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Epigenetic genome-wide association methylation in aging and longevity

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Abstract

The aging phenotype is the result of a complex interaction between genetic, epigenetic and environmental factors. Evidence suggests that epigenetic changes (i.e., a set of reversible, heritable changes in gene function or other cell phenotype that occurs without a change in DNA sequence), may affect the aging process and may be one of the central mechanisms by which aging predisposes to many age-related diseases. The total number of altered methylation sites increases with increasing age, such that they could serve as marker for chronological age. This article systematically highlights the advances made in the field of epigenomics and their contribution to the understanding of the complex physiology of aging, lifespan and age-associated diseases.

Keywords

aging; EW AS; longevity; methylation

Aging has emerged as a major global public health issue [1]. Worldwide, the number of individuals older than 65 years of age are projected to increase from 420 million in the year 2000 to as many as 973 million by 2030 [2]. In the USA, this same age group presently consists of 36 million individuals and could increase to nearly 80 million by 2050 [3]. Aging is generally accompanied by age-associated chronic diseases, diminishing quality of life and placement of additional burden on the healthcare system. These factors necessitate identifying biological components that influence human aging and age-related diseases. Both genome-wide association studies and candidate gene approaches have provided valuable information on the role of DNA in aging and disease risk. These approaches,

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however, do not provide information on differential genetic expression due to developmental or epigenetic changes. The latter relates to a series of histone modifications and DNA methylations, which in turn affect gene expression. Examples of epigenetic regulation of genome architecture and gene expression span across the evolutionary lineage, including sirtuins [4], p66Shc [5], monozygotic twins [6], cloned animals [7] and the maternal effect of agouti mice [8]. Inheritance of epigenetic modifications has been reported in a variety of taxa, ranging from plants [9], yeast [10] and flies [11,12] as well as vertebrates such as mice [13–15] and humans [16]. In general, DNA hypermethylation leads to gene silencing, and DNA hypomethylation endorses gene activation. DNA hypermethylation causing silencing of genes involved in the cell cycle, apoptosis, detoxification and cholesterol metabolism [17] has been reported. Methylation changes may lead to alterations in gene expression and thus contribute to the phenotype of decreased incidence or delayed onset of age-related diseases. In addition, epigenetic changes in specific gene regions have been associated with cancer risk [18]. Epigenetic modification has been linked to many genetic syndromes such as Prader–Willi [19], Angelman [20], Beckwith–Wiedemann [21,22] and Rett syndromes [23,24]. The agouti mouse model provides an example of epigenetic variability and inheritance of a pleiotropic trait with pathological effects [8].

Technological advances in the field

The concept of performing an unbiased approach to reveal age-associated methylation changes and to quantify it using high-throughput machinery has only recently been explored only recently through the use of newly developed high-throughput technologies. Genome-wide DNA methylation and its various effects have been investigated using high-throughput DNA methylation-profiling techniques. In the majority of epigenomic studies, BeadArray™ (Illumina) platform (with bisulfite-treated DNA) has been utilized to analyze a moderate number of CpGs across the genome in a large number of samples [25]. Another strategy has been the use of microarrays with restriction enzyme or affinity-based enrichment methods to provide relative levels of methylation across the genome [25]. More recently, next-generation sequencing-based methods have been implemented to assess tens of millions of DNA fragments, allowing for detection of DNA methylation across the entire genome, including interspersed repeat sequences that are inaccessible using microarrays. Sequencing has several advantages over array platforms, particularly since array restricts the profiling data to specific annotations and content [25]. A variety of platforms have been developed to increase the number of CpGs for which methylation can be assessed. Table 1 reviews the most current DNA methylation platforms. There is an increasing demand for understanding of the genome-wide methylation status via high-throughput techniques for assessment of methylation profiling. This review describes the challenges of applying epigenomic unbiased screening, and reports on the most recent studies carried out in this area.

Epigenomic modification with age-empirical support

■ Humans

Evidence in literature suggests that epigenetic changes occur as a function of age in many tissues and may serve as a marker of chronological age. An insight into the epigenetic

changes that occur with aging was provided by Bocker *et al.*; the detailed methylation profile of $CD34^+$ hematopoietic progenitor cells demonstrated that epigenetic changes occur with aging and there is *de novo* methylation of Polycomb chromatin genes [26]. Using a different type of blood cell, $CD4^+$, and utilizing the Infinium HumanMethylation27 BeadChip platform, 360 CpG sites (labeled as aging-associated differentially methylated regions) that were either hypo- or hyper-methylated with age have been identified. In total, 60% of the hypermethylation sites were replicated in $CD14^+$ of independent cohorts. The aging-associated differentially methylated regions signature, especially hyper-methylation of chromatin domain promoters, has been replicated in buccal cells [27].

