Facing up to the global challenge of ageing

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Abstract

Longer human lives have led to a burden of late-life disease. However, some people experience little late-life ill-health, and this trait should be extended to the general population. Interventions into lifestyle, including increased exercise and reduction in food intake and obesity, can help to maintain healthspan. Altered gut microbiota, removal of senescent cells, young blood factors and drugs can all improve late-life health in animals. Application to humans will require better biomarkers of disease risk and of responses to interventions, closer alignment of work in animals and humans, and increased use of electronic health records, biobanks and cohort studies.
During the last 200 years, average human life expectancy has doubled in most developed countries\(^1\) (Figure 1). Better quality of water, food, hygiene, housing and lifestyle, immunisation against infectious disease, antibiotics, and improved medical care, first reduced mortality in early life\(^2,3\) and, after about 1950, in 70+ year olds\(^1,4\). Whether there will be a limit to human life expectancy is vigorously debated\(^5\), but survival rates in the elderly and mean life expectancy are generally projected to continue to increase\(^6\). In parallel with longer lives, most aspects of age-specific health have also improved, with increases in both physical and cognitive functioning during ageing in successive birth cohorts\(^7,8\).

Recent increases in human life expectancy have been much too rapid for genetic change to have played a significant role. In contemporary populations, individuals who survive to great ages are particularly common in the so-called ‘Blue Zones’ of the world, Okinawa in Japan, part of Sardinia in Italy, Ikaria in Greece, Nicoya in Costa Rica and Loma Linda in CA, USA. These populations have not been found to be genetically distinct from their neighbours, suggesting that environment and lifestyle play important roles\(^9\). Factors such as diet, education and physical activity throughout postnatal life have a cumulative effect on mortality\(^10\), with early life conditions and parental health playing a prominent role\(^3\).

Improved health of people of all ages, including older people, and the consequent increase in life expectancy, are to be celebrated as achievements of civilisation. However, healthy, disease-free lifespan (healthspan) has not increased as much as lifespan\(^11\). A global increase of 5 years in total life expectancy between 2000 and 2015 has been accompanied by only 4.6 years of healthy life expectancy\(^12\). An average 16-20% of life is now spent in late-life morbidity\(^13\), longer in females than in males, in individuals of low socioeconomic status and with obesity\(^13-15\). Most of us now live far longer than in our evolutionary past, to ages that have not been moulded by natural selection. Advancing adult age is hence the major risk
factor for chronic killer diseases, including cancer, cardiovascular and neurodegenerative
disease\textsuperscript{16} (Figure 2). Ageing impairs sensory, motor and cognitive function, and hence lowers
quality of life. The burden of these conditions is now falling mainly on older people.
Reduction in the length and severity of late-life morbidity should therefore be a major aim in
civilised societies for the future. We shall refer to this goal as ‘compression of morbidity’.

Compression of morbidity should be achievable. Individuals who survive to over 100, 105 or
110 years show progressively greater compression of late-life morbidity\textsuperscript{17,18}. Thus, a
relatively healthy end to life is physiologically feasible and, if we could find the mechanisms
at work, it might be possible to extend the trait to the general population. Second,
experimental work with laboratory animals, mainly yeast, nematode worms, fruit flies and
mice, has revealed the remarkable malleability of ageing. Genetic, environmental and
pharmacological interventions can extend lifespan, ameliorate the loss of function and
diseases of ageing and, in some cases, compress late-life morbidity\textsuperscript{19-21}. Although laboratory
animals live less long than humans, ageing has underlying mechanisms that are conserved
over the long evolutionary distances, and that provide potential targets to maintain human
health at older ages\textsuperscript{22}. Indeed, similar life-extending interventions are effective in different
laboratory species\textsuperscript{19,21}.

We here address the opportunities and challenges for discovering the genetic and
environmental determinants of human lifespan and healthspan, and in translating results of
discoveries in animals into health improvements for ageing humans. We will not to be able to
abolish ageing, but we do expect to be able to attenuate the process and greatly ameliorate its
effects.
Genetics of human lifespan and healthspan

Genetic determinants of the marked individual variation in human lifespan have been assessed using several approaches (Box 1 and Table 1). Twin studies have suggested that human lifespan is ~25% heritable\(^\text{23}\). However, a recent study in a population of millions of individuals, using the population pedigree, showed a heritability of only 12%\(^\text{24}\). The variation in these figures is probably due to the difficulty of accurately estimating common environmental effects of factors such as diet and smoking within families. The heritability of lifespan is minimal for parents who die between puberty and age 60, and then increases progressively with death at later ages\(^\text{25}\). Different measures, including overall lifespan, healthspan, and survival to exceptionally late ages (often termed longevity) have been used in different studies. Multiple genome-wide association studies (GWAS), and the only genetic locus to show robust, genome-wide significance across studies is apolipoprotein E (APOE)\(^\text{26}\), a cholesterol carrier in peripheral tissues and the brain, also involved in the risk of cardiovascular and Alzheimer’s disease\(^\text{27}\) (Box 1). The general lack of replication of findings in independent studies may be attributable to different measures of survival and health, the age-specificity of genetic effects\(^\text{28}\), and different allele frequencies of lifespan-associated genetic variants in different birth cohorts\(^\text{29}\).

Survival to advanced ages, particularly the 1-10% longest lived of the generation, is enriched in families\(^\text{23}\), and members of these families show a lifelong survival advantage, with lower risk of coronary artery disease, cancer and type 2 diabetes\(^\text{30-32}\) and better immune and metabolic health in middle and old age\(^\text{32-34}\). Neither familial nor sporadic long-lived individuals\(^\text{35,36}\) display a decreased load of common genetic risk variants for age-related disease\(^\text{36,37}\). However, any common protective genetic variant responsible for familial longevity has eluded detection. Replicated studies based upon candidate genes emerging from studies in model organisms can also be informative\(^\text{38,39}\), and have identified the
FOXO3A\textsuperscript{40} locus, encoding a transcription factor, whose equivalents in model organisms play a consistent role in healthy ageing\textsuperscript{19,41,42}.

Since genetic approaches have yielded only limited insights into exceptional longevity, attention has turned to the characteristics (phenotypes) of ageing in humans, and their potential underlying mechanisms.

**Phenotypes and mechanisms of human ageing**

Human mortality rates reach a minimum at around puberty and increase roughly exponentially thereafter (Figure 1). Initially, the ageing process is manifested sub-clinically, in various types of physiological deterioration. From the third decade, age-related changes in body composition occur, including loss of bone, cartilage, muscle mass and strength and gain of abdominal fat\textsuperscript{43,44}. Systemic changes follow, for instance in the endocrine system with altered hormone levels and changes in blood pressure and blood lipids. The responses of tissues to hormones can also be affected, as in insulin resistance\textsuperscript{45}. Mechanical and structural changes, including vascular stiffness, affect heart and brain functions\textsuperscript{46}. Eventually, these continuous sub-clinical changes can culminate in a range of medically defined disease conditions in middle age, with the co-existence of two or more chronic health conditions in an individual being defined as multimorbidity. People with higher levels of markers of disease risk in their blood (Box 2), and those with multimorbidity, die up to 20 years younger than those with lower levels\textsuperscript{47,48}. Late ages are frequently accompanied by frailty\textsuperscript{49}, a composite index of ill-health, functional and psychosocial deficits\textsuperscript{50}, which increases the risk of falls, fractures, hospitalization, organ failure, disability and death\textsuperscript{51} (Figure 3). The largest medical challenges in treating the growing number of elderly patients are multimorbidity, present in at least half of the elderly over 70 years\textsuperscript{47,52}, and the related use of five or more types of medication (polypharmacy), which occurs in over 10\% of the general population\textsuperscript{53,54} and 30\%
of the elderly. Up to 12% of all hospital admissions of older patients can be attributed to adverse drug reactions, most commonly involving anticoagulants, blood pressure lowering and hypoglycaemic drugs, antiplatelet agents (aspirin) and nonsteroidal anti-inflammatory drugs (NSAIDs), the latter two most frequently contributing to death after admission. Behavioural risk factors play a major role. Large multi-cohort studies in high-income countries have indicated that the years lost to smoking, physical inactivity and high alcohol intake (>21 units per week for men, >14 per week for women) are, on average, 4.8, 2.4 and 0.5 years, respectively. In addition, older people can spend, on average, almost 10 waking hours in an immobile posture. Thus, the way that people age is malleable, and sensitive to modulation throughout life. The World Health Organization is therefore targeting the major risk factors so far identified, with the overall aim of reducing premature mortality from noncommunicable diseases by 25% by 2025.

Further progress in preventing late-life ill-health will come from better predictors of its occurrence, and from understanding how to intervene to block the mechanisms at an early stage. Different measures (biomarkers) can indicate the aetiology of ageing and its progress to disease states. Physiological decline can partly be recorded by standardized measures of physical, respiratory and cognitive capacity, blood pressure and circulatory markers. Poor performance on these indicators in midlife associates with increased morbidity and mortality risk over time. These markers can also monitor health improvement in response to interventions, but do not yet robustly reflect all relevant aspects of ageing. The generation of comprehensive biomarker profiles that are capable of doing so is therefore important, and this field is progressing rapidly.

Preventative interventions into lifestyle aimed at slowing or halting the progress of specific effects of ageing have already been successful. For instance, treating adults at risk of diabetes
by diet alterations, increased physical activity or both, can be as effective as by medication, and with better continued benefit during 7.5 years of follow up\(^{62}\). Reductions in hypertension, diabetes and brain atrophy, improved cognitive performance and reduction in mortality due to cancer and cardiovascular disease, have all been achieved by alterations to lifestyle. Specific diets\(^{63,64}\), exercise\(^{65}\), the two combined\(^{66}\), cognitive training and vascular risk management\(^{67}\), caloric restriction\(^{68}\) and intermittent fasting\(^{69}\) and supplementation of vitamin D\(^{70}\) have all been reported as effective for specific conditions. Responses to these interventions usually show marked individual variation, and it may become possible to target them to those individuals who will benefit most when robust biomarkers of the variation become available. For instance, the optimal amount of protein in the diet may vary, with older people suffering from frailty benefitting from more protein to combat traits such as muscle wasting and weakness (sarcopenia)\(^{71}\) and middle-aged people benefitting from less protein to combat cancer. Similarly, a combined intervention of dietary restriction and physical exercise can be more beneficial in subjects with elevated systolic blood pressure at baseline\(^{72}\). Lifestyle interventions, while often beneficial, can be insufficient to prevent the progress of age-related problems, partly because of failures in compliance, and also because of limited and variable responses. Drugs are also already in widespread use for prevention of cardiovascular disease (CVD), by pharmacological lowering of hypertension\(^{73}\) and LDL cholesterol\(^ {74,75}\). In primary prevention the drug is prescribed to at risk individuals (with, for example, high blood pressure or serum lipids), while in secondary prevention to patients (e.g. individuals with type 2 diabetes) to lower the risk of multimorbidity (e.g. the manifestation of cardiovascular disease and retinopathy in individuals with type 2 diabetes). The principle of pharmacological prevention of age-related diseases is thus well established in clinical practice.

The ageing process in animals has evolutionarily conserved, parallel and interacting mechanisms, known as hallmarks\(^{22}\), that have proved to be modifiable, and several of these
are also well documented in humans \((\text{Table 2})\). They eventually lead to unrepaired damage in DNA\(^76\), accumulation of misfolded and aggregated proteins (e.g. in the brain and the retina) and senescent cells (e.g. in joints and kidneys)\(^77\) and to an inappropriate and persistent activation of stress responses\(^78\), for instance in the innate immune system (inflammaging\(^79\)). To develop further interventions to compress morbidity, including drugs, we need a better understanding of the roles of individual ageing mechanisms in different tissues and at different stages in life, and their contributions to the aetiology of age-related diseases. Here, animal studies are useful to inform more targeted studies in humans.

**Translating discoveries from studies of ageing in animals**

The model organisms commonly used in ageing research are much shorter lived than humans, but they recapitulate many features of human ageing\(^80\). Furthermore, like many humans, their culture and care regimes in the laboratory largely protect them against infectious disease, provide them with abundant high quality food, restrict their exercise and remove many physical challenges. In consequence they, too, live to much greater ages than in their evolutionary past. Conservation of mechanisms of ageing between animals and humans extends both to ageing hallmarks\(^22\) and to genes involved in ageing and age-related diseases\(^16,20\). Different model organisms best recapitulate specific aspects of human age-related problems. Work across different model organisms has yielded biomarkers (Box 2) that predict remaining lifespