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Review Paper

A Systematic Review of the Possibility of Determining Age Based on DNA Methylation of the ELOVL2 Gene in Human Samples

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ABSTRACT

Background: In forensic medicine, predicting the age of a victim or suspect can be a clue to solving a crime. Epigenetics has recently played a vital role in age prediction in forensic medicine. Cytosine methylation at cytosine and guanine separated by phosphate (CpG) sites is well recognized as a novel epigenetic marker for age estimation. This study aimed to summarize the information obtained from previous studies to determine age by evaluating DNA methylation in the ELOVL2 gene.

Methods: In this systematic review, all related articles published between 2012 and 2022 were extracted by searching reputable scientific databases, such as ISI Web of Science, Science Direct, PubMed, and Scopus. After selecting the appropriate articles, the full text of the articles was prepared and fully evaluated by the researchers. The protocol of this study was carried out based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement.

Results: Out of 307 articles, 5 articles were eligible for review according to the study protocol. The strongest correlation between DNA methylation and age was observed at sites 11044644 and 11044634 on chromosome 6 in the living cases. The relationship between the chronological age and the age calculated through DNA methylation was above 90% with an approximate error ranging from 7.5 to 10.4. However, the relationship between the chronological age and the age calculated through DNA methylation was above 90% in the multivariate analysis of sites 11044624 and 11044634 on chromosome 6. In this case, the calculation error reached approximately 6.9 years. Hence, considering a combination of multiple cytosine and guanine separated by phosphate (CpG) sites improves the calculation accuracy and reduces the error percentage. The relationships between DNA methylation and the age at sites 11044880 and 11044640 on chromosome 6 were significantly less reported in the blood samples taken from the dead and in those taken from the living (nearly 64%–78.5%).

Conclusion: The results of this study indicated that DNA methylation in the ELOVL2 gene could help predict a person's biological age.

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1. Introduction

Morphological traits and skeletal characteristics are usually analyzed to estimate the age of human remains [1]. Nevertheless, these investigations can only be conducted on skeletal and dental tissues, and such methods cannot always predict the deceased's accurate age [2]. Aging is a complicated process characterized by a general decline in physiological abilities. So far, many attempts have been made to determine age by finding reliable biomarkers, such as the telomere lengths of leukocytes, the disappearance of mitochondrial DNA, variations in the expression of some specific proteins, and genetic examinations. Moreover, epigenetics has been among the most promising research areas [3–5]. DNA profiling has now helped forensic pathologists solve many problems to identify unknown corpses in crucial legal cases; however, DNA profiling techniques are adopted in forensic investigations due to how accurate these techniques are [6]. Age estimation at the molecular level can be acquired by telomere length, mRNA markers, and signal joint T-cell receptor rearrangement excision circle (sjTREC)-based methods, but their accuracy is not sufficient for forensic purposes. DNA methylation is a proper solution to this problem which can estimate the age at the time of death with a mean absolute deviation of about 3 to 5 years from the predicted age [7]. According to the research literature, serious remodeling of DNA methylation patterns in the human genome can be identified as a function of the chronological age in some tissues. Therefore, it is possible to make a rather reliable age estimate by combining cytosine and guanine separated by phosphate (CpG) sites in which the methylation status is related to the age [8].

The CpG sites are the dinucleotides that can be methylated by adding a methyl group derived from S-adenosylmethionine on the residual cytosine C5 site. The human CpG methylation patterns are often analyzed by using specific DNA arrangements across the genome via Illumina Infinium human-methylation45 BeadChip [9]. In 2012, the Infinium 450K platform was even used for the 1st time in a study to analyze age-related variations in the DNA methylation of blood samples. Two genome regions were identified at CpG sites about the gene drives of ELOVL2 and FHL2, the methylation status of which was closely correlated with age [10]. Different studies showed that the ELOVL2 gene shows a progressive increase in methylation in the 1st stage of life, and this locus is a good blood source for estimating chronological age and is almost stable at room temperature for 4 weeks [7, 10]. In recent years, various research groups have employed the Illumina BeadArray technology to identify CpG sites. According to their results, age-related hyper-methylation in CpG islands often targets the genes that are not expressed in blood tissues, whereas age-related hypo-methylation targets the highly expressed genes [11]. Recent studies indicated that the use of DNA methylation biomarkers is better than the analysis of other molecular biomarkers for age examination [12–16].

