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Age prediction using ADN methylation

Review Article

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SUMMARY

Chronologicasl age estimation is a fundamental part for forensic science; it is used to establish a narrower profile for the search of suspects or for the identification of a corpse. In several articles based on the study of the genome it has been reported that ADN methylation promises to be a high precision marker for chronological age estimation. In the present study, articles that designed experimental models of age prediction were analyzed, including methylation patterns of characteristic genes related to the aging. In spite of the few analyzes that have focused on ADN methylation to determine age, in the great majority of them favorable results were obtained with a multivariate regression statistic; some of the hundreds of CpG sites were studied; although a greater number of CpG sites can provide greater accuracy than fewer CpG sites, such an approach can not be applied since the samples commonly found at the crime scene are usually limited.

Keywords: *ADN, Forensics, Methilation, Age, Saliva, HumanMethylation450.*

INTRODUCTION

Age is considere done of the most important parameter for a wide range of clinical conditions; it is undeniable that the survival of old aged individuals has increased considerably during the last century (Murgatroyd, 2010; Marioni, 2018; Bell, 2012).

Human aging is a complex process characterized by the overall decrease in physiological functions (Garagnani, 2012; Bjornsson, 2008) and can not be completely understood in terms of genetic configuration; epigenetics is derived as an alternative means to explain the alterations associated with age (Heyn, 2012; Teschendorff, 2018).

Various studies on insects, particularly honey bees, have revealed that the same genome can be programmed to produce short-lived workers or long-lived queens. Little by little, evolution has made us realize that the characteristic features of organisms are basically formed by the interaction of intrinsic and extrinsic factors (Murgatroyd, 2010, Meissner, 2010).

EPIGENETICS AND METHYLATION

Epigenetics has come to prevail as a discipline that plays an important role in predicting age in areas of forensic medical importance (Hamano, 2017). At present the external characteristics that manifest phenotypically and that are observed with the naked eye are those related to pigmentation, such as the color of eyes and hair; however, age is usually a characteristic worth mentioning since it can be related to ethnicity and predict appearance. (Sae, 2017; Steegenga, 2014).

To determine the age of a person based on Deoxyribonucleic Acid (DNA), molecular methods have been mentioned, all of them based on the analysis of telomere shortening and mitochondrial

DNA deletion; recently, research have included cells of cellular signaling of the T cell receptor (sjTRECs) and DNA methylation as biomarkers that promise a high prediction accuracy (Sae, 2017; Ward, 2018; Jung, 2017).

Globally, several studies have shown that DNA methylation decreases with aging in human tissues as a consequence of a progressive loss of DNA methyltransferase type 1a (DNMT1a). However, loci of specific genes that undergo hypermethylation during aging have also been reported. (McEwen, 2018).

On the other hand, methylation associated with age is not only based on random deterioration during dental development; researchers have been able to relate chronological age to changes in DNA methylation and some markers of methylation have been recorded that promise to be good epigenetic predictors of aging (Shao, 2014, Bocklandt, 2011). In general, it is believed that the presence of methylated DNA causes the silencing of genes that affect the chromatic structure, so that sites where methylation is observed are critical for cellular functions; on the other hand, there are also loci that are less stable or that their expression is no longer necessary; these loci are usually more prone to changes in their methylation status over time and due to environmental factors and spontaneous epigenetic modifications (Alghanim, 2017; Rakyan, 2018).

Until now, methylation is the best characterized epigenetic mechanism; methylation is carried out by binding a methyl group (-CH3) to the cytosine nucleotide of the DNA strand to 5-methylcytosine; this process usually occurs in sites rich in cytosine and guanine linked by a phosphate group (CpG); this union is established during the ontogeny of the mammal and can be replicated during cell division by maintenance of DNA methyltransferases type 1 (Bork, 2010, Hernandez, 2011).

FORENSIC IMPLICATIONS

In the forensic area, age estimation is one of the most important keys for investigators when it comes to the monitoring and resolution of a crime; for forensic medicine, the exact age estimation has a value similar to the externally visible or biogeographic characteristics (Zbiec, 2015; Vidaki, 2017).

Age changes the appearance of an individual noticeably, which influences and provides information for the recreation of sketches of unknown suspects; changes such as male pattern baldness or a slight change in hair color are often of importance in narrowing the search range in criminal cases (Zbiec, 2015; Kayser, 2015).

The estimation of human age has been the subject of research for many years and the arrival of molecular methods has achieved a great forensic impact; this is due to the fact that conventional methods for age estimation are based on anthropological techniques that analyze bone markers such as bones and teeth, with an acceptable margin of error; however, estimates are limited to the existence of the skeleton, discarding samples commonly found in crime scenes such as body fluids or hair (Zbiec, 2015; Sae, 2018; Eipel, 2016).