Epigenetic changes in several CpG loci, mostly in CpG islands, assessed by Infinium HumanMethylation27 BeadChip were associated with age in different parts of 387 humans (1–102 years old) brains. This central effect of methylation, especially in genes associated with DNA binding and transcription regulation, reemphasizes the importance of methylation in the mechanism of aging [28].

Using the powerful tool of homozygote twins, Bocklandt *et al*. expanded on an observation that was first reported by Fraga *et al* [6]. Studying the epigenetic changes with age in 21–55-year-old homozygous twins, they showed that 88 methylation sites, representing nearly 80 genes, demonstrate significant changes with age. The association of those loci with age were further replicated in independent cohort aged 18–70 years old [29] and a regression model built based on this observation could predict an individual's age with an accuracy of 5.2 years [29]. Changes in epigenomic modification such as methylation can vary substantially between tissues and through the aging process. Christensen *et al*. examined a panel of 1413 autosomal CpG loci in 217 nonpathologic human tissues from ten anatomic sites and demonstrated that age-associated epigenomic changes occur with age in multiple tissues, and that the methylation pattern with aging can predict the tissue of origin [30]. Aiming to detect an aging signature, Koch and Wagner used five different cell types from four tissues, a total of 110 samples. The commercial DNA methylation platform initially employed to screen contained 27,578 CpG sites. Five genes demonstrated methylation changes with age across the board. These genes were further validated in eight more cell types representing eight tissues, in a total of 766 samples leading authors to conclude that this assay can serve as a predictor of donor age [31]. Using the same platform, Bork *et al*. studied mesenchymal stromal cells (MSCs) in old (52–83 years of age) and young (21–50 years of age) donors [32]. Significant methylation with age has observed in homeobox genes and genes involved in cell differentiation. Further validation of the epigenome using an independent technology (pyrosequencing) resulted in the discovery that six selected CpG sites demonstrated the same trend. These results further support the role of epigenetics in regulating replicative senescence and aging [32]. In a longitudinal study performed over 8 years in 1097 subjects aged 55–92 years old, the Alu elements demonstrated decline in methylation with age, while the LINE-1 repetitive elements did not change dramatically, emphasizing the role of methylation loss with cellular senescence and aging [33].

Gentilini *et al.* screened the *HUMARA* locus for heterozygosity to test the hypothesis that this locus is relevant for lifespan [34]. Using 50 female centenarians and three groups of controls, authors screened 1085 CpG sites across the X chromosome on top of the *HUMARA*

locus for methylation changes, and found no difference between the groups. They concluded that although skewing of X-chromosome inactivation has been observed with aging, there were no associated epigenetic modifications [34].

■ Animal models

There are very few animal studies that have assessed global methylation changes with age. Genomic methylation changes were demonstrated with age using the HELP assay in liver and visceral adipose tissues from young and old rats. These methylation changes were validated with an independent technology (luminometric methylation assays) showing that these changes are tissue dependent. While the pattern of methylation and expression of some of the genes were similar in both the tissues, subsets of the genes that are associated with metabolism and metabolic regulation were differentially expressed with age [35].

