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Review article

A review of epigenetics and its association with ageing of muscle and bone

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ABSTRACT

Ageing is defined as the 'increasing frailty of an organism with time that reduces the ability of that organism to deal with stress'. It has been suggested that epigenetics may underlie the observation that some individuals appear to age faster than others. Epigenetics is the study of changes which occur in an organism due to changes in expression of the genetic code rather than changes to the genetic code itself; that is, epigenetic mechanisms impact upon the function of DNA without changing the DNA sequence. It is important to recognise that epigenetic changes, in contrast to genetic changes, can vary according to different cell types and therefore can demonstrate significant tissue-specificity. There are different types of epigenetic mechanisms: histone modification, non-coding RNAs and DNA methylation. Epigenetic clocks have been developed using statistical techniques to identify the optimal combination of CpG sites (from methylation arrays) to correlate with chronological age. This review considers how epigenetic factors may affect rates of ageing of muscle and bone and provides an overview of current understanding in this area. We discuss studies using first-generation epigenetic clocks, as well as the second-generation iterations, which appear to show stronger associations with the ageing muscle phenotype. We also review epigenome-wide association studies that have been performed in various tissues examining relationships with osteoporosis and fracture. It is hoped that an understanding of this area will lead to interventions that might prevent or reduce rates of musculoskeletal ageing in later life.

1. Introduction

The aim of older adults is often to maintain their autonomy. Several studies have reported that most adults in later life prefer to live in a familiar environment (typically their family home) rather than in residential care homes [1]. However, an increase in life expectancy and a subsequent ageing population has led to a growth in syndromes such as musculoskeletal diseases, frailty, and falls [2], which might hamper older adults' ability to self-care and maintain coveted independence. It is imperative that we understand mechanisms behind musculoskeletal ageing, to identify individuals at highest risk of adverse clinical events and to initiative preventative and therapeutic strategies to prevent musculoskeletal ageing. Given the potential importance of this topic area for medicine, this review considers how epigenetic factors may affect rates of musculoskeletal ageing and provides an overview of current understanding in this area.

2. Methods

Literature searches were performed on PubMed. Although a formal systematic review was not performed, the structure of the literature searches for each of the aims is described below. The following terms were employed via PubMed: ("epigenetic age acceleration" OR "epigenetic age" OR "methylation age acceleration" OR "methylation age" OR "clock" OR "epigenetic clock" OR "methylation clock" OR "DNAm clock" OR "biological clock") AND/OR ("musculoskeletal" OR "muscle" OR "bone" OR "sarcopenia" OR "osteoporosis" OR "grip" OR "grip strength" OR "bone mineral density" OR "BMD" OR "DXA" "bone microarchitecture" OR "HR-pQCT" OR "gait" OR "body composition" OR "ageing" OR "ageing"). For the examination of the literature for epigenome-wide association studies of grip strength and bone mineral density, searches were performed on PubMed for ("EWAS" OR "epigenome-wide" OR "epigenome-wide association study" OR "methylation" OR "GWAS" OR "genome-wide association study") AND ("grip strength" OR "sarcopenia" OR "strength" OR "BMD" or "bone mineral density" OR "osteoporosis"). Additional searches for relevant outcomes including "grip strength" and " bone mineral density" were performed using the National Human Genome Research Institute and European Bioinformatics Institute Genome wide association study (GWAS) catalogue and the MRC Integrative Epidemiology Unit Epigenome wide

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association study (EWAS) catalogue. After reviewing titles and abstracts full manuscript reviews were performed and relevant information and data collected. References were examined for further relevant titles and literature which were then reviewed.

2.1. Ageing – an introduction

Ageing is defined as the 'increasing frailty of an organism with time that reduces the ability of that organism to deal with stress' [3]. As suggested by the increased susceptibility to many diseases with increasing age, ageing affects all tissues. It is characterized by cellular features including altered telomere length, deterioration in the function of antioxidants, reduced somatic repair, alterations in stress response and chronic inflammation. Some individuals age at a faster rate, with the associated impact on physical performance and ability to live independently [4]. This review considers ageing as applied to bone and muscle health, and associated frailty. We include frailty as it is so closely related to sarcopenia, often considered the end phenotype [5].

