

2. Materials and methods

All experimental protocols were approved by the epidemiological

* Corresponding author at: Center for Cause of Death Investigation Research and Education, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan.
E-mail address: okhiroko@hiroshima-u.ac.jp (H. Oka).

https://doi.org/10.1016/j.legalmed.2022.102042
Received 23 October 2021; Received in revised form 23 January 2022; Accepted 7 February 2022
Available online 9 February 2022

research ethics review committee of Hiroshima University (no. E-1073). We used donated saliva samples and opt-out residual specimens from other clinical tests. A written informed consent for donation was signed either by the donors themselves or the next of kin.

2.1. Sample collection, DNA extraction, and bisulfite conversion

Saliva samples from 79 healthy Japanese donors and 34 healthy Indonesian donors were collected in plastic tubes following paraffin wax chewing stimulation. Ethnicity information was collected through interviews. Donors were defined as "Japanese" if both donor and the donor's parents had Japanese nationality and had lived in Japan most of their life. Similarly, donors were defined as "Indonesian" if both donor and the donor's parents had Indonesian nationality and had lived in Indonesia most of their life. Age was calculated from the date (year and month) of birth. Age and sex information for the 113 samples is shown in Table 1. All samples were stored in a − 20 °C freezer within 12 h after collection for further studies. DNA was extracted from the saliva samples using QIA amp DNA Investigator Kit (Qiagen, Hilden, Germany) and treated with EpiTect Fast Bisulfite Conversion Kit (Qiagen) for bisulfite conversion according to the manufacturer's protocol.

2.2. High-resolution melting

For the PCR we used the following previously reported [12,14] pairs of primers: for *ELOVL2*, forward primer 5′-CGATTTGTAGGTTTAGT-3′ and reverse primer 5′-ACTACCAATCTAAACAA-3′ (91 bp, 10 CpG sites); for *EDARADD*, forward primer 5′-AGAAGGTTTGATTTTGGTTAGAT-3′ and reverse primer 5′-CCTCTCCCCATCTATTTAAT-3′ (139 bp, 4 CpG sites); for *FHL2*, forward primer 5′-TTTACCAAAACTCCTTTCTT-3′ and reverse primer 5′-GTGGGTAGATTTTTGTTATT-3′ (133 bp, 14 CpG sites). PCR amplification was carried out with a StepOne™ Plus (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with the HRM Software (version 3.1, Thermo Fisher Scientific). A total volume of 20 μL containing 1 × MeltDoctor HRM Master Mix (Thermo Fisher Scientific), 300 nM of each primer, and 40 ng of bisulfite-modified template was used. The anneal/extend temperatures of 50, 52, and 60 °C were used for *ELOVL2*, *EDARADD*, and *FHL2*, respectively. When HRM analysis was performed, the pre-melt and post-melt temperature regions were respectively set as follows: 68–69 °C and 82–83 °C for *ELOVL2*; 65–66 °C and 80–81 °C for *EDARADD*; and 68–69 °C and 82–83 °C for *FHL2*. The samples were analysed using HRM in duplicate. Other variables were set appropriately according to the manufacturer's protocols.

2.3. Calculating methylation scores

Fully methylated/unmethylated and bisulfite converted control DNA were purchased from Qiagen and mixed in appropriate ratios to make 0%, 1%, 10%, 25%, 50%, 65%, 80%, 90%, and 100% methylated control DNA. These were then used to prepare standard curves for each HRM measurement. The Df value of each control sample obtained by HRM was plotted, and standard curves for *ELOVL2* (non-linear), *EDARADD* (linear), and *FHL2* (non-linear) were developed in Excel (Microsoft Excel for Microsoft Office 365) to determine the value of the methylation score for the saliva samples.

Table 1
Age and sex information for samples used in this study.

