Ageing, genes, environment and epigenetics: what twin studies tell us now, and in the future

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Abstract

Compared with younger people, older people are much more variable in their organ function, and these large individual differences contribute to the complexity of geriatric medicine. What determines this variability? Is it due to the accumulation of different life experiences, or because of the variation in the genes we are born with, or an interaction of both? This paper reviews key findings from ageing twin cohorts probing these questions. Twin studies are the perfect natural experiment to dissect out genes and life experiences. We discuss the paradox that ageing is strongly determined by heritable factors (an influence that often gets stronger with time), yet longevity and lifespan seem not to be so heritable. We then focus on the intriguing question of why DNA sequence-identical twins might age differently. Animal studies are increasingly showing that epigenetic modifications occurring in early development and adulthood, might be key to ageing phenomena but this is difficult to investigate longitudinally in human populations, due to ethical problems of intervention and long lifespan. We propose that identical twin studies using new and existing cohorts may be useful human models in which to investigate the interaction between the environment and genetics, mediated by epigenetic modifications.

Keywords: ageing, twin study, epigenetics, genetic susceptibility, older people

Internal medicine and traditional epidemiology focus on separating the ‘diseased’ from the unaffected. However, geriatric medicine and ageing relate to continuums of function. Some ageing researchers focus on continuous traits known to change with age, others have studied functional thresholds and ageing-related diseases. Many groups research lifespan or longevity, as well as liability to death (one definition of ‘frailty’). There have been attempts to find age-related traits (biomarkers) that will predict lifespan or risk of death but without clear consensus.

Continuous measures can demonstrate population variability and many change with age, for example lung function, speed of cognitive processing, grip strength and lens keratography (cataract). In most age-related traits, there is more variation in an older population. This concept underlies the therapeutic complexity of geriatric medicine. The purpose of this paper is to discuss the findings of twin studies concerning the aetiology of this variability that may be of interest to practicing geriatricians—i.e. why might two 80 year olds be so different? We focus on an area of ageing research, which shows particular promise—epigenetics, and give an outline of how twin studies may be useful to this research area particularly in the search for modifiable causes of ageing.

Epigenetic mechanisms involve molecular markers that alter the expression of genes—indicating which ‘books’ should be referenced from the genomic ‘library’, explaining, for example, why our 200 tissues can develop from a single cell and yet have the same DNA structure. Although monozygotic (MZ) twins have the same library, they may have different reference patterns, leading to different phenotypes or traits. Epigenetics involves any modification of the chromosome changing transcription, from modification of chromatin structure to methylation of DNA itself.
Classic twin studies and variability in ageing

Twin studies can investigate the causes of variation in a population. MZ twins share virtually 100% of their variable DNA sequence, whereas dizygotic (DZ) twins, like any siblings, share on average only half. Classical twin studies compare the variability in MZ pairs compared with DZ pairs, and then estimate the extent attributable to genetic factors, environmental factors shared by the twin pair, and that which is unique to each twin.

Twin studies are generalisable. While twins are born smaller than singletons, this has no demonstrable effect when they are adult. Overall and cardiovascular mortality are comparable with same-aged singleton populations [1]. The TwinsUK cohort is comparable with a similar singleton population in many continuous measures [2].

What is heritability?

Heritability is typically described as the proportion of total phenotypic variation attributable to genetic effect, and twin studies indicate substantial heritability in most age-related diseases (Table 1) and continuous age-related traits (Table 2). It may be surprising to geriatricians that frailty and falls are more heritable than breast cancer. Heritability has been important in spurring scientists to search for the specific common genes involved in ageing traits using genome-wide association studies (GWAS). Such studies were expected to identify DNA changes, whose effects add up to explain most of the heritability. However, except for a few ageing traits (notably macular degeneration) the polymorphisms found only reflect a tiny fraction of the heritability estimated in twin studies. This has led to investigators re-evaluating the traditional understanding of heritability. Twin studies usually have higher estimates of heritability than family studies, but are preferable because they estimate the impact of shared environment and are not confounded by cohort differences. Epigenetic mechanisms may account for some of this ‘heritability.’ Epigenetic modifications are partially stable and are influenced by a wide variety of factors. Where these factors are more shared by MZ twins than DZ [e.g. genes themselves, interaction of shared (e.g. early life) environment and their DNA] heritability will be increased [3]. This applies to epigenetic mechanisms working across the lifespan. However, where these factors are unshared by the MZ pairs, (for example in later life environment or stochastic changes with ageing) ‘heritability’ will be reduced. Overall, it is likely that epigenetics contributes to heritability because MZ twins (particularly dichorionic who have split earlier in development) have less DNA methylation difference than DZ twins [4].