miRNA & longevity

miRNAs are small ncRNAs that were initially discovered in *Caenorhabditis elegans* and since reported across the animal kingdom. In humans, thousands of miRNAs have been demonstrated in a variety of tissues with major impact on transcription and translational repression or gene silencing. The role of miRNAs in aging was demonstrated recently in *C. elegans* and in mice [36,37]. miRNAs affect gene expression during the aging process in mice and modulate senescence in human cell lines [38]. Studies in *C. elegans* and mice have resulted within some important observations, such as: miRNAs work in groups (packs) by coordinating and regulating gene expression/silencing resulting in age-dependent disease states or alternatively with longevity [39]; inherited epigenetic effects in miRNA loci lead to changes in gene expression that modulate longevity [40]; and miRNAs that target members of the insulin/IGF-1 pathway (a known target for genetic disruption that leads to life extension) can predict up to 47% of lifespan differences [36]. This observation on the role of *IGF-1* was further supported by Liang *et al*. in studies in long-lived mutant mice, higher expression of three miRNAs altered *IGF-1* signaling that in turn promotes long-lived phenomenon [41]; and de Lencastre *et al*. demonstrated that miRNAs could affect lifespan through disruption of multiple loci that are not necessarily associated with the insulin/IGF-1 pathway. Some loci illustrate positive effects on lifespan, promoting longevity, and some however demonstrate the opposite effect leading to a shorter lifespan [42]. Such observations are also reported by Ugalde *et al*.; altered expression of two miRNAs promoted progeroid phenotype in a mouse model for a progeria syndrome through the effect on key components of the DNA-damage response pathways [43]. Human studies are limited; however, a genome-wide miRNA screen for differential expression between long-lived individuals and controls revealed that 10% of the miRNA microarray (863 miRNAs) demonstrated significant alterations in expression, of which only 16 were upregulated in the exceptional long-lived individuals. Most of these differentially expressed miRNAs have been associated with genes linked to major age-associated diseases, suggesting under-regulation of key genes by miRNAs could promote longevity in humans [44].

The role of the epigenomic modification in aging & age-related diseases

Aging is a complex physiological process that results in compromise of biological functions, increased susceptibility to age-related diseases and eventually death [45]. It is well recognized that human aging and longevity are influenced by both genetic and environmental factors. Inherited genetic mutations and polymorphisms resulting in alterations in gene function can explain some features of aging and age-related diseases [46]. However, in addition to inherited genetic factors, aging is influenced by the gradual accumulation of molecular alterations after birth. Environmentally induced perturbations in the epigenetic processes that involve alterations of gene expression without a change in DNA sequence can determine different aspects of aging, as well as etiology and pathogenesis of age-related diseases. Epigenetic changes can specifically play a role in the modulation of aging processes and healthy life extension [47]. In particular, promoter DNA methylation changes and associated gene silencing are the epigenetic changes seen in age-related diseases [46].

Epigenetic mechanisms have been established to play a major role in aging at both cellular and organism level [48,49]. In addition to DNA methylation, one of the well-characterized epigenetic processes, several types of histone modifications have been demonstrated to occur both globally and at gene-specific loci during aging [49]. Other epigenetic processes include histone modifications of Polycomb group proteins, chromosomal position effects and methylation of ncRNAs [49–52].

Common human age-related diseases are accompanied by a loss of genomic DNA methylation. Progressive loss of genomic DNA methylation has been demonstrated throughout the human genome [33], in mice and in cell lines [53–55], although this decrease may be tissue and/or gene specific [56–58]. Age-related epigenetic changes have also been demonstrated in sperm cells; however, the direction of change over time appears to be gene specific [59]. Within pairs, differences in DNA methylation are greater in older than in younger monozygotic twins [6].

DNA methylation dynamics can inf luence brain function. 5-hydroxymethylcytosine (5-hmC) is a newly described epigenetic modification generated by the oxidation of 5-methylcytosine by the ten–eleven translocation family of enzymes [60–62]. An increase of 5-hmC with age in the mouse brain as well as an age- and gene-expression level related enrichment of 5-hmC in genes implicated in neurodegeneration have been demonstrated. Many 5-hmC-regulated regions are dynamically modified during neurodevelopment and aging [61], suggesting that 5-hmC may play an important role in the etiology and course of age-related neurodegenerative disorders [60].

With aging, there is a decrease in long-term synaptic plasticity, especially long-term potentiation (LTP), manifesting as cognitive decline. This decrease in LTP is linked to histone acetylation, and BDNF/trkB signaling. Indeed, treatment with histone deacetylase inhibitors or a neurotrophin receptor B agonist restores LTP in the hippocampus of old animals. These studies suggest that epigenetic changes may play a significant role in age-related diseases [63].