	Japanese N = 79		Indonesian N = 34	
	Male	Female	Male	Female
Age < 10	4	4	0	0
Age 10–19	2	3	0	0
Age 20–29	9	9	12	15
Age 30–39	11	6	2	3
Age 40–49	3	10	0	0
Age 50–59	6	4	0	1
Age > 60	5	3	0	1
Total	40	39	14	20

2.4. Statistical analysis

Spearman's rank correlation coefficient (ρ) was used to measure the correlation between each of the methylation scores (*ELOVL2, EDARADD*, and *FHL2*) and age. According to the results of preliminary experiments from 48 Japanese samples (Supplementary Table 1), we did not find any significant correlation between chronological age and methylation score of *ELOVL2* in our experimental conditions (Supplementary Fig. 1). For this reason, we used the score of *EDARADD* and *FHL2* for the following multiple linear regression (MLR) analysis. An MLR analysis with reference to chronological age was done with the methylation scores of *EDARADD* and *FHL2*, sex (1 = male, 2 = female), and nationality (1 = Japanese, 2 = Indonesian) as the explanatory variables. Non-parametric Mann-Whitney's *U* test was used to compare the methylation scores between Japanese and Indonesian saliva samples. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [16]. More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics. A p-value lower than 0.05 was considered statistically significant.

3. Results

In the "Age 23–25" group, there were significant differences in the methylation scores of *EDARADD* between Japanese and Indonesian saliva samples (Fig. 1a, d). In contrast, there were no significant differences in the methylation scores in the "Age 26–28" group (Fig. 1a, b). With respect to the methylation scores of *FHL2* among these groups, there were no significant differences (Fig. 1a, c, e). Considering methylation scores, sex, and nationality, we found that the *EDARADD* ($p = 0.000$), *FHL2* ($p = 0.000$), and nationality ($p = 0.005$) coefficients were significant for age estimation (Table 2). Contrastingly, the sex coefficient was not significant for the estimation of age (Table 2). Variance inflation factor (VIF) of the methylation scores of *EDARADD* and *FHL2* and sex and nationality coefficients, were 1.385, 1.372, 1.043, and 1.032, respectively. The regression models excluding sex from the explanatory variables are shown in Figs. 2 and 3. The mean absolute deviation (MAD) of a prediction model with nationality was 10.8 years (Fig. 2b), while that of a model without this factor was 11.1 years (Fig. 3b).

4. Discussion

In the present study, we uncovered a significant difference in age prediction among Japanese and Indonesian saliva samples while considering the MS-HRM *EDARADD* and *FHL2* methylation scores. Age prediction methods based on DNA methylation levels of saliva samples were previously reported [12,13]. However, to the best of our knowledge, no information is available on the possible influence of interethnic differences. For this reason, we carefully verified the nationalities of donors upon sample collection. In the present study, of the 113 samples used, we compared the methylation scores of groups "Age 23–25" and "Age 26–28", which have similar distributions between Japanese and Indonesians. Then, we found significant differences in the *EDARADD* methylation scores in the "Age 23–25" group between Japanese and Indonesian saliva samples. Although our prediction model did not present enough accuracy (Fig. 2: MAD = 10.8 years), our results suggest that, in some cases, we need to consider the population of origin in existing age predicting DNA methylation methods and adapt them in both forensic and clinical settings.

