

Epigenetic Approach in Forensic Age Estimation

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Abstract: Age estimation study is a very important research area that contributes to the solution of the forensic case by helping to identify the identity in forensic sciences. Human age estimation in the traditional way is performed by analysis of bony marks on bones and teeth. An analysis of the age estimation of biological samples from the use of genetic analysis has not yet become part of routine practice. The use of genetic analyses for forensic purposes started with the Restriction Fragment Length Polymorphism (RFLP) analysis in the late 1980s and developed with Short Tandem Repeats (STR) analysis. Along with the technological developments in forensic genetics, progress has continued with single nucleotide polymorphism (SNP) analysis, which enables the identification of hair, eye and skin color and geographic infrastructure of an unknown sample in forensic case resolution. However, recent studies in forensic genetics have focused on epigenetic mechanisms and it has been discovered that DNA methylation can be used in case resolution for forensic age estimation. With the development of DNA methylation studies, a quantitative statistical relationship has been established between DNA methylation and different age groups. The results have been obtained with $\pm 3-4$ age prediction accuracy using DNA methylation markers (CpG regions) tested to date with different methodological approaches. Thus, with the advancement of epigenetic studies in the fields of forensic sciences, the phenotypic features of the DNA of the evidence samples have been estimated with some error rates. The aim of this study is to reveal the latest developments in the field of epigenetics and evaluation of the use of epigenetic-based age estimates for forensic purposes.

Keywords: *Forensic genetic; epigenetic; DNA methylation; age estimation*

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1. Introduction

1.1. Epigenetic and Age

Age estimation is one of the important subjects of forensic sciences used to determine the identity of suspects. Age estimation of people with traditional methods, bone and teeth are made by the analysis of bony traces (Hillewig et al., 2013; Olze et al., 2006). However, this approach is limited to cases of a skeleton. On the other hand, biomarkers in which 4977-bp was detected in this area, which was removed from the mitochondrial DNA (mtDNA) at the molecular level (Meissner et al., 1997), d-aspartic acid ratios in proteins (Helfman et al., 1976) and length of leukocyte telomeres (Blasco, 2005) could be used to age estimation. However, the age estimates made by these biomarkers defined so far have significant limitations (because of the poor understanding of the mechanisms that cause the deletion of mtDNA) (Meissner et al., 2010).

Epigenetics is a rapidly developing field as a result of new technological developments and the increase of biological discoveries. The relationship between epigenetic and environmental factors is not well defined in mammals, however, it was found that hypo and hypermethylation levels in the methylation regions of DNA were related to aging (Day et al., 2013; Fraser et al., 2012; Tammen et al., 2013). Thus, DNA methylation levels can be used in case of solutions for forensic age estimation. Researchers working on this subject have discovered the age-related specific methylation sites in the human genome with aging. In this context, it has been observed that forensic sciences experts have increased their interest in age-related DNA methylation markers in the last decade. $\pm 3-4$ year prediction accuracy results were obtained from biological samples using DNA methylation markers (CpG sites) tested to date with different methodological approaches. Thus, with the advancement of genetic and epigenetic studies in the field of forensic science, the phenotypic features of the DNA of the evidence samples have been estimated with certain error rates. In recent years, the estimation and analysis of new multiple CpG regions with a linear correlation between DNA methylation levels and biological age for forensic age estimation and error rates in age estimates tend to decrease. The aim of this study is to reveal the recent developments in this field and to evaluate the use of epigenetic-based age estimates for forensic purposes.

2. DNA Methylation

DNA methylation occurring in the 5' position of cytosine in CpG dinucleotide is a genetically programmed type of DNA modification in mammals (Kohli et al., 2013). DNA methylation analysis has proven to be a reliable method in estimating age in forensic sciences (Vidaki et al., 2013; Yi et al., 2014; Yi et al., 2015; Zbiec-Piekearska et al., 2015) since the relationship of DNA methylation with age has been proven (Hernandez et al., 2011; Johansson et al., 2013; Steegenga et al., 2014).

2.1. DNA Methylation and Environment

Studies in identical twins have focused on identifying links between environmental or aging and long-term epigenetic effects on the phenotype. Recent studies support the hypothesis that DNA methylation

acts as a cross between the constant genome and the dynamic environment. In contrast to changes in DNA methylation patterns, environmental stress during the first years of life or throughout life serves as a lifelong genome adaption mechanism and activates the same genome to express different phenotypes (Szyf, 2011). Many studies examining identical twins have determined the relationship between environmental and aging by means of phenotypic persistent epigenetic effects (Fraga et al., 2005; Wong et al., 2005).

Because DNA base sequences are the same, the investigation of identical twins for forensic case solutions is one of the most troublesome subjects of forensic sciences. Because identical twins share the same genetic basis serves as an ideal system for epigenetic research. In a study that examined DNA methylation levels in monozygotic twins, while there was almost no difference between twins in the early stages of life, there were significant differences between twins over 28 years of age (Fraga et al., 2005). Scientists thought it could be explained in a wide spectrum on non-genetic age-related differences on 'gene traces', anthropomorphic characteristics, usually observed in identical twins or disease predisposition (Fraga et al., 2005).

2.2. DNA Methylation as a Biological Indicator

Genes that show high levels of DNA methylation in regulatory regions are usually not expressed in the transcription phase and DNA methylation accumulates in the long term leading to silencing of the genes. Epigenetic inaccurate programming, which leads to abnormal DNA methylation patterns (hyper/hypo-methylation) is significantly observed in many diseases and malignant tumors. Hypermethylation generally occurs in CpG regions in promoter regions of cancer-specific genes and is associated with gene inactivation. Hypomethylation throughout the genome is associated with the progression of cancer through different mechanisms (Wild et al., 2010). Interestingly there is evidence supporting two different hypotheses about how hypomethylation occurs. One involves 'passive' loss of DNA methylation in cell division and the other involves the 'active' and potentially much faster loss of methylation independent of DNA replication.

2.3. DNA Methylation Analysis

There are many techniques to measure the level of DNA methylation (Kristensen et al., 2009); but most of these techniques require a large amount of DNA or a control experiment. However, in recent years the developed method of pyrosequencing provides the opportunity to work with less biological samples and is relatively suitable for use in forensic laboratories (Park et al., 2014; Zbiec-Piekarska et al., 2015). In addition, this technique is considered to be the most reliable method to determine the level of DNA methylation (Tost et al., 2007). Until today, more than 1000 Human Methylation 450 Bead Chip data sets are stored in the National Center for Biotechnology Information Gene Expression Omnibus-GEO in the USA. Detection of DNA methylation patterns in a single CpG region, gene, or whole methyloma has recently become a growing value, particularly in the field of medical diagnosis. Recommended methods for methylation detection:

a) Chemical modification of unmethylated cytosine residues

b) Protein interaction with 5-methyl cytosine

c) Methylation sensitive restriction enzymes

However, in some cases a combination of techniques may be used, depending on the scientific purpose (Ammerpohl et al., 2009).

2.4. Forensic Science and Age Estimation by DNA Methylation Levels

The relationship between the levels of DNA methylation and age, which is the subject of this review, has been shown in studies conducted since 2000s. Along with aging, both increases and decreases in DNA methylation levels occur depending on the tissue and gene (Richardson et al., 2003). These changes lead to the development of malignancy with aging, and may also have pathological consequences that contribute to other diseases (Richardson et al., 2003). Considering the global genome, it is observed that DNA methylation levels mostly decrease with aging (Gentilini et al., 2013; Peng et al., 2012). However, DNA methylation levels have been reported to increase with age in some specific CpG regions (Beerman et al., 2013; Samuel et al., 2012).

In forensic sciences, determining the age of individuals in some cases is an important problem. When a skeleton is uncovered, age estimation (biological age) of the dead can be done by examining various morphological changes related to age in bones or teeth (Lynnerup et al., 2010). These methods produce a relative estimation with a wide variety. Moreover, it is not possible to use these methods on biological samples obtained from the scene and without skeleton or skeleton fragments.

A number of changes in the natural process tissues and organs, which cause aging, can be examined at the molecular level. So far, several methods have been developed for the aging process except from the DNA methylation studies at the molecular level. But despite all the efforts of scientists, all these proposed methods have limitations and causes loss of material in most cases and exhibit low accuracy (Meissner et al., 2010). Studies by Zubakov et al. based on the rearrangement of DNA T-cells in blood have attracted attention as a reliable age estimation method (Zubakov et al., 2010). However, researches have reported error rates of ± 8.9 years. Therefore, this method is more suitable for use only in recommended age groups, rather than full age. It is also necessary to pay attention to the diversity of gender and population groups in the implementation of the method. And people with pathological blood disease are not suitable for this template.

Epigenetic analysis, DNA methylation, which is one of the mechanisms of cell differentiation and aging, may serve as age prediction/detection method. Cells and tissues differentiate during growth and this process may involve changes in gene expression and DNA mutation. Considering that the identical twins start with almost the same methylation patterns, it is seen as an ideal model for age-related DNA methylation differences (Bocklandt et al., 2011). There are many studies examining epigenetic status of identical twins in aging studies (Li et al., 2011; Sahin et al 2011).

Bocklandt et al. used genomic size methylation analysis from the saliva sample of 34 pairs of identical twins (21-55 years old) using Illumina Human Methylation microarrays. This indication was not duplicated after advanced statistical analysis in Bocklandt's study. But a subset of 88 new loci was highly correlated with the age detected (Bocklandt et al., 2011). Based on 3 CpG regions (NPTX2, EDARADD, TOM1L1), a regression model was created with average ± 5.2 accuracy for the person's age estimate.

Koch and Wagner analyzed several data sets from 13 different cell types or tissues from local data stores (Koch et al., 2011). First, they identified age-related hypermethylated 431 and age-related hypomethylated 25 CpG regions. Then, they chose a subset from the 5 CpG regions to integrate into the epigenetic aging mark (TRIM58, KCNQ1DN, NPTX2, BIRC4BP and GRIA2). Especially, one of these CpG regions (NPTX2) was used by Bocklandt et al. in their own work. Based on the selected CpG region, estimates can be made with average ± 9.3 year accuracy (Bocklandt et al., 2011).

Bekaert et al. selected 4 genes (ASPA, PDE4C, ELOVL2, and EDARADD) related to age and identified CpG methylation levels on 206 blood samples from living and dead people (age range: 0-91). They investigated the estimation accuracy of this data with linear and non-linear regression models (Bekaert et al., 2015). They achieved high levels of accuracy with ELOVL2 methylation levels in the quadratic regression model. They found an average of 3.75 years difference between chronological age and predicted age and $R^2=0.95$ correlation. No difference for the samples obtained between two genders in terms of accuracy. There were no differences in the accuracy rate of samples from people living and dead in both sexes. Researchers also published estimation accuracy of their results according to age groups (Table 1) (Bekaert et al., 2015).

Table 1. Number and percentage of accurate and inaccurate age estimates

Age	0-19	20-39	40-59	60-91	Total
Total inaccurate	1 (3.6%)*	4 (6.6%)	14 (21.9%)	23 (45.1%)	42 (20.4%)
Total accurate	27 (96.4%)	57 (93.4%)	50 (78.1%)	28 (54.9%)	162 (79.6%)
Total	28	61	64	51	204

*Estimated and chronological age was considered correct prediction when matched as ± 5 years.

Weidner et al. used Human Methylation 450 Bead Chip technique to identify age-related DNA methylation markers in blood. In this study, pyrosequence analysis based on the combination of 3 DNA methylation markers yielded ± 5 years of accuracy in estimating age (Weidner et al., 2014).

In forensic areas, the ELOVL2 promoter is considered the most promising locus for age prediction (Garagnani et al., 2012; Florath et al., 2014; Johansson et al., 2013). Yi et al. found an average of 4 years between predicted and actual age with estimating using multiple linear regression models on blood samples (Yi et al., 2014). Likewise, Zbiec-Piekarska et al. used a multiple linear regression model based on the simultaneous analysis of the 5 CpG regions they tested in the blood and found an error rate of ± 3.9 years (Zbiec-Piekarska et al., 2015).

Horvath, a German scientist who has extensive work on DNA methylation and age, has created a large database on the computer to allow online age estimation (Horvath, 2013).

Andrew et al. studied the mtDNA control region and reported no significant difference between genders on the accuracy of age estimates based on DNA methylation analysis (Andrew et al., 2011). Bakeart et al. studied 4 age-associated genes (ASPA, PDE4C, ELOVL2, and EDARADD). Similarly, Bakeart et al. reported in their study, an average of 3.53 years for men, and an average age of 3.95 years for women and there is no statistical difference between estimates for both genders (Bakeart et al., 2015).

Li et al. have formed DNA methylation algorithm for age estimation. They stated that it is not possible to clarify the complex relationship between DNA methylation and age using a simple linear model. They used the Gradient Enhancer Regressor (GER) model to minimize the estimation error and increase the accuracy of the model. It is also examined the methylation data of 278 saliva samples to test their strength when selected age-dependent CpG regions were applied to non-blood body fluids. And it is stated that they found the correlation coefficient as 0.85 between the predicted age and the actual age (Li et al., 2018).

The most widely used DNAm microarray, Illumina Infinium Human Methylation 450 (450K sequence), has recently been replaced with the Illumina Infinium Human Methylation EPIC (EPIC sequence). Thus, the number of targeted CpG fields nearly doubled. Mc Even et al. Infinium Methylation provided support for the use of age acceleration residual metric measure in their work using EPIC Bead Chip technology (Mc Even et al., 2018).

Conclusion

In recent years, the importance of biological samples obtained from the scene increased with increasing crime incidents and the introduction of new techniques. It is not easy to make a simple and precise estimation of the molecular level from biological materials, because the aging process is quite biologically complex. However, current and future research associated with age-related epigenetic patterns may have the potential to change our understanding of aging not only in health but also in disease. Studies have shown that DNA methylation assays are a reliable and effective method for the estimation of forensic age on postmortem and anthropological samples and for the differentiation of identical twins. In addition, it can be used for age estimation in some forensic cases, especially those without material integrity, especially for the crime scene analysis (age estimation of culprit/victim). However, although significant results have been obtained in studies conducted so far, further studies are needed on population and tissue-specific DNA methylation characteristics.

Conflict of interest

The authors declare that they have no conflict of interest.

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