Conclusion & future perspective: exciting directions in epigenomic research in aging

Nutritional epigenetics has emerged as a novel mechanism underlying gene–diet interactions, elucidating the modulatory role of nutrition in aging and age-related disease development. Nutrients can regulate the placement of these epigenetic modifications [64–67]. Nutrients involved in one-carbon metabolism, namely folate, vitamin B_{12}, vitamin B_6, riboflavin, methionine, choline and betaine, are involved in DNA methylation by regulating levels of the universal methyl donor *S*-adenosylmethionine and methyltransferase inhibitor *S*-adenosylhomocysteine. The effects of folate on DNA methylation patterns have recently been investigated in prenatal and early-postnatal life and aging. Folate exposure in the intrauterine environment and during the aging process may have profound effects on DNA methylation with significant functional ramifications [68]. In addition to folate and vitamin B_{12}, other nutrients and related compounds such as retinoic acid, resveratrol, curcumin, sulforaphane and tea polyphenols can modulate epigenetic patterns by altering the levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine or directing the enzymes that catalyse DNA methylation and histone modifications [64]. Indeed, sirtuin 1 may mediate some of the effects of dietary restriction through effects on DNA methylation [66].

The field of epigenomics is exciting, new and is rapidly evolving. Although a nascent field, significant progress and advancement of technology offer the promise of better understanding of the role of epigenetics in the complex process of aging, age-related diseases and, therefore, lifespan.

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References

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. World Health Statistics Annual 1996. Geneva, Switzerland: WHO; 1998.
2. From the Centers for Disease Control and Prevention. Public health and aging: trends in aging – United States and worldwide. JAMA. 2003; 289(11):1371–1373. [PubMed: 12636453]
3. Locher JL, Kilgore ML, Morrisey MA, Ritchie CS. Patterns and predictors of home health and hospice use by older adults with cancer. J. Am. Geriatr. Soc. 2006; 54(8):1206–1211. [PubMed: 16913986]
4. Porcu M, Chiarugi A. The emerging therapeutic potential of sirtuin-interacting drugs: from cell death to lifespan extension. Trends Pharmacol. Sci. 2005; 26(2):94–103. [PubMed: 15681027]
5. Ventura A, Luzi L, Pacini S, Baldari CT, Pelicci PG. The *p66Shc* longevity gene is silenced through epigenetic modifications of an alternative promoter. J. Biol. Chem. 2002; 277(25):22370–22376. [PubMed: 11948181]
6. Fraga MF, Ballestar E, Paz MF, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc. Natl Acad. Sci. USA. 2005; 102(30):10604–10609. [PubMed: 16009939]

7. Rideout WM 3rd, Eggan K, Jaenisch R. Nuclear cloning and epigenetic reprogramming of the genome. Science. 2001; 293(5532):1093–1098. [PubMed: 11498580]
8. Morgan HD, Sutherland HG, Martin DI, Whitelaw E. Epigenetic inheritance at the agouti locus in the mouse. Nat. Genet. 1999; 23(3):314–318. [PubMed: 10545949]
9. Hollick JB, Patterson GI, Coe EH Jr, Cone KC, Chandler VL. Allelic interactions heritably alter the activity of a metastable maize pl allele. Genetics. 1995; 141(2):709–719. [PubMed: 8647404]
10. Grewal SI, Klar AJ. Chromosomal inheritance of epigenetic states in fission yeast during mitosis and meiosis. Cell. 1996; 86(1):95–101. [PubMed: 8689692]
11. Cavalli G, Paro R. The *Drosophila Fab-7* chromosomal element conveys epigenetic inheritance during mitosis and meiosis. Cell. 1998; 93(4):505–518. [PubMed: 9604927]
12. Jensen S, Gassama MP, Heidmann T. Taming of transposable elements by homology-dependent gene silencing. Nat. Genet. 1999; 21(2):209–212. [PubMed: 9988275]
13. Allen ND, Norris ML, Surani MA. Epigenetic control of transgene expression and imprinting by genotype-specific modifiers. Cell. 1990; 61(5):853–861. [PubMed: 2111735]
14. Hadchouel M, Farza H, Simon D, Tiollais P, Pourcel C. Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with *de novo* methylation. Nature. 1987; 329(6138):454–456. [PubMed: 2443854]
15. Roemer I, Reik W, Dean W, Klose J. Epigenetic inheritance in the mouse. Curr. Biol. 1997; 7(4): 277–280. [PubMed: 9094308]
16. Franklin TB, Mansuy IM. Epigenetic inheritance in mammals: evidence for the impact of adverse environmental effects. Neurobiol. Dis. 2010; 39(1):61–65. [PubMed: 19931614]
17. Burzynski SR. Aging: gene silencing or gene activation? Medical Hypotheses. 2005; 64(1):201–208. [PubMed: 15533642]
18. Lu Q, Qiu X, Hu N, Wen H, Su Y, Richardson BC. Epigenetics, disease, and therapeutic interventions. Ageing Res. Rev. 2006; 5(4):449–467. [PubMed: 16965942] ▪Excellent review summarizing the role of epigenetic changes in aging.
19. Smith EY, Futtner CR, Chamberlain SJ, Johnstone KA, Resnick JL. Transcription is required to establish maternal imprinting at the Prader-Willi syndrome and Angelman syndrome locus. PLoS Genet. 2011; 7(12):e1002422. [PubMed: 22242001]
20. Mabb AM, Judson MC, Zylka MJ, Philpot BD. Angelman syndrome: insights into genomic imprinting and neurodevelopmental phenotypes. Trends Neurosci. 2011; 34(6):293–303. [PubMed: 21592595]
21. Niederhoffer KY, Penaherrera M, Pugash D, et al. Beckwith-Wiedemann syndrome in sibs discordant for IC2 methylation. Am. J. Med. Genet. A. 2012; 158A(7):1662–1669. [PubMed: 22615066]
22. Hirasawa R, Feil R. Genomic imprinting and human disease. Essays Biochem. 2010; 48(1):187–200. [PubMed: 20822494]
23. Yasui DH, Scoles HA, Horike S, et al. 15q11.2–13.3 chromatin analysis reveals epigenetic regulation of *CHRNA7* with deficiencies in Rett and autism brain. Hum. Mol. Genet. 2011; 20(22): 4311–4323. [PubMed: 21840925]
24. Sanchez-Mut JV, Huertas D, Esteller M. Aberrant epigenetic landscape in intellectual disability. Prog. Brain Res. 2012; 197:53–71. [PubMed: 22541288]
25. Fouse SD, Nagarajan RO, Costello JF. Genome-scale DNA methylation analysis. Epigenomics. 2010; 2(1):105–117. [PubMed: 20657796] ▪Well written and comprehensive review of currently used methylation detection reagents and their application to microarray and sequencing platforms.
26. Bocker MT, Hellwig I, Breiling A, Eckstein V, Ho AD, Lyko F. Genome-wide promoter DNA methylation dynamics of human hematopoietic progenitor cells during differentiation and aging. Blood. 2011; 117(19):e182–e189. [PubMed: 21427290]
27. Rakyan VK, Down TA, Maslau S, et al. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res. 2010; 20(4):434–439. [PubMed: 20219945]
28. Hernandez DG, Nalls MA, Gibbs JR, et al. Distinct DNA methylation changes highly correlated with chronological age in the human brain. Hum. Mol. Genet. 2011; 20(6):1164–1172. [PubMed: 21216877]