Disasters and crimes are a global occurrence and the victims are

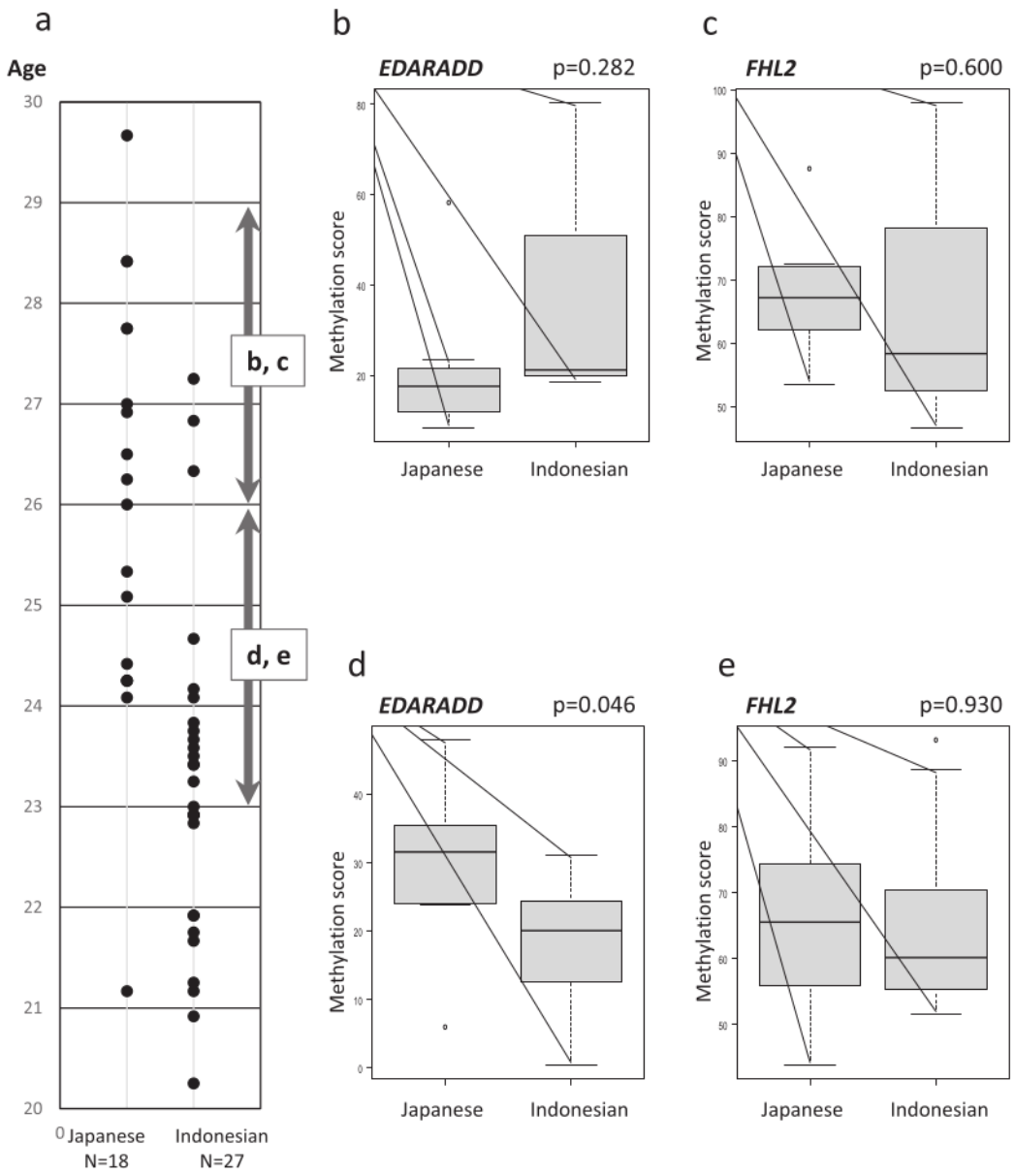

Fig. 1. Differences in the methylation scores between Japanese and Indonesian saliva samples. Mann–Whitney's *U* test. (a) Age distribution of participants in their 20 s; (b) *EDARADD* of Age 26–28; (c) *FHL2* of Age 26–28; (d) *EDARADD* of Age 23–25; (e) *FHL2* of Age 23–25.

Table 2
Estimated regression coefficients. A multiple linear regression (MLR) analysis; ***p < 0.001. The result of the MLR analysis of chronological age considering the methylation scores of *EDARADD* and *FHL2*, sex, and nationality as the explanatory variables (79 "Japanese" and 34 "Indonesian" saliva samples).

	Regression coefficient (estimate)	95% CI lower	95% CI upper	S.E.	P-value	
Intercept	21.180	4.739	37.620	8.294	0.012	*
EDARADD	−0.329	−0.471	−0.188	0.072	0.000	***
FHL2	0.494	0.299	0.690	0.099	0.000	***
Sex	−1.740	−6.980	3.500	2.644	0.512	
Nationality	−8.174	−13.852	−2.497	2.864	0.005	**
				$R^2 = 0.246$	**p-value = 0.000**	

a

	Estimate	S.E.	t value	Pr (>|t|)
Intercept	19.108	7.654	2.497	0.014
EDARADD	-0.321	0.070	-4.571	0.000
FHL2	0.486	0.098	4.978	0.000
Nationality	-8.331	2.847	-2.926	0.004
			R^2=0.25	**p-value=0.000**