Table 1. Heritabilities of common dichotomous ageing traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Heritability estimate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death from CHD</td>
<td>Men 57%, women 38%</td>
<td>[5]</td>
</tr>
<tr>
<td>Alzheimer dementia (aged &gt;65)</td>
<td>79%</td>
<td>[6]</td>
</tr>
<tr>
<td>Parkinson disease (aged &gt;50)</td>
<td>0–6%</td>
<td>[7, 8]</td>
</tr>
<tr>
<td>Hip fracture (aged &lt;69)</td>
<td>68%</td>
<td>[9]</td>
</tr>
<tr>
<td>Hip fracture (aged 69–79)</td>
<td>47%</td>
<td>[9]</td>
</tr>
<tr>
<td>Hip fracture (aged &gt;79)</td>
<td>3%</td>
<td>[9]</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>60–85%</td>
<td>[10]</td>
</tr>
<tr>
<td>Knee osteoarthritis</td>
<td>39%</td>
<td>[11]</td>
</tr>
<tr>
<td>Age-related macular degeneration</td>
<td>45%</td>
<td>[12]</td>
</tr>
<tr>
<td>Age-related hearing impairment</td>
<td>Men 47%, women 71%</td>
<td>[13, 14]</td>
</tr>
<tr>
<td>Falls</td>
<td>35%</td>
<td>[15]</td>
</tr>
<tr>
<td>Frailty</td>
<td>43%</td>
<td>[16]</td>
</tr>
<tr>
<td>Urge incontinence (men)</td>
<td>49%</td>
<td>[17]</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>35%</td>
<td>[18]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>42%</td>
<td>[18]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>27%</td>
<td>[18]</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>26%</td>
<td>[18]</td>
</tr>
</tbody>
</table>

Heritability estimates have been taken from the largest available samples. Note that heritability estimates can change with age, and population, as detailed in the text.

Are the changes in traits that occur with age also heritable?

Some informative longitudinal studies have investigated the heritability estimates within the same population as it ages. In some traits, although total variation increases with age, the proportions of variation attributable to genes and environment stay the same, so the heritability remains constant at each age (blood pressure: [23]; lung function and motor function [19], show heritability which increases with age—supporting, in these cases, the old adage that the older we are the more we resemble our parents. Table 2 shows the heritabilities of level and slope, or intra-individual change in various ageing traits. Early research of cognitive function suggested that genetic effects

Table 2. Heritabilities of common continuous age-related traits in longitudinal older cohorts

<table>
<thead>
<tr>
<th>Trait</th>
<th>Heritability estimate for general level (intercept) (%)</th>
<th>Heritability estimate for trajectory of change (slope) (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1 (men)</td>
<td>54</td>
<td>100</td>
<td>[19]</td>
</tr>
<tr>
<td>Grip strength (men)</td>
<td>72</td>
<td>0</td>
<td>[19]</td>
</tr>
<tr>
<td>Grip strength (women)</td>
<td>42</td>
<td>0</td>
<td>[19]</td>
</tr>
<tr>
<td>General cognitive function</td>
<td>91</td>
<td>1</td>
<td>[20]</td>
</tr>
<tr>
<td>Post menopausal bone density (whole body)</td>
<td>—</td>
<td>44</td>
<td>[21]</td>
</tr>
<tr>
<td>Mean arterial blood pressure</td>
<td>82</td>
<td>63</td>
<td>[19]</td>
</tr>
<tr>
<td>BMI (women)</td>
<td>60</td>
<td>64</td>
<td>[19, 22]</td>
</tr>
</tbody>
</table>

FEV1, forced expiratory volume in 1 s; BMI, body mass index.
become increasingly important over time [25]. While this may be true from adolescence to late adulthood, more recent studies of much older longitudinal cohorts have found that while the level of cognitive function in normal older adults is largely genetically determined, change in cognitive function is ‘environmentally’ mediated [20, 26]. A study of word recall scores found a significant peak in non-shared environmental influences between 70 and 75 [27]. This pattern is also seen in hip fracture where in the eighth decade and beyond, environmental factors become much more important than genetic factors [9]. What are the unique environments at older ages, which may be promoting or hindering healthy ageing?

In other organs, genetic factors remain important in determining change in function, e.g. lumbar disc degeneration [28], lung function and motor function [19]. Change in physical functioning and bone density is more heritable in women than men, indicating that the aetiologies of ageing may be different in the two sexes [21, 29, 30].

Do specific genes become important as we age in adulthood?

This is a philosophically important question, which informs debates about whether human ageing is programmed [31, 32]. Non-stable genetic influences suggest the emergence of new genetic effects at different points along the lifespan (genes turned off or on). Few quantitative genetic studies have investigated the stability of genetic effects longitudinally, but those that have, have shown the same genetic factors are important over time [5, 22, 33]. If this is confirmed in other studies it is further evidence against theories of programmed ageing in humans.

To what extent are the genes influencing one trait shared with other ageing phenomena?

Clinicians see daily that not all physiological systems change at the same rate as each other within an individual and this is confirmed by the organ differences discussed above, and this may explain why it has been difficult to find biomarkers that capture ageing as a whole. Twin studies can look for shared genetic aetiology between ageing traits. Recently, the Danish twin registry published on the interactions between self-reported physical functioning and cognitive function in older twins. They found shared causes underpinning both [34]. There is also evidence of shared aetiology of hypertension, migraine and Raynauds [35], but this research area is just beginning: multivariate analysis of the shared aetiologies of different organ ageing could identify systems that would fail in parallel. Clinically this might help in the structuring of the medical assessment of older people—targeting aetologically related problems first.

Why is longevity not as strongly heritable as organ function?

Some European twin cohorts sufficiently old to ascertain mortality have estimated that 25% of the variability in age of death is due to genetic differences [29]. A recent study found little evidence suggesting genetic effects on age of death before 60, and moderate but increasing influence of genetic effects from 60 onwards [36]. Frailty, defined in this case as a composite of physical and cognitive weakness, low self-rated health and reduced activity of daily living, (a good predictor of mortality risk), has indeed been found to be substantially heritable (43%) and further, the influence of genetic factors increases with age [16]. Survival into old age is clearly multi-factorial, depending on multiple chance and non-chance events. Cardiovascular disease, hypertension and angina are all highly heritable [5], but cancer, due in part to a variety of chance mutation or epigenetic events, is less so [18]. This may explain why longevity has a lower heritability compared with conceptually separated biological traits. On the other hand, survival given old age may be more influenced by genetic factors, albeit each of small effect size [37].

From heritability to genes

Twin cohorts have contributed to vast collaborative exercises searching for genetic determinants to age-related traits. A review of all the ‘ageing’ associated genes and single nucleotide polymorphisms found by studies incorporating twin data is out of the scope of this review but has been reviewed recently by Fallin and Matteini [38]. Studies have shown that a high heritability does not necessarily mean that GWA studies can find the genes responsible. This is probably due to the extensively polygenic nature of most ageing traits. GWAS can only detect common variants of the moderate effect size and a highly heritable trait may not have any such common variants of discoverable effect.

So if it is not all genetic: what is this so-called ‘environment’?

Despite the importance of genetics, there still remains a significant amount of variability in all ageing traits that is not attributable to heritable factors. In quantitative genetic twin models, environment and error unique to each individual are inseparable, so the greater difficulty in measuring the trait of interest, the more heritability could be underestimated. However, in many cases, measurement error, as assessed by reliability rates or agreement with other tests, is quite small and cannot account for the magnitude of the ‘environmental’ term. Greater understanding of this environmental influence will be useful in determining potentially modifiable factors leading to ageing. There is, however, some blurring of what is called environmental factors—exercise, smoking and diet all have major genetic components.
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To what extent are MZ twins discordant in ageing diseases?

Identical twins do not necessarily succumb to the same diseases, and age differently. For example, even for the most heritable forms of cancer: breast, colorectal and prostate, the absolute risk of the same disease occurring before 75 for a MZ twin whose twin had the cancer, was only 9–21% [18]. We know there is a much stronger genetic component to coronary heart disease; however, a 36-year follow-up study showed that the risk of a man dying of coronary heart disease like his co-twin was less than 50% [5]. The point is, that even in very heritable disease, there is an important non-genetic component influencing disease outcome, which separates DNA-identical twins.

Why might identical twins age differently?

Studies have focussed on two distinct sources of discordance. The first is early developmental differences and the second involves the body's response to environmental factors in adulthood. However, it is also possible that the lack of complete concordance between MZ twins is not due to conventional 'exposures' to measured or unmeasured external environments, but due to chance events in the endogenous remodelling cycles that continue over life, leading to accumulated 'errors' that have consequences for the internal 'environment'. One example is somatic gene mutation but other 'mistakes' on an epigenetic, telomeric, proteomic or even cellular level are also possible. For example, genetically identical marbled crayfish raised under identical environmental conditions still differ in development, lifespan and epigenetic changes—indicating a stochastic element to developmental variation, which may also be mediated through epigenetics [39].

The fact that MZ twins can differ in epigenetic markers, has led to the investigation of the stochastic and environmental arguments. These are attractive because, at least environmental factors might be modified, or if not, the path by which they, or error, lead to ageing could be altered if the mechanism can be interrupted. For example, post-mortem methylation differences have been found in hippocampal cells in MZ twins discordant for Alzheimer's disease (AD). These patterns, and mounting evidence of the importance of epigenetics in AD, have been replicated [40]. What are the aetiologies of these potentially reversible changes, which could enable us to modify the outcome for these individuals?

The influence of early developmental events

Twin studies have been able to shed further light on the foetal origins of disease hypothesis [41] especially with regard to birth weight. They add interest because they can separate genetic disposition to lower birth weight from early environmental effects. The association between low birth weight and adult hypertension is greater in MZ twins than DZ, indicating that environmental (possibly placental or uterine) factors may be more important than genetic causes [42, 43]. The story is more complicated with other traits. A Danish team found that there is a significant and 'dose-dependent' effect of lower birth weight on body fat, insulin sensitivity [45] and muscle glycogen synthase levels in older MZ twins [46] suggesting that, despite the same genotype, birth weight produces life long effects on metabolism. The same team also found that difference in birth weight is inversely associated with glucose tolerance in MZ twins, more-so than DZ twins, again supporting a non-genetic causation using this continuous measure. However, against this conclusion, a large twin study from Sweden has shown that the association seen between birth weight and the categorical outcomes of cardiovascular disease [43] and diabetes [47] in the population as a whole disappear when considering just discordant MZ twins. They argue that genetic factors may therefore be a common cause explaining the birth weight and later disease, but concede that this could also be due to placental function, as MZ have greater placental anastamoses.

Epigenetics and early developmental hypotheses of ageing

Of epigenetic mechanisms, DNA methylation has been the most studied, due to the relative ease of measurement. The most common and probably functionally important methylation of DNA occurs to cytosine nucleotides adjacent to guanine (CpGs). It appears that the CpGs clusters of CpG repeats, located upstream of gene promoters, are the most consistently linked to changes in gene expression. Hypermethylation leads to gene silencing through a complex cascade [48]. Epigenetic alteration of gene expression is the main mechanism of tissue differentiation [49, 50]. A recent study, using twins to identify methylation sites associated with ageing, has shown that just two methylation markers in human saliva are so linearly associated with age, they can predict someone's age to within 5.2 years, explaining 73% of the variance in age [51].

Developmental animal studies have shown changes in methylation patterns in specific genes in response to dietary intake at certain crucial points of development. This has been postulated to be the stable effect that influences disease/ageing much later in life. Perinatal environment—especially excess fat in the maternal diet—seems to alter liver histone modification and predispose offspring to obesity in monkeys [52]. Likewise, over feeding in the neonatal period in rats led to hyper-methylation of the pro-opiomelanocortin gene and a permanent disposition to obesity [53]. Neonatal stress can also lead to longstanding methylation changes, which may influence ageing [54]. These processes may be mediated through glucocorticoids: for example, differential methylation up stream of the
glucocorticoid receptor promoter in the hippocampus was seen in rat offspring treated differently by their mothers [55].

Such changes are more difficult to replicate in humans due to the ethics of interventions, length of lifespan and difficulty accessing tissue—particularly of the brain. However, individuals in utero during the Dutch famine of 1944–45 were found to have less methylation of the IGF2 gene 60 years later, compared with their same sexed siblings showing the effect of early life nutritional adversity [56]. Their offspring had higher rates of schizophrenia and cardiac problems.

Twins may offer a unique study population in which to test hypotheses regarding epigenetic mechanisms in development. Current cohorts which should deliver this include the Twins Early Development study at the Institute of Psychiatry, London [57], and a Melbourne cohort of newborn twins [58].

Environmental factors and epigenetic changes in adulthood

There is some evidence that 50-year-old twins who spent less time together show more epigenetic difference [59]. However, so far no twin studies have investigated precisely what environmental changes contribute to epigenetic change. Literature on epigenetic changes in cancer have indicated that part of the effect of some carcinogens such as asbestos and cigarette smoke are mediated though epigenetic alterations. Moreover, these changes may be mitigated by diets rich in leafy green vegetables and multivitamins [60, 61].

Folate has long been implicated in mitigating epigenetic drift, and in pregnant women can alter offspring methylation [62]. However, decreased folate levels with age lead to reduced global methylation [63], which seems to be less important in altering gene expression than hypermethylation of ageing implicated gene promoter regions. Environmental factors implicated in modifying such regions include smoking (detrimental) and green tea and physical exercise, which may protect [61].

Future of twin research into epigenetics and ageing: what are the unanswered questions?

Two urgent questions could be answered by twin research into epigenetics. Firstly whether MZ twins discordant for ageing-related traits are also discordant in replicable epigenetic differences. A large-scale study (EpiTwin) currently underway aims to identify methylated genes responsible for 10 age-related traits, using longitudinal data to test for direction of causality [64].

The second is to define the relationship between environmental factors and epigenetic changes using twins discordant for specific environments. Do human studies uphold what has been described above in animal studies—that environment alters epigenetic signals which in turn contribute to ageing traits? These studies will provide a new paradigm for nature–nurture interactions in ageing.

Conclusion

Increasing variability in organ function is seen with age, and individual factors—be they genetic or environmental—become much more important with increasing age. Contrary to expectation, this increasing variability is not simply a snowballing of environmental effects. The effect of genes is important in determining both ageing-related measures at a single time point, and how they change over time. Studies so far do not seem to suggest that new genes become switched on in later life, rather that the same genes are important over adulthood. Time amplifies the effect of both genes and environment making us increasingly different from each other.

Twin studies have much more to offer the study of ageing than simply providing heritability estimates. Further quantitative genetic study of the interaction between ageing traits and their development over time is needed. It is also now possible to incorporate DNA and epigenetic information in twin studies, and test hypotheses from animal models about the molecular mechanisms leading to ageing phenotypes. The insights into ageing to be gained from the ‘natural experiment’ of twinning have only begun to be realised.

Key points

• Variability in organ and system function increases with age.
• Genes remain important in determining function at advanced age, and change in function with age.
• Studies of identical twins can shed light on environmental causes of ageing, including the effects of very early life events.
• Epigenetics is a promising research area, which may explain how environments and chance lead to difference in ageing trajectories.

Conflicts of interest

None declared.

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Supplementary data

Supplementary data mentioned in the text is available to subscribers in *Age and Ageing* online.

References

The very long list of references supporting this review has meant that only the most important are listed here and are represented by bold type throughout the text. The full list of references is available in the Supplementary material.