29. Bocklandt S, Lin W, Sehl ME, et al. Epigenetic predictor of age. PLoS ONE. 2011; 6(6):e14821. [PubMed: 21731603]
30. Christensen BC, Houseman EA, Marsit CJ, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet. 2009; 5(8):e1000602. [PubMed: 19680444]
31. Koch CM, Wagner W. Epigenetic-aging-signature to determine age in different tissues. Aging. 2011; 3(10):1018–1027. [PubMed: 22067257]
32. Bork S, Pfister S, Witt H, et al. DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. Aging Cell. 2010; 9(1):54–63. [PubMed: 19895632]
33. Bollati V, Schwartz J, Wright R, et al. Decline in genomic DNA methylation through aging in a cohort of elderly subjects. Mech. Ageing Dev. 2009; 130(4):234–239. [PubMed: 19150625]
34. Gentilini D, Castaldi D, Mari D, et al. Age-dependent skewing of X chromosome inactivation appears delayed in centenarians' offspring. Is there a role for allelic imbalance in healthy aging and longevity? Aging Cell. 2012; 11(2):277–283. [PubMed: 22292741]
35. Thompson RF, Atzmon G, Gheorghe C, et al. Tissue-specific dysregulation of DNA methylation in aging. Aging Cell. 2010; 9(4):506–518. [PubMed: 20497131]
36. Pincus Z, Smith-Vikos T, Slack FJ. MicroRNA predictors of longevity in Caenorhabditis elegans. PLoS Genet. 2011; 7(9):e1002306. [PubMed: 21980307]
37. Inukai S, de Lencastre A, Turner M, Slack F. Novel microRNAs differentially expressed during aging in the mouse brain. PLoS ONE. 2012; 7(7):e40028. [PubMed: 22844398]
38. Smith-Vikos T, Slack FJ. MicroRNAs and their roles in aging. J. Cell Sci. 2012; 125(Pt 1):7–17. [PubMed: 22294612]
39. Lanceta J, Prough RA, Liang R, Wang E. MicroRNA group disorganization in aging. Exp. Gerontol. 2010; 45(4):269–278. [PubMed: 20034554]
40. Pang S, Curran SP. Longevity and the long arm of epigenetics: acquired parental marks influence lifespan across several generations. Bioessays. 2012; 34(8):652–654. [PubMed: 22674543]
41. Liang R, Khanna A, Muthusamy S, et al. Post-transcriptional regulation of IGF1R by key microRNAs in long-lived mutant mice. Aging Cell. 2011; 10(6):1080–1088. [PubMed: 21967153]
42. de Lencastre A, Pincus Z, Zhou K, Kato M, Lee SS, Slack FJ. MicroRNAs both promote and antagonize longevity in C. elegans. Curr. Biol. 2010; 20(24):2159–2168. [PubMed: 21129974]
43. Ugalde AP, Espanol Y, Lopez-Otin C. Micromanaging aging with miRNAs: new messages from the nuclear envelope. Nucleus. 2011; 2(6):549–555. [PubMed: 22064465]
44. Elsharawy A, Keller A, Flachsbart F, et al. Genome-wide miRNA signatures of human longevity. Aging Cell. 2012; 11(4):607–616. [PubMed: 22533606]
45. Berdasco M, Esteller M. Hot topics in epigenetic mechanisms of aging: 2011. Aging Cell. 2012; 11(2):181–186. [PubMed: 22321768]
46. Kim J, Kim J, Issa JJ. Aging and DNA methylation. Curr. Chem. Biol. 2009; 3:321–329.
47. Vaiserman AM. Epigenetic engineering and its possible role in anti-aging intervention. Rejuvenat. Res. 2008; 11(1):39–42.
48. Gonzalo S. Epigenetic alterations in aging. J. Appl. Physiol. 2010; 109(2):586–597. [PubMed: 20448029] ▪Thorough and detailed review on the three major epigenetic mechanisms, DNA methylation, histone modifications and ncRNAs, and their involvment in aging.
49. Trygve, O. Epigenetics of Aging. Tollefsbol, TO., editor. NY, USA: Springer; 2010.
50. Klauke K, De Haan G. Polycomb group proteins in hematopoietic stem cell aging and malignancies. Int. J. Hematol. 2011; 94(1):11–23. [PubMed: 21523335]
51. Krishnan V, Chow MZ, Wang Z, et al. Histone H4 lysine 16 hypoacetylation is associated with defective DNA repair and premature senescence in Zmpste24-deficient mice. Proc. Natl Acad. Sci. USA. 2011; 108(30):12325–12330. [PubMed: 21746928]
52. So AY, Jung JW, Lee S, Kim HS, Kang KS. DNA methyltransferase controls stem cell aging by regulating BMI1 and EZH2 through microRNAs. PLoS ONE. 2011; 6(5):e19503. [PubMed: 21572997]
53. Singhal RP, Mays-Hoopes LL, Eichhorn GL. DNA methylation in aging of mice. Mech. Ageing Dev. 1987; 41(3):199–210. [PubMed: 3431172]

54. Wilson VL, Smith RA, Ma S, Cutler RG. Genomic 5-methyldeoxycytidine decreases with age. J. Biol. Chem. 1987; 262(21):9948–9951. [PubMed: 3611071]
55. Wilson VL, Jones PA. DNA methylation decreases in aging but not in immortal cells. Science. 1983; 220(4601):1055–1057. [PubMed: 6844925]
56. Richardson BC. Role of DNA methylation in the regulation of cell function: autoimmunity, aging and cancer. J. Nutr. 2002; 132(Suppl. 8):S2401–S2405.
57. Richardson B. Impact of aging on DNA methylation. Ageing Res. Rev. 2003; 2(3):245–261. [PubMed: 12726774]
58. Zhang Z, Deng C, Lu Q, Richardson B. Age-dependent DNA methylation changes in the *ITGAL* (*CD11a*) promoter. Mech. Ageing Dev. 2002; 123(9):1257–1268. [PubMed: 12020947]
59. Flanagan JM, Popendikyte V, Pozdniakovaite N, et al. Intra- and interindividual epigenetic variation in human germ cells. Am. J. Hum. Genet. 2006; 79(1):67–84. [PubMed: 16773567]
60. Van Den Hove DL, Chouliaras L, Rutten BP. The role of 5-hydroxymethylcytosine in aging and Alzheimer's disease: current status and prospects for future studies. Curr. Alzheimer Res. 2012; 9(5):545–549. [PubMed: 22272626]
61. Szulwach KE, Li X, Li Y, et al. 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nat. Neurosci. 2011; 14(12):1607–1616. [PubMed: 22037496]
62. Van Den Hove DL, Chouliaras L, Rutten BP. The role of 5-hydroxymethylcytosine in aging and Alzheimer's disease: current status and prospects for future studies. Curr. Alzheimer Res. 2012; 9(5):545–549. [PubMed: 22272626]
63. Mendelsohn AR, Larrick JW. Epigenetic-mediated decline in synaptic plasticity during aging. Rejuvenat. Res. 2012; 15(1):98–101.
64. Park LK, Friso S, Choi SW. Nutritional influences on epigenetics and age-related disease. Proc. Nutr. Soc. 2012; 71(1):75–83. [PubMed: 22051144]
65. Li Y, Daniel M, Tollefsbol TO. Epigenetic regulation of caloric restriction in aging. BMC Med. 2011; 9:98. [PubMed: 21867551]
66. Ford D, Ions LJ, Alatawi F, Wakeling LA. The potential role of epigenetic responses to diet in ageing. Proc. Nutr. Soc. 2011; 70(3):374–384. [PubMed: 21781363]
67. Mckay JA, Mathers JC. Diet induced epigenetic changes and their implications for health. Acta Physiol. 2011; 202(2):103–118.
68. Ly A, Hoyt L, Crowell J, Kim YI. Folate and DNA methylation. Antioxidants Redox Signal. 2012; 17(2):302–326.
69. Eckhardt F, Lewin J, Cortese R, et al. DNA methylation profiling of human chromosomes 6, 20 and 22. Nat. Genet. 2006; 38(12):1378–1385. [PubMed: 17072317]
70. Meissner A, Mikkelsen TS, Gu H, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature. 2008; 454(7205):766–770. [PubMed: 18600261] ▪▪Reduced representation bisulfite sequencing maps of 12 different mouse cell types.
71. Hodges E, Smith AD, Kendall J, et al. High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. Genome Res. 2009; 19(9):1593–1605. [PubMed: 19581485]
72. Ball MP, Li JB, Gao Y, et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. Nat. Biotechnol. 2009; 27(4):361–368. [PubMed: 19329998]
73. Deng J, Shoemaker R, Xie B, et al. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. Nat. Biotechnol. 2009; 27(4):353–360. [PubMed: 19330000]
74. Bibikova M, Fan JB. Golden Gate assay for DNA methylation profiling. Methods Mol. Biol. 2009; 507:149–163. [PubMed: 18987813]
75. Weber M, Davies JJ, Wittig D, et al. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat. Genet. 2005; 37(8):853–862. [PubMed: 16007088] ▪▪First paper to describe the use of the methylcytosine antibody to immunoprecipitate DNA.

76. Weber M, Hellmann I, Stadler MB, et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat. Genet. 2007; 39(4):457–466. [PubMed: 17334365]
77. Zhang X, Yazaki J, Sundaresan A, et al. Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis. Cell. 2006; 126(6):1189–1201. [PubMed: 16949657] ▪▪First high-resolution mapping of a eukaryotic methylome using methylated DNA immunoprecipitation coupled with tiling microarray analysis.
78. Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S. Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. Nat. Genet. 2007; 39(1):61–69. [PubMed: 17128275]
79. Yuan E, Haghighi F, White S, et al. A single nucleotide polymorphism chip-based method for combined genetic and epigenetic profiling: validation in decitabine therapy and tumor/normal comparisons. Cancer Res. 2006; 66(7):3443–3451. [PubMed: 16585166]
80. Bird AP, Taggart MH, Smith BA. Methylated and unmethylated DNA compartments in the sea urchin genome. Cell. 1979; 17(4):889–901. [PubMed: 487434]
81. Lippman Z, Gendrel AV, Black M, et al. Role of transposable elements in heterochromatin and epigenetic control. Nature. 2004; 430(6998):471–476. [PubMed: 15269773]
82. Oda M, Greally JM. The HELP assay. Methods Mol. Biol. 2009; 507:77–87. [PubMed: 18987808]
83. Rauch T, Li H, Wu X, Pfeifer GP. MIRA-assisted microarray analysis, a new technology for the determination of DNA methylation patterns, identifies frequent methylation of homeodomain-containing genes in lung cancer cells. Cancer Res. 2006; 66(16):7939–7947. [PubMed: 16912168] ▪Describes the use of high affinity complex to methylated DNA that increases DNA microarrays hybridization efficiency.
84. Leamon JH, Lee WL, Tartaro KR, et al. A massively parallel PicoTiterPlate based platform for discrete picoliter-scale polymerase chain reactions. Electrophoresis. 2003; 24(21):3769–3777. [PubMed: 14613204]
85. Down TA, Rakyan VK, Turner DJ, et al. A Bayesian deconvolution strategy for immunoprecipitation-based DNA methylome analysis. Nat. Biotechnol. 2008; 26(7):779–785. [PubMed: 18612301]
86. Mckernan KJ, Peckham HE, Costa GL, et al. Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding. Genome Res. 2009; 19(9):1527–1541. [PubMed: 19546169]
87. Cokus SJ, Feng S, Zhang X, et al. Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. Nature. 2008; 452(7184):215–219. [PubMed: 18278030] ▪▪First description of whole-genome bisulfite sequencing.
88. Lister R, O'Malley RC, Tonti-Filippini J, et al. Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. Cell. 2008; 133(3):523–536. [PubMed: 18423832]
89. Li Y, Zhu J, Tian G, et al. The DNA methylome of human peripheral blood mononuclear cells. PLoS Biol. 2010; 8(11):e1000533. [PubMed: 21085693]
90. Clarke J, Wu HC, Jayasinghe L, Patel A, Reid S, Bayley H. Continuous base identification for single-molecule nanopore DNA sequencing. Nat. Nanotechnol. 2009; 4(4):265–270. [PubMed: 19350039]
91. Suzuki M, Jing Q, Lia D, Pascual M, Mclellan A, Greally JM. Optimized design and data analysis of tag-based cytosine methylation assays. Genome Biol. 2010; 11(4):R36. [PubMed: 20359321] ▪▪Describes the use of MPS-based assays such as HELP-tagging for epigenome-wide association studies in human disease.

Executive summary

- Epigenetic changes occur with age.
- Advances in the field of epigenetics have increased our understanding of the association between disease states and methylation patterns.
- Technology to detect global epigenetic changes has made tremendous strides.
- Unique ‘epigenetic signatures’ may be detected in different tissues.
- The environment can contribute to the diversity in individual epigenetic changes.
- New epigenetic mechanisms such as miRNAs play a role in gene regulation.
- Nutritional epigenomics is an exciting and emerging field with translational potential.
- Evidence is accumulating that the presumed ‘junk DNA’ is indeed functional with a role in epigenetics.
- Finally, altered epigenetic patterns could play a role in cell maintenance, delay age-associated diseases and improve life expectancy.

Table 1

Epigenomic platforms.

Approach	Coverage of CpGs (%)	Resolution	Platform	Tools	Method
Candidate	0.1–1	Single db	Sequencing	Bs-seq [69] RRBS [70] Array capture [71] Padlock probes [72,73]	
		Single db	Bead arrays	Golden Gate [74]	
Genome wide	1–10	~50 bp	Targeted arrays	CpG island microarrays [75] Promoter microarrays [76] Tiling microarrays [77,78] SNP arrays [79] Restriction enzymes [80] Microarrays and McrBC [81] Miseq	HELP assay [82] Microarrays and methyl sensitive MeDIP [75] MIRA [83]
		Single db	Bead arrays	Infinium [74]	
Whole genome	10–100	1–100 bp (depends on the tool)	Sequencing	Roche – 454 [84] Illumina – Solexa (MeDIP-seq) [75,85] Applied Biosystems – SOLiD [86] Bs-Seq [87–89] Nanopore-seq [90] HiSeq2000,1000	HELPtag assay [91]

Bs-seq: Bisulfite DNA sequencing; HELP: *Hpa*II tiny fragment enrichment by ligation-mediated PCR; MeDIP-seq: Methylated DNA immunoprecipitation sequencing; MIRA: Methylated CpG island recovery assay; RRBS: Reduced representation bisulfite sequencing; SOLiD: Sequencing by oligo ligation detection.