b

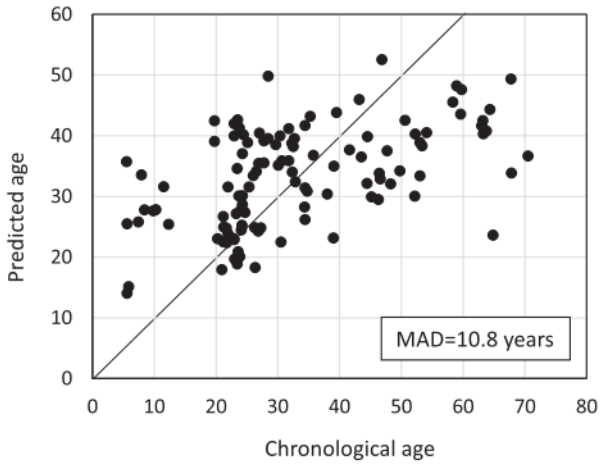

Fig. 2. Validation of the predicted age through multiple linear regression analysis considering the methylation scores of *EDARADD* and *FHL2* and information on nationality (79 "Japanese" and 34 "Indonesian" saliva samples). (a) Coefficients in this study. (b) Relation between predicted and chronological ages.

diverse. In Japan, the number of foreign residents is increasing every year. At the end of 2019, the number of foreign residents was ~ 2.9 million, mostly from Asian countries [17]. In our study, identifying the donors' nationality purely based on appearance would have been challenging. In the current scenario, our population consists of a mixture of people from multiple regions of different countries, therefore, we need to confirm if it is reasonable to include biochemical indicators for age estimation.

In the present study, all saliva samples were collected following paraffin wax chewing stimulation. It is known that saliva contains oral epithelial cells, lymphocytes, and neutrophils [18]. Several studies have reported age-correlated methylation sites of *EDARADD* in buccal epithelial cells [19] and of *FHL2* in blood cells [14,19]. Therefore, we can reasonably say that in the present study there is a correlation between chronological age and each methylation score detected in the saliva sample. However, in our preliminary examination with 48 Japanese samples, methylation of *ELOVL2*, the well-known age-correlated factor in saliva [12,19], did not correlate with the chronological age significantly. Even though we used the same primer sequences that were used in a previous study that estimated age using saliva samples [12], some of our processes, such as the real-time PCR machine and chemical regents, were different. Thus, we adjusted the protocol for PCR and MS-HRM analysis to suit our conditions. However, the methylation scores of *EDARADD* and *FHL2* in 79 Japanese samples were obtained from appropriate real-time PCR reactions, but we failed to obtain appropriate reactions for *ELOVL2* in some samples (n = 31). In addition, although we used chewing stimulation (a gum test method) to collect the saliva samples in this study, there was no information about the saliva collection method (stimulated or non-stimulated) in the previous study. Salivary DNA has cellular heterogeneity and there is a possibility that the proportion of cells differs among collection methods. These factors can explain the low correlation observed in the present study compared with previous ones.

Our study, however, presents some limitations. First, the sample size was not large and focused on Japanese and Indonesian participants alone. In addition, Indonesian participants were aged between 20 and 50 years, with a particular bias towards the 20 s. This was due to the difficulty in collecting Indonesian saliva samples with reliable interview

a

	Estimate	S.E.	t value	Pr (>|t|)
Intercept	6.075	6.434	0.944	0.347
EDARADD	-0.307	0.072	-4.243	0.000
FHL2	0.511	0.100	5.090	0.000
			R^2=0.198	**p-value=0.000**

b

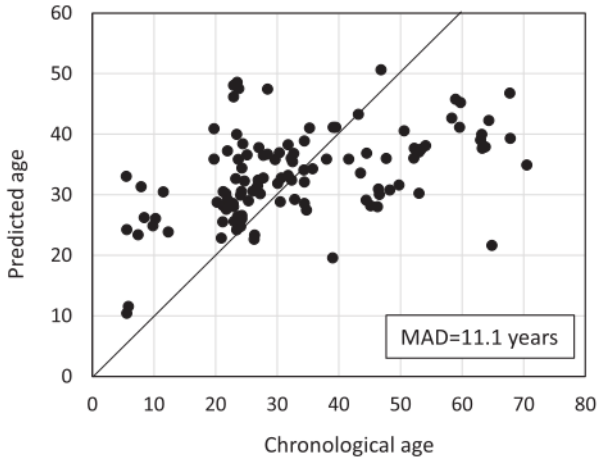

Fig. 3. Validation of the predicted age through multiple linear regression analysis considering the methylation scores of *EDARADD* and *FHL2* only (79 "Japanese" and 34 "Indonesian" saliva samples). (a) Coefficients in this study. (b) Relation between predicted and chronological ages.

results from young and elderly donors in Japan. To develop the accurate age estimation model, we need more samples. Second, we could not include *ELOVL2* in the main analysis. Adding *ELOVL2* to the analysis would probably improve the accuracy of the prediction formula. Third, the saliva samples were frozen immediately after collection. In our preliminary results, we observed that methylation was subject to variation in a 4 °C refrigerator (Supplementary Fig. 2). As mentioned in a previous study [14], storage conditions of samples must be maintained during examination to measure saliva DNA methylation. Further investigations are needed to define the effects of prolonged storage and preservation methods on saliva samples.