This study aimed to summarize and conclude the previous research findings to determine age by evaluating the DNA methylation in the ELOVL2 gene.

2. Materials and Methods

This systematic review aimed to find relevant evidence for age estimation based on the DNA methylation in the ELOVL2 gene by searching reputable scientific databases, such as ISI Web of Science, ScienceDirect, PubMed, and Scopus. For this purpose, all papers published in the period 2012–2022 were extracted by using keywords, such as DNA methylation, age estimation, age predictor, age marker, aging, chronological age, biological age, epigenetic clock, epigenotyping, epigenetic, ELOVL2 gene, ELOVL2 marker, ELOVL2 promoter, and CpG site.

After the initial search process was finalized, two of the authors (FF and SMM) started to analyze the titles and abstracts of papers, regardless of the journals and the authors' names. The only papers included were the authentic research papers with available full texts, and written in English. The included papers reported the results of changes to ELOVL2 and indicated the ages of participants. Zoonotic studies, irrelevant studies, letters to chief editors, and review studies were excluded and archived. After the appropriate papers were selected, their full texts were prepared and fully evaluated by two researchers. The protocol of this systematic study was based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) declaration [17]. Before the search process started, the research protocol was registered at: PROSPERO; The International prospective register of systematic reviews [18].

The extracted references were analyzed independently by two reviewers (SMM and FF). If removed, they were archived for specific reasons. When the two reviewers differed in selecting papers, a 3rd party (MA) was asked to help select articles. After the articles were reviewed, the necessary data were inserted in certain tables designed for this purpose. The data included the authors' names, publication years, research sites, sample sizes, techniques of methylation investigation, number and type of examined

samples, variations in ELOVL2, and the mean absolute value of age deviation based on DNA methylation.

The papers were qualitatively evaluated based on the types of studies by strengthening the reporting of observational studies in epidemiology (STROBE) [19]. Before the study started, it was approved by the Ethics Committee of Iran University of Medical Sciences (Code: IR.IUMS.FMD.REC.1401.250).

3. Results

In the first search attempt, 307 studies were extracted from March to July 2022 and entered into EndNote software. In the first step, 8 repetitive papers were determined and excluded. The remaining 299 papers were screened by the two reviewers based on titles and abstracts. After that, 43 papers were qualified for the next step. The full texts of those papers were then analyzed for selection, and 38 papers were excluded for various reasons (i.e. one paper was excluded due to lack of peer-reviewed; 7 papers were excluded due to their limited populations; 5 papers were excluded because the research papers were not valid, and 23 other papers were excluded due to the interference of other genes with ELOVL2. Finally, seven papers met the inclusion criteria. Figure 1 demonstrates a PRISMA chart indicating the operational schematic for the search and selection of papers. Their results were extracted and analyzed by strengthening the reporting of observational studies in epidemiology (STROBE). Table 1 presents data regarding the direct effects of DNA methylation variations on ELOVL2 based on age.

In all selected studies, the bisulfite conversion and pyrosequencing method were used to check the methylation status in the ELOVL2 gene. Nevertheless, liver tissue, dental pulp, dentine, cementum, buccal swabs, saliva, and blood samples were reported to be investigated in different studies, only those articles that were conducted on blood samples fulfilled the inclusion criteria. Though this is the first review to examine DNA methylation of the ELOVL2 gene for age estimation into a predictive model, it is limited by the heterogeneity of studies, and statistical analysis is not possible.

Anaya et al. analyzed the DNA methylation levels of Chromosome 6 at Site 11044880 (CpG3) in 232 blood samples taken from deceased individuals aged between three months and 93 years. Using the linear univariate regression analysis, they reported a significant relationship (P=0.00). According to their results, CpG3 methylation for the ELOVL2 had a positive correlation with the