Most of the articles analyzed are based on the prediction of chronological age by means of fluids that are commonly

found in crime scenes (semen, blood and saliva). Sae et al (2017) conducted a predictive study based on saliva samples from 54 individuals, of which CpG markers were identified that showed a high correlation between methylation and age. Because the markers associated with the age of saliva differed from those of blood, the DNA methylation patterns of 6 CpG markers (cg00481951, cg19671120, cg14361627, cg08928145, cg12757011, and cg07547549 of the SST genes, were investigated. CNGA3, KLF14, TSSK6, Tbr1, and SLC12A5 respectively) in an independent set of saliva samples from 226 individuals aged 18 to 65 years. The saliva was collected with the Orangene self-collection kit, stored at room temperature; DNA extraction was performed using the QlAmp1 DNA Mini Kit to subsequently perform DNA methylation of the whole genome of saliva using the HumanMethylation450 BeadChip matrix (Sae, 2017).

Shao et al (2014) identified 8 fragments of genes obtained from blood samples, in which the degree of methylation was significantly related to age; samples analyzed were provided by 40 male voluntary donors and 25 voluntary female donors with an age range of between 11 and 72 years. The isolated DNA fragments were cloned into pMD 19-T Vector and subsequently quantified by bisulfite sequencing using the EZ DNA methylation kit and sequenced with a 3730 genetic analyzer. For each donor, 10 cloned segments of each fragment were isolated and sequenced; the methylated fraction of each CpG was averaged among the 10 cloned segments.

Heyn et al (2012) raised a reasonable doubt: Do individuals at the most extreme points of their lives have differences in DNA methylation? Due to this, they carried out the sequencing with bisulfite of the newborn and centenarian genomes with a CpG microarray 450000. Blood samples in newborns were obtained from the umbilical cord using DNA extracted from CD4 T cells processed through a genomic analyzer Ilumina Human -Omni5 Quad BeadChip. In this sense, Hamano et al (2017) reported an age predictive model based on methylation of cytosine at CpG sites obtained from blood samples with 71 methylation markers from Illumina Infinitum HumanMethylation450 BeadChip. Garagnani et al (2012) reported a predictive model based on Illumina Infinitum HumanMethylation450 BeadChip with a small cohort of 64 individuals of different ages.

In the literature we can find articles including several samples that mostly belong to body fluids; the samples were obtained by voluntary donors collecting oral cells using cotton swabs, finger prick blood by collecting blood cells with cotton swabs, or semen samples from male volunteers (Silva, 2016; Jenkins, 2018). In studies where blood samples were collected exclusively, as reported by Park et al (2016), samples were obtained from a cohort composed of more than 300 men and 300 women with a distribution of age groups of 10 years each group (range between 11 and 90 years). To demonstrate the versatility of molecular analyzes based on DNA methylation, articles where mesenchymal stem cells and fibroblasts were analyzed to determine changes associated with age in primary tissues including dermis, epidermis or cervical cytology (Koch, 2011).

To select a set of variables for the use of the construction of an age predictive model, data from 62 CpG sites are usually used. The regression model explains 96.9% of the total variance in 54 males with an error of 3.83 years. A further

analysis of the ptpn7 gene reported that this gene shows very low predictive value in blood, but a very high value in buccal epithelial cells (0.05 and 0.82, respectively).

In an independent set of 226 individuals using a SNaPshot multiplex methylation assay, a methylation pattern highly related to age was reported, which can explain 63.5% and 54.9% of the age variance. The most correlated CpG sites were found in the KLF14 and SLC12A5 gene (Sae, 2017). Heyn et al (2012) reported that samples from elderly individuals showed a lower correlation in terms of CpG methylation status compared to newborn samples as they were more homogeneously methylated at CpG. From the point of view of regulatory genes, one of the main characteristics of centenary samples is their low density of DNA methylation in promoters poor in CpG; The findings of Heyn et al were validated with a broader cohort of newborn and nonagenarian individuals using a CpG K microarray 450 DNA. The results were clear evidence of the significant difference between the methylation patterns of the extremes of human life.

In the studies by Hamano et al (2017) it was observed that the methylation profiles of the ELOVL2 and EDARADD genes have a high correlation with age and were used to predict age with a regression of support vectors. It is reported for the first time that the ELOVL2 gene is a predictive marker of age for saliva samples. The prediction model showed a high precision of prediction of age with an absolute mean deviation from the chronological age of 5.96 years among 197 samples. The model was validated with an additional study of 50 samples with an absolute mean deviation of 6.25 years. The age prediction model was tested again on samples of saliva obtained from cigarette butts, representing a recurring sample collection at a real crime scene with an absolute mean deviation of 7.65 years; for these samples a slightly higher result was obtained than for the saliva samples that were collected intact.