3. Epigenetics

It has been suggested that epigenetics may underlie the observation that some individuals appear to age faster than others. Epigenetics is the study of changes which occur in an organism due to changes in expression of the genetic code rather than changes to the genetic code itself i.e. epigenetic mechanisms impact upon the function of DNA without changing the DNA sequence [6]. It is important to recognise that epigenetic changes, in contrast to genetic changes, can vary according to different cell types and therefore can demonstrate significant tissue-specificity [7]. There are different types of epigenetic mechanisms (Fig. 1): histone modification, non-coding RNAs and DNA methylation, which will be described below.

Histones are basic proteins which act as spools, around which DNA is wrapped and, as such, fulfil the function of both efficiently packing, and providing protection to DNA strands [8]. A nucleosome is a unit formed of DNA wound around a histone (octamer) core and is the subunit of chromatin. Histones possess N-terminal tails of amino acids which are usually positively charged but can undergo neutralisation via post-translational modifications leading to reduced binding to (negatively charged) DNA [8,9]. This results in unwinding of DNA and provides a platform for transcription factors to bind and express genes.

Gene expression can also be negatively regulated by non-coding ribonucleic acids (RNAs). These silencing RNAs include microRNAs and short-interfering (si)RNAs which are usually 20–50 nucleotides in length and act in the cytoplasm to block the transcription of messenger (m)RNA [10]. There are also long non-coding RNAs which are larger at over 200 nucleotides in length and modulate the structure and function of chromatin in order to control the transcription of genes and influence RNA translation [11].

3.1. Methylation

Methylation of the fifth carbon of the aromatic ring of cytosine bases (forming 5-methylcytosine) can theoretically affect any cytosine in the genome [12], but frequently occurs where a cytosine abuts a guanine base (named a CpG site with 'C' standing for cytosine, 'G' standing for guanine and 'p' referring to the phosphate backbone of DNA which joins the two bases).

The purposes of DNA methylation are the subject of ongoing research, but findings so far suggest that the process plays a role in genomic imprinting (where DNA methylation is differentially present in the paternal and maternal germlines leading to differential gene expression in those genes inherited from each parent), X chromosome inactivation [13] and, potentially, in the regulation of gene expression.

CpG sites are under-represented in the mammalian genome with 5-fold fewer than would be expected by chance alone [6]. This under-representation may be due to the fact that a methylated cytosine can undergo deamination to become thymine and thereby predispose to mutation [14]. CpG sites cluster in regions known as CpG islands [15]. These islands are bordered by CpG shores (which extend for <2 kilobases), which in turn are bordered by CpG shelves (which extend for <2 kilobases). Over 50 % of genes in vertebrate genomes have CpG islands [16]; most CpG islands remain unmethylated in somatic cells [13], and over 90 % in normal cells and tissues [15]. On the contrary, the majority (80 %) of CpG sites (as a whole) are methylated in human embryonic stem cells [17].

This emphasises that the process of DNA methylation is dynamic. Cytosine methylation is performed by DNA methyltransferases (DNMT), a family of enzymes with various functions including the maintenance of DNA methylation through mitotic division [18] and de novo methylation at particular sites [19]. Demethylation is performed by demethylases [20,21]. The dynamic process of cytosine methylation and demethylation is important when we consider the potential impact on gene expression.

CpG islands are found in transcription start sites and, less commonly, in the body of genes [16]. Methylation of cytosines has been shown to be

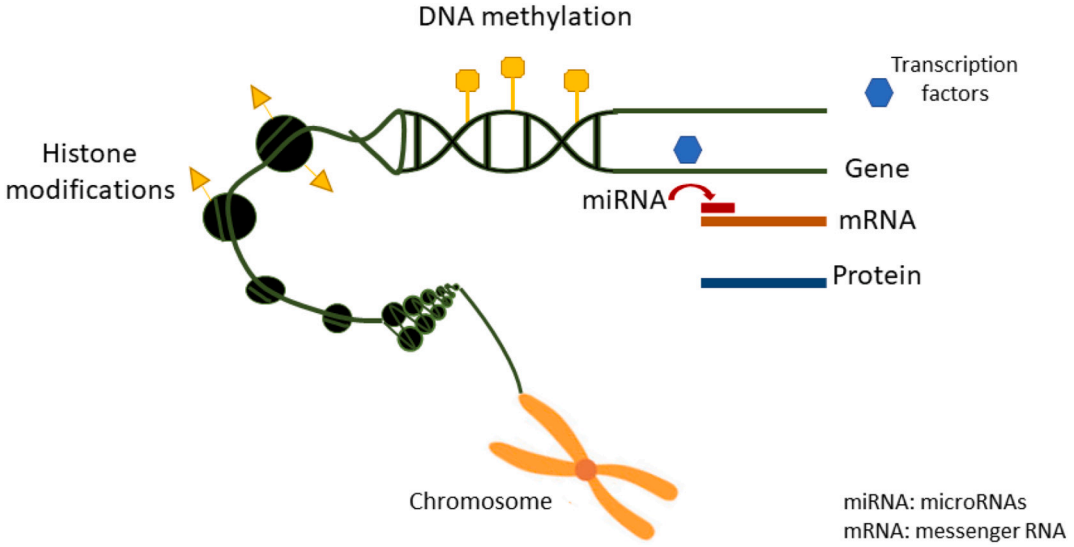

Fig. 1. Different types of epigenetic mechanisms: histone modification, non-coding RNAs and DNA methylation.

more prevalent in the promoter regions of silenced genes compared to those genes which are actively transcribed [22] and methylated cytosines have been demonstrated to block the binding of transcription factors (required for gene expression) [23,24]. These associated discoveries suggest that the presence of methylation at transcription start site is associated with silenced genes. However, it is not established whether the methylation of CpG islands initiates gene silencing or is a method of 'locking in' the repression of the gene [6,16,25].

CpG sites also occur within gene bodies, although most gene bodies are 'CpG-poor' and those that do occur are often methylated [16]. CpG islands also occur within gene bodies [26]; however, methylation at these locations within the body does not appear to repress gene transcription [27] and, in fact, methylation appears to be positively correlated with gene expression [26]. The differential relationship between methylation and gene expression at these two genomic locations (promoter regions and gene bodies) is important to consider when contextualising the findings from epigenome-wide association studies.

Global alterations in methylation levels can occur across the genome in response to particular environmental exposures and nutritional stimuli [16]. Hypermethylation can occur with age and loss of methylation has the potential to lead to genomic instability, unregulated gene expression and cancer [28].

With technological advancement including methylation microarrays, methylation at particular loci could be investigated [29]. There are data to suggest that methylation can accumulate across the lifecourse in response to environmental triggers [30]. Indeed, there is evidence from twin studies to support the hypothesis that increased variation in methylation with ageing is due to environmental stimuli [31]. In this study of 230 monozygotic twin pairs, older twins had greater between-twin variation in methylation than younger twins. The increased variability of methylation associated with ageing has led to interest in the ability of 'the methylome' to correlate with chronological age in so-called 'Epigenetic Clocks'.

3.2. Epigenetic clocks

These tools have been developed using statistical techniques to identify the optimal combination of CpG sites (from methylation arrays) to correlate with chronological age. There are 3 main permutations which are summarised in Table 1 below.

The HorvathAge clock [27] was designed as a multi-tissue clock, being trained on 51 different cell and tissue-types. In human biology the term age acceleration refers to the residual of the regression of chronological age and a calculated biological age. Age acceleration is positive if the predicted biological age is greater than the chronological age, and negative (or age decelerated) if the predicted biological age is less than the chronological age.

The profile of the clocks through the lifecourse has been investigated with longitudinal studies (using the Horvath clock) and suggest that methylation age is stable throughout adulthood [28] with increases in 'ticking rate' during periods of rapid growth, for example in childhood [29]. There are significant differences in epigenetic age acceleration between the sexes, with men consistently being 'more accelerated' than women (consistent with epidemiological findings regarding life expectancy) [30,31]. Long-living individuals (e.g., centenarians) are age decelerated compared to controls [32] and, even the offspring of semi-supercentenarians have lower epigenetic age than their age-matched controls [32].

Since Horvath's initial DNA methylation 'HorvathAge' clock, further methylation clocks have been devised second-generation methylation clocks (including GrimAge and PhenoAge) which aim to utilise CpG sites which could be more causative in the ageing process.

The GrimAge [33] instrument aimed to improve the prediction of lifespan; it uses methylation from 1030 CpG sites to predict time to death (all-cause mortality) and so give a measure of lifespan. The PhenoAge [34] clock aimed to correlate with clinical characteristics which are known features of phenotypic ageing; composed of 513 CpG sites it significantly outperformed previous predictors of cancer, 'healthspan', physical functioning and Alzheimer's disease in studies [34].

Table 1
The characteristics of the Horvath, GrimAge and PhenoAge epigenetic clocks.

Clock	Array type	Number of CpG sites	Tissue type	Correlation with chronological age
HorvathAge, 2013	27 k/ 450 k array	353	Multiple tissues	96 % correlation 3.6 year mean absolute difference
Horvath GrimAge	450 k/ 850 k arrays	1030	Whole blood leukocytes	Not applicable
Horvath PhenoAge	450 k/ 850 k arrays	513	Whole blood leukocytes	71 % correlation

3.3. Associations of epigenetic age acceleration with musculoskeletal health

While the Horvath clocks have been investigated in cohort studies and associations demonstrated with mortality [34], cancer incidence [35], lung cancer [36], and a host of other cancer outcomes, diseases of ageing and mental health phenomena, there are relatively few studies investigating associations with ageing of muscle or bone.

In a cross-sectional, observational study of 1820 older individuals in their early 60s in Germany, Horvath methylation age acceleration was significantly associated with a comprehensive frailty measure, 'The Frailty Index' [37]. This index was based on 34 deficits in health status (including the presence of 11 diseases, self-rated health, 6 symptoms and difficulties in performing 16 particular activities of daily living). Each of these scores a single 'deficit' in the Frailty Index with the maximum being 34. There was an approximate increase of half a deficit per 6 years of epigenetic age acceleration ($p = 0.0004$).

The relationship between peripheral blood epigenetic age acceleration and frailty (according to the Fried Criteria [38]) has also been investigated in the Lothian Birth Cohort 1936 [39]. This study found that a greater Extrinsic Epigenetic Age Acceleration (EEAA) (which incorporates the changes in white cell composition associated with age) was associated with a higher risk of being frail (for one year increase in epigenetic age, RR 1.06, 95 % CI 1.02–1.10). No associations were noted with Horvath or Intrinsic Epigenetic Age Acceleration (IEAA) (age acceleration adjusted for white cell composition).

Physical activity has been investigated in both the Women's Health Initiative, which found a weak correlation between EEAA and physical inactivity (assessed by self-report) [40] and in the Lothian cohort, which found that neither EEAA or IEAA were associated with sedentary or walking behavior (which had been objectively measured) [41]. Increased epigenetic age acceleration was associated with reduction in balance, motor coordination, self-reported physical limitation, cognitive abilities, self-rated health, facial ageing but not grip strength [42]. It should be noted that effect sizes were modest.

Marioni and colleagues [43] investigated the associations between the Horvath epigenetic clock-based age acceleration and four mortality-linked markers of fitness including general cognitive ability, gait speed, lung function and grip strength. Epigenetic age acceleration from blood was acquired, and the above markers were assessed in 920 members of the Lothian Birth Cohort at age 70, age 73 (in 299 participants) and age 76 (in 273 participants). Significant cross-sectional associations (after adjustment for leukocyte composition, height, and smoking status) were observed between age acceleration and cognitive ability ($\beta = -0.07$, $p = 0.024$), grip strength ($\beta = -0.05$, $p < 0.01$), Forced Expiratory Volume in one second (FEV1) ($\beta = -0.06$, $p < 0.01$), though not with 6-m gait speed ($\beta = 0.03$, $p = 0.45$). There was no significant change in DNA methylation age acceleration over time (supporting previous data

suggesting that it is stable in adulthood [28]) and only longitudinal change in FEV1 was associated with baseline age acceleration, though with a clinically insignificant effect size ($\beta = 7.8 \times 10^{-4}$, $p = 0.05$). The lack of association may have been due to insufficient tissue specificity, the relatively short follow-up time (6 years) and a potential lack of statistical power.

Simpkin and colleagues [44] investigated associations between Horvath age acceleration and three measures of physical capability: grip strength, standing balance time and chair rise speed. Participants were drawn from females enrolled in the National Survey for Health and Development (NSHD) and included 152 who had blood samples. Age at baseline was 53 years and follow-up was performed at age 60–64 years. Baseline epigenetic age acceleration was significantly associated with a greater decrease in grip strength from baseline to follow-up (0.42 kg decrease in grip strength per year increase in age acceleration, 95 % CI 0.82 kg; $p = 0.03$), though this was not then demonstrated in replicate work in The Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. No associations were observed with standing balance time or chair rise speed.

HorvathAge acceleration was investigated in 48 monozygotic twins in Finland with the finding that an increase in Horvath age acceleration was associated with a reduction in grip strength ($\beta = -5.3$, SE 1.9, $p = 0.01$), though no association was observed with knee extension or 10 m gait speed [45].

Taken together, the studies that have been performed have been too few and perhaps underpowered to be able to state conclusively the association between epigenetics and ageing of muscle and bone. However since the above studies [43–45] which focused on the first-generation epigenetic clocks were published, further studies have been performed to examine the second-generation iterations, which appear to show stronger associations with the ageing muscle phenotype, and these are discussed here.

McCrory and colleagues found significant associations between GrimAge acceleration and grip strength and gait speed in 490 community-dwelling older adults who were part of The Irish Longitudinal Study of Ageing (TILDA) [46]. Maddock and colleagues also found significant associations for GrimAge and PhenoAge in a meta-analysis of studies including the National Survey for Health and Disease (NSHD) over 13 years of follow-up [47].

Although a few associations have been established with grip strength, none of the associations with muscle outcomes have been particularly strong. However, a recent paper reports the development (using elastic net regression) of a clock to provide a biological age of human skeletal muscle tissue [48]. Whereas the original Horvath clock contained 353 CpG sites, this new muscle clock is composed of 200 CpG sites (16 of which overlap with the original clock). This suggests that there may be utility in more 'tissue-specific' clock development correlated to musculoskeletal outcomes.

Finally, no studies to date have reported associations between epigenetic age acceleration and bone outcomes; one study in 32 individuals with osteoporosis and 16 controls found no association between bone parameters and HorvathAge acceleration [49]. This may be due to a lack of power or the use of a first-generation rather than second-generation epigenetic clock. Taken together, these studies highlight a need for further research in this area, including bone outcomes.

3.4. Epigenome-wide association studies

In addition to individual studies of candidate genes, EWAS studies consider the association between a phenotype and epigenetic variants, typically methylation. As the cost of such tests has decreased in recent times, the number of such studies has increased significantly, and some of these that consider bone and muscle phenotype in later life are discussed here.

3.5. Bone mineral density

Epigenome-wide association studies (EWAS) have been performed in various tissues examining relationships with osteoporosis and fracture. Some studies have focussed on the methylation profile of femoral head tissue collected from hip fractures compared to hip replacements [50]. An alternative approach examined associations between osteoporosis and methylation (via 450 k array) at 2529 CpG sites in 100 genes previously identified to be associated with bone mineral density in postmenopausal women [51].

Cheishvili and colleagues compared 22 osteoporotic postmenopausal women against 22 non-osteoporotic controls and found 1233 differentially methylated CpG sites (using 450 k array) with a false discovery rate (FDR) of 5 % in peripheral blood leukocytes. They then subdivided the osteoporotic group into early and advanced osteoporosis (using a T-score threshold of $\geq$3.0 for advanced disease) and selected a panel of 5 of the most significantly associated, biologically plausible genes to create a polygenic risk score to predict the development of early osteoporosis [52]. Interestingly the panel of genes included Programmed Cell Death Protein 1 (PDCD1), which plays a role in T- and B- cell regulation.

A large, collaborative EWAS of peripheral blood leukocyte methylation and BMD, including a 4616 individual discovery dataset identified one CpG site, cg23196985, associated with BMD in females. However, this association was not reproduced in the 901 individual validation dataset [53].

3.6. Grip strength

Compared to bone mineral density, there is a relative paucity of grip strength EWAS studies. Soerensen and colleagues investigated associations between grip strength and differentially methylated CpG sites in blood samples from a cohort of 672 twins from the Study of Middle-Aged Danish Twins (MADT) and the Longitudinal Study of Ageing Danish Twins (LSADT) [54]. They found that no CpG sites met statistical significance (adjusted for sex, age, height, cell composition); however, two CpG sites implicated a role of the immune system. Two further EWAS studies of grip strength have demonstrated no significant associations; one in 172 female twins [55] and the other in the Lothian Birth Cohort [44].

4. Conclusion

There remains a considerable research agenda around the important of epigenetics to musculoskeletal ageing. This includes a need to undertake more studies that include muscle and bone outcomes, and ideally both measured in individual cohorts. Sample sizes for such studies needs to be adequate to allow meaningful conclusions to be derived. A particular challenge of work in this area is tissue specificity – epigenetic modification may be tissue specific meaning that it is hard to obtain samples from large numbers of participants in community based cohorts. An awareness of ongoing research in this field is important for all clinicians in training, as future personalised medicine may include consideration of epigenetic factors.

Contributors

NR Fuggle contributed to the literature search and review, preparation of the original draft, and revision of the draft.

F Laskou contributed to the literature search and review revision of the draft.

NC Harvey contributed to the literature search and review revision of the draft.

EM Dennison contributed to the literature search and review, preparation of the original draft, and revision of the draft.

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