In conclusion, we believe that DNA phenotyping has considerable potential in age prediction and resolving forensic issues. However, there was a significant difference in methylation scores between Japanese and Indonesian participants of early 20 s group. Additionally, considering methylation scores, sex, and nationality, we found that the nationality coefficient was significant for age estimation when applying the existing method for the analysis of the methylation status of *EDARADD* and *FHL2*. Therefore, when focusing on the development of improved age prediction models using certain biological indicators, ethnic information should be considered.

Funding

This work was supported by JSPS KAKENHI grant number JP18K09910 and JP21K10253 (to Hiroko Oka).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank all the donors in this study. We also wish to acknowledge the work of the members of the Oral Clinical Examination Center Hiroshima University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.legalmed.2022.102042.

References

[1] X.-L. Ou, J. Gao, H. Wang, H.-S. Wang, H.-L. Lu, H.-y. Sun, R. Lafrenie, Predicting human age with bloodstains by sjTREC quantification, PLoS One 7 (8) (2012) e42412, https://doi.org/10.1371/journal.pone.0042412.

[2] F. Ren, C. Li, H. Xi, Y. Wen, K. Huang, Estimation of human age according to telomere shortening in peripheral blood leukocytes of Tibetan, Am. J. Forensic Med. Pathol. 30 (2009) 252–255, https://doi.org/10.1097/PAF.0b013e318187df8e.

[3] A.B. Márquez-Ruiz, L. González-Herrera, A. Valenzuela, Usefulness of telomere length in DNA from human teeth for age estimation, Int. J. Legal Med. 132 (2) (2018) 353–359, https://doi.org/10.1007/s00414-017-1595-5.

[4] D. Zubakov, F. Liu, M.C. van Zelm, J. Vermeulen, B.A. Oostra, C.M. van Duijn, G. J. Driessen, J.J.M. van Dongen, M. Kayser, A.W. Langerak, Estimating human age from T-cell DNA rearrangements, Curr. Biol. 20 (22) (2010) R970–R971, https://doi.org/10.1016/j.cub.2010.10.022.

[5] S. Bocklandt, W. Lin, M.E. Sehl, F.J. Sánchez, J.S. Sinsheimer, S. Horvath, E. Vilain, N. Landsberger, Epigenetic predictor of age, PLoS ONE 6 (6) (2011) e14821, https://doi.org/10.1371/journal.pone.0014821.

[6] G. Hannum, J. Guinney, L. Zhao, L.i. Zhang, G. Hughes, S. Sadda, B. Klotzle, M. Bibikova, J.-B. Fan, Y. Gao, R. Deconde, M. Chen, I. Rajapakse, S. Friend, T. Ideker, K. Zhang, Genome-wide methylation profiles reveal quantitative views of human aging rates, Mol. Cell 49 (2) (2013) 359–367, https://doi.org/10.1016/j.molcel.2012.10.016.

[7] S. Horvath, DNA methylation age of human tissues and cell types, Genome Biol. 14 (10) (2013) R115, https://doi.org/10.1186/gb-2013-14-10-r115.

[8] R. Zbieć-Piekarska, M. Spólnicka, T. Kupiec, A. Parys-Proszek, Ż. Makowska, A. Paleczka, K. Kucharczyk, R. Ploski, W. Branicki, Development of a forensically useful age prediction method based on DNA methylation analysis, Forensic Sci. Int. Genet. 17 (2015) 173–179, https://doi.org/10.1016/j.fsigen.2015.05.001.

[9] S.R. Hong, S.E. Jung, E.H. Lee, K.J. Shin, W.I. Yang, H.Y. Lee, DNA methylation-based age prediction from saliva: High age predictability by combination of 7 CpG markers, Forensic Sci. Int. Genet. 29 (2017) 118–125, https://doi.org/10.1016/j.fsigen.2017.04.006.

[10] A. Vidaki, M. Kayser, Recent progress, methods and perspectives in forensic epigenetics, Forensic Sci. Int. Genet. 37 (2018) 180–195, https://doi.org/10.1016/j.fsigen.2018.08.008.

[11] A.E. Field, N.A. Robertson, T. Wang, A. Havas, T. Ideker, P.D. Adams, DNA methylation clocks in aging: Categories, causes, and consequences, Mol. Cell 71 (6) (2018) 882–895, https://doi.org/10.1016/j.molcel.2018.08.008.

[12] Y. Hamano, S. Manabe, C. Morimoto, S. Fujimoto, K. Tamaki, Forensic age prediction for saliva samples using methylation-sensitive high resolution melting: Exploratory application for cigarette butts, Sci. Rep. 7 (2017) 10444, https://doi.org/10.1038/s41598-017-10752-w.

[13] S.E. Jung, S.M. Lim, S.R. Hong, E.H. Lee, K.J. Shin, H.Y. Lee, DNA methylation of the ELOVL2, FHL2, KLF14, C1orf132/MIR29B2C, and TRIM59 genes for age prediction from blood, saliva, and buccal swab samples, Forensic Sci. Int. Genet. 38 (2019) 1–8, https://doi.org/10.1016/j.fsigen.2018.09.010.

[14] Y. Hamano, S. Manabe, C. Morimoto, S. Fujimoto, M. Ozeki, K. Tamaki, Forensic age prediction for dead or living samples by use of methylation-sensitive high resolution melting, Leg. Med. (Tokyo) 21 (2016) 5–10, https://doi.org/10.1016/j.legalmed.2016.05.001.

[15] M. Kondo, H. Aboshi, M. Yoshikawa, A. Ogata, R. Murayama, M. Takei, S. Aizawa, A newly developed age estimation method based on CpG methylation of teeth-derived DNA using real-time methylation-specific PCR, J. Oral Sci. 63 (1) (2021) 54–58, https://doi.org/10.2334/josnusd.20-0138.

[16] Y. Kanda, Investigation of the freely available easy-to-use software "EZR" (Easy R) for medical statistics, Bone Marrow Transplant. 48 (2013) 452–458, https://doi.org/10.1038/bmt.2012.244.

[17] Portal site of Official Statics of Japan, https://www.e-stat.go.jp. Table 19-12-01-1.

[18] C.M. Cianga, I. Antohe, M. Zlei, D. Constantinescu, P. Cianga, Saliva leukocytes rather than saliva epithelial cells represent the main source of DNA, Rev. Rom. Med. Lab. 24 (2016) 31–44, https://doi.org/10.1515/rrlm-2016-0011.

[19] A. Woźniak, A. Heidegger, D. Piniewska-Róg, E. Pośpiech, C. Xavier, A. Pisarek, E. Kartasińska, M. Boroń, A. Freire-Aradas, M. Wojtas, M. de la Puente, H. Niederstätter, R. Ploski, M. Spólnicka, M. Kayser, C. Phillips, W. Parson, W. Branicki, VISAGE Consortium, Development of the VISAGE enhanced tool and statistical models for epigenetic age estimation in blood, buccal cells and bones, Aging 13 (5) (2021) 6459–6484.

Some tips on age estimation using DNA methylation in saliva samples as an index across the Japanese and Indonesian ethnicities

ORIGINALITY REPORT

20%	14%	17%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Yuya Hamano, Sho Manabe, Chie Morimoto, Shuntaro Fujimoto, Keiji Tamaki. "Forensic age prediction for saliva samples using methylation-sensitive high resolution melting: exploratory application for cigarette butts", Scientific Reports, 2017 Publication	2%
2	www.alexandria.unisg.ch Internet Source	2%
3	www.lib.kobe-u.ac.jp Internet Source	2%
4	Shuntaro Fujimoto, Yuya Hamano, Kentaro Ichioka, Sho Manabe, Eriko Hirai, Osamu Ogawa, Keiji Tamaki. "Rapid semen identification from mixed body fluids using methylation-sensitive high-resolution melting analysis of the DACT1 gene", Legal Medicine, 2021 Publication	1%

5	hdl.handle.net Internet Source	1 %
6	www.frontiersin.org Internet Source	1 %
7	Kuzmina, Nina S., Nellya Sh. Lapteva, and Alexander V. Rubanovich. "Hypermethylation of gene promoters in peripheral blood leukocytes in humans long term after radiation exposure", Environmental Research, 2016. Publication	1 %
8	link.springer.com Internet Source	1 %
9	trisomie21.de Internet Source	1 %
10	www.jstage.jst.go.jp Internet Source	1 %
11	0-www-mdpi-com.brum.beds.ac.uk Internet Source	1 %
12	www.mdpi.com Internet Source	1 %
13	Hitoshi Komatsuzawa, Kouji Ohta, Tamaki Fujiwara, Gil H Choi, Harald Labischinski, Motoyuki Sugai. " Cloning and sequencing of the gene, , which affects oxacillin resistance in	<1 %

methicillin-resistant ", FEMS Microbiology Letters, 2001
Publication

14	Ziwei Ye, Lirong Jiang, Mengyao Zhao, Jing Liu, Hao Dai, Yiping Hou, Zheng Wang. "Epigenome-wide screening of CpG markers to develop a multiplex methylation SNaPshot assay for age prediction", Legal Medicine, 2022 Publication	<1 %
15	umpir.ump.edu.my Internet Source	<1 %
16	Kiyoharu Shimizu, Takashi Sadatomo, Takeshi Hara, Hideo Ohba, Kiyoshi Yuki, Kaoru Kurisu. "Frequency and Predicting Factors on Chronic Expanding Intracerebral Hematoma in Spontaneous Intracerebral Hemorrhage", Journal of Stroke and Cerebrovascular Diseases, 2017 Publication	<1 %
17	apsjournals.apsnet.org Internet Source	<1 %
18	waseda.pure.elsevier.com Internet Source	<1 %
19	www.spandidos-publications.com Internet Source	<1 %

20	A Heidegger, A Pisarek, M de la Puente, H Niederstätter et al. "Development and inter-laboratory validation of the VISAGE enhanced tool for age estimation from semen using quantitative DNA methylation analysis", Forensic Science International: Genetics, 2021 Publication	<1 %
21	Evelina Björkegren, Helena Svaleryd. "Birth order and health disparities throughout the life course", Social Science & Medicine, 2023 Publication	<1 %
22	docksci.com Internet Source	<1 %
23	pubmed.ncbi.nlm.nih.gov Internet Source	<1 %
24	coek.info Internet Source	<1 %
25	eprints.lib.okayama-u.ac.jp Internet Source	<1 %
26	govdocs.nebraska.gov Internet Source	<1 %
27	www.science.gov Internet Source	<1 %
28	www.tmd.ac.jp Internet Source	<1 %

29	Zbieć-Piekarska, Renata, Magdalena Spólnicka, Tomasz Kupiec, Żanetta Makowska, Anna Spas, Agnieszka Parys-Proszek, Krzysztof Kucharczyk, Rafał Płoski, and Wojciech Branicki. "Examination of DNA methylation status of the ELOVL2 marker may be useful for human age prediction in forensic science", Forensic Science International Genetics, 2015. Publication	<1 %
30	A. Freire-Aradas, C. Phillips, A. Mosquera-Miguel, L. Girón-Santamaría et al. "Development of a methylation marker set for forensic age estimation using analysis of public methylation data and the Agena Bioscience EpiTYPER system", Forensic Science International: Genetics, 2016 Publication	<1 %
31	Helena Correia Dias, Eugénia Cunha, Francisco Corte Real, Licínio Manco. "Age prediction in living: Forensic epigenetic age estimation based on blood samples", Legal Medicine, 2020 Publication	<1 %
32	Yuya Hamano, Sho Manabe, Chie Morimoto, Shuntaro Fujimoto, Munetaka Ozeki, Keiji Tamaki. "Forensic age prediction for dead or	<1 %

living samples by use of methylation-sensitive high resolution melting", Legal Medicine, 2016
Publication

33 Alan Tomusiak, Ariel Floro, Ritesh Tiwari, Rebeccah Riley, Hiroyuki Matsui, Nicolas Andrews, Herbert G. Kasler, Eric Verdin. "Development of a novel epigenetic clock resistant to changes in immune cell composition", Cold Spring Harbor Laboratory, 2023 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

Some tips on age estimation using DNA methylation in saliva samples as an index across the Japanese and Indonesian ethnicities

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6