In most of the studies analyzed, a high correlation was observed between the methylation patterns and the prediction of chronological age with an absolute mean deviation not exceeding 9.0 years in the prediction models (Table 1)

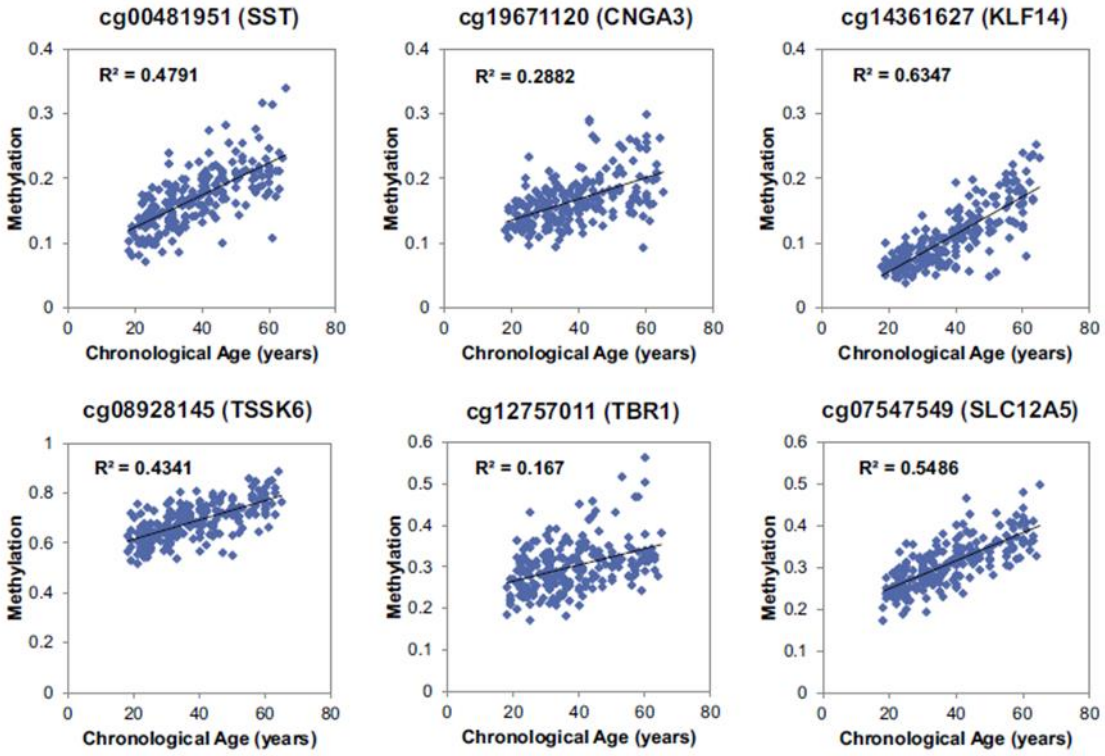

Table 1. Correlation between age and methylation in 6 CpG sites, one in each of the SST genes, CNGA3, KLF14, TSSK6, TBR1 and SLC12A5 in 226 saliva samples. The level of DNA methylation was determined by the SNaPshot reaction of multiplexed methylation (taken from Sae et al, 2017).

CONCLUSION

Based on the favorable and promising results regarding the correlation of methylation patterns and chronological age, we can conclude that advances in technology and molecular biology can become very important tools for forensic studies.

In general, forensic investigations depend on the tools that can provide information regarding the evidence found at the crime scene; in many cases where corpses are not identified by the state, it is impossible to perform common age prediction techniques.

DNA profiles are compared with suspects; however, in many countries there is no database and where they exist they are usually incomplete. That is why molecular techniques are used as epigenetic markers to provide information to help the resolution of forensic cases.

DNA methylation has proven to be a promising epigenetic marker, with high precision in DNA prediction models, which has the versatility to adapt for a wide range of recurring crime scene samples. However, it is also important to mention that the process of age prediction models by DNA methylation requires techniques and equipment with which forensic laboratories do not usually count and / or perform on a daily basis, which implies additional costs and adequate staff training.

Another important condition that must be taken into account is that since methylation is an epigenetic mechanism that regulates gene expression, age prediction analysis can not be based completely on the results obtained from previous studies, since the population groups are exposed to various environmental factors that could change gene regulation and therefore methylation patterns. Each population group must be sequenced and analyzed to standardize not only the technique, but also the reference parameters used for the results interpretation.

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