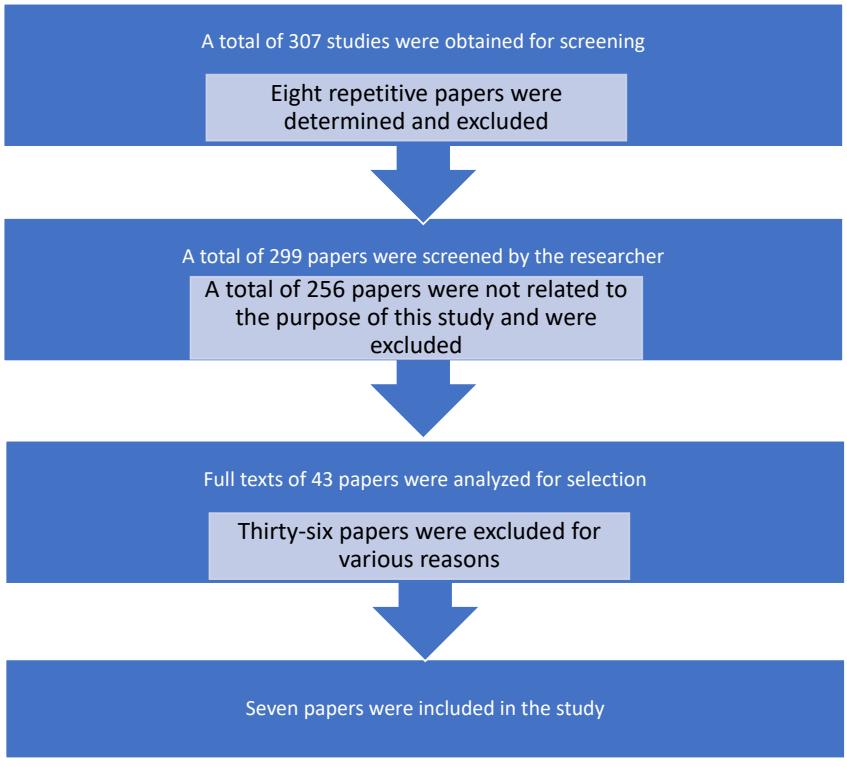

Figure 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) chart, the operational schematic for the search and selection of papers

Table 1. Characteristics of the samples included in the study (in all of the following studies, the method based on bisulfite conversion and pyrosequencing was used to check the methylation status in the ELOVL2 gene).

Reference (Published Year)	Country	Blood Sample	Age (y)	Number of Samples	Gene Location on Chromosome 6	R	R^2	SE	P	MAD
Anaya (2021) [19]	U.S.A	Deceased individuals	93-0	232	11044880	0.643	0.412	14.08	0.00	64.8
Dias (2020) [20]	Portugal	Living individuals	95-1	53	11044644	0.936	0.874	10.41	7.97×10^{-25}	01.8
Dias (2020) [21]	Portugal	Deceased individuals	86-24	51	11044640	0.785	0.608	10.2	2.39×10^{-11}	89.8
Dias (2020) [22]	Portugal	Living	94-1	56	11044628	0.951	0.904	-	3.58×10^{-29}	-
		Deceased individuals	86-28	62	11044628	0.791	0.626	-	2.04×10^{-14}	-
		Deceased and living individuals	94-1	118	11044628	0.919	0.845	-	7.60×10^{-49}	-
Jung [15]	Korea	Living individuals	18-74	150	11044628	0.879	0.773	-	<0.001	-
Cho [34]	Korea	Living individuals	20-74	100	11044628	0.921	0.848		<0.001	10.33*
Zbiec-Piekarska (2015) [13]	Poland	Living individuals	75-2	303	11044661	0.828	0.685	10.23	1.17^{-77}	-
					11044655	0.798	0.635	11.01	4.53^{-68}	-
					11044647	0.813	0.660	10.62	9.10^{-73}	-
					11044644	0.844	0.711	9.80	2.32^{-83}	-
					11044642	0.882	0.778	8.60	1.71^{-100}	-
					11044640	0.859	0.737	9.35	1.90^{-89}	-
					11044634	0.913	0.883	7.46	4.34^{-119}	-
					11044642	0.927	0.859	6.85	4.91^{-13}	5.03
					11044634				9.93^{-32}	

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Abbreviations: MAD: Mean absolute deviation; SE: Standard error. * This value is calculated based on the model by Cho et al.

chronological age of participants (R=0.643). They reported a high accuracy in predicting the ages of participants (R^2=0.412). It covered 41.2% of the age dispersion of research samples. The standard error and the mean absolute deviation were 14.08 and 8.64, respectively, compared to the chronological age, indicating many outliers in the blood samples taken from corpses aged 0–93 years [5].

Dias et al. analyzed the DNA methylation level of chromosome 6 at site 11044644 (CpG6) in 53 blood samples taken from living participants aged 1–95 years. They reported a significant relationship ($P=7.97\times10^{-25}$). In the linear univariate regression analysis, CpG6 methylation for ELOVL2 had a strong correlation with the chronological age among participants (R=0.936) and covered 87.4% of age dispersion in the research sample (R^2=0.874). The standard error and the mean absolute deviation were reported 10.41 and 8.01, respectively, as opposed to the chronological age. Hence, many outliers were not observed in the blood samples of the living individuals aged 1–95 years. The results also indicated no significant differences between male and female participants in terms of DNA methylation at CpG6 sites depending on the ELOVL2 gene with increasing age (no data reported in this case) [20].

In the other study, Dias et al. analyzed the DNA methylation levels of chromosome 6 at site 11044640 (CpG6) in 51 blood samples of deceased individuals aged 24–86 years. Using the linear univariate regression analysis, they reported a significant relationship ($P=2.39\times10^{-11}$). According to their results, CpG6 methylation for the ELOVL2 site had a positive correlation with the chronological age of the samples (R=0.785) and had a high accuracy in age prediction ($R^2=0.608$). It covered 60.8% of the age dispersion among participants. The standard error and the mean absolute deviation were reported 10.2 and 8.89, respectively, as opposed to the chronological age, indicating that many outliers were not observed in the blood samples taken from the living participants aged 1–95 years [21].

In conducting another study, Dias et al. measured the DNA methylation levels of chromosome 6 at site 11044628 in 56 blood samples taken from living participants aged 1–94 years and in 62 blood samples taken from the decreased individuals aged 28–86 years. Using the linear univariate regression analysis, they reported a significant relationship. According to their results, DNA methylation at the ELOVL2 site had a positive correlation with chronological age, although this correlation was stronger in the blood samples of the living (r=0.951) than in deceased individuals (R=0.791). Moreover, the correlation covered 90.4% and 62.6% of the age dispersion among the samples taken from living and the decreased, respectively. According to the results, no significant differences were observed between men and women in terms of DNA methylation at the ELOVL2 site (no data were reported in this case) [22].

Zbiec–Piekarska et al. analyzed the DNA methylation levels of chromosome 6 at sites 11044661 (CpG1), 11044655 (CpG2), 11044647 (CpG3), 11044644 (CpG4), 11044642 (CpG5), 11044640 (CpG6), and 11044634 (CpG7) in 303 blood samples taken from the living participants aged 2–75 years. The strongest correlation was found at CpG7 (R=0.913) which covered 83.3% of the age dispersion in the research samples. In the multivariate analysis, CpG7 and CpG5 were selected for the final linear regression evaluation. They covered nearly 86% of the age dispersion in the research sample ($R^2=0.859$). The mean error and the mean absolute deviation in this case were reported at 6.85 and 5.03 years, respectively [13].

4. Discussion

Various methylation sites have been found in the human gene that is related to chronological age. Researchers have become interested in these sites to conduct scientific evaluations and develop age prediction models, especially in legal medicine investigations. In this regard, the best biomarkers are those that can make significant changes in methylation across the human age range with the lowest error rate. An effective method is based on bisulfite conversion and pyrosequencing to analyze the methylation status of the ELOVL2 gene [13]. This gene expresses the information about an enzyme that plays a role in producing a very long polyunsaturated fatty acid chain [23]. This method is based on increasing the segments containing 308 open pairs or sequencing shorter segments containing 7 CpG sites on chromosome 6 at sites 11044634 to 11044661 [13]. Garagnani et al. reported for the 1st time that the methylation levels of CpG sites in the promoter ELOVL2 gene had a significant correlation with age to the point that aging would increase it from 7% to 91% [13]. After that, different studies indicated the possibility of analyzing DNA methylation in the ELOVL2 gene to develop age prediction models [10, 15]. Although it has been recommended to measure methylation variations in different genes to design age prediction models [24], ELOVL2 variations appear to be the best option to complete the age evaluation model based on genetic variations due to its strong correlation and a wide variety of changes as a result of aging [25, 26].

According to the literature, the strongest correlation between DNA methylation and age was observed at sites 11044644 and 11044634 on chromosome 6 in the living cases. The relationship between the chronological age and the age calculated through DNA methylation was above 90% with an approximate error ranging from 7.5 to 10.4. However, the relationship between the chronological age and the age calculated through DNA methylation was above 90% in the multivariate analysis of sites 11044624 and 11044634 on chromosome 6. In this case, the calculation error reached approximately 6.9 years. Hence, considering a combination of multiple CpG sites improves the calculation accuracy and reduces the error percentage. Nevertheless, the relationships between DNA methylation and the age at sites 11044880 and 11044640 on chromosome 6 are significantly less reported in the blood samples taken from the dead and in those taken from the living (nearly 64%–78.5%). The calculation error ranged approximately from 14 to 10.2 years. Dias et al. [22] evaluated the DNA methylation levels of blood samples taken from the living and deceased cases and reported that the correlation with the chronological age was higher in the living cases than in the dead ones. Postmortem changes in DNA methylation levels appear to be responsible for these differences. Nonetheless, Bekaert et al. [11] reported no differences

between the two groups. Furthermore, Vilahur et al. [27] indicated that DNA methylation does not experience any variations, at least in biological samples kept at room temperature.

According to this study, the lowest standard error (nearly 7.5 years) in age calculation based on DNA methylation was observed at site 11044634 on chromosome 6, whereas the highest standard error (nearly 14 years) was observed at site 11044800 on chromosome 6 (the mean absolute value of deviation was approximately 8.6 in the latter). Moreover, Bekaert [11] and Zbiec–Piekarska [13] reported that the highest error rate was recorded in the samples taken from the elderly age group (above 60 years). According to Zbiec–Piekarska [13], DNA methylation was 26% at site 11044634 on chromosome 6 at the age of two years and reached nearly 80% until the age of 76 years. Variations in DNA methylation occur very fast in childhood and increase significantly until adolescence, after which this process decelerates [28, 29]. Hamano reported very low error rates in calculating the ages of samples before the age of 20 years and the findings should be interpreted carefully for the participants above 50 years [30].

According to the findings, no significant differences were observed between male and female participants in terms of DNA methylation at ELOVL2 following the ages of the living and the dead individuals. Similar results were also reported in other studies [9, 30]. Huang et al. designed a model for age estimation in male and female samples; however, they reported no significant changes in their proposed model [31]. Nevertheless, differences existed between the two genders in terms of DNA methylation levels in gender-dependent genes (e.g. G6PD, ENFB1, ELK1, and GPC3) [30].

Zibec–Piekarska et al. [13] reported the durability of DNA methylation in blood stains after one month at room temperature. They also indicated that their method can also be used to predict age using 15-year-old blood stains. Nevertheless, 308 open pairs for pyrosequencing may not be required to reach accurate predictions in very old samples due to DNA decay. This finding is consistent with the results reported by Vilahur [27] and An [32]. Nonetheless, Antunes et al. [33] evaluated DNA methylation-based on bisulfite conversion and pyrosequencing and reported similar methylation levels in the samples kept for 20 years in proper conditions compared to the fresh samples.

5. Conclusion

The results of this study indicated that DNA methylation in the ELOVL2 gene can help predict a person's biological age. However, this estimation may not be consistent with the chronological age. Since fewer findings are available about age estimation based on autopsy examinations usually after maturity, the epigenetic changes, such as DNA hyper-methylation evaluations in this gene, which is quite sustainable in human samples, can be effective in identifying unknown individuals. Moreover, simultaneous evaluations of CpG sites can be used in forensic investigations to predict age that is possibly close to the chronological age. This can be a new field of research in forensic science applied to samples obtained from a crime scene or mass fatalities. Given the novelty of this research area, conducting such studies in Iranian society and proposing an appropriate model to predict age with acceptable accuracy in Iran's legal genetic laboratories can reduce the costs of genetic identification and provide better services for Iranians.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles are considered in this article. This study was approved by the Ethics Committee of the Iran University of Medical Sciences (Code: IR.IUMS.FMD.REC.1401.250). This article is a systematic review with no human or animal sample.

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Authors' contributions

Conceptualization and Supervision: Sayed Mahdi Marashi and Foroozan Faress; Methodology: Maryam Ameri; Investigation, Writing-original draft, and Writing -review & editing: All authors; Data collection: Sayed Mahdi Marashi, and Foroozan Faress; Data analysis: Sayed Mahdi Marashi, and Foroozan Faress; Funding acquisition and Resources: Foroozan Faress, and Maryam Ameri.

Conflict of interest

The authors declared no conflict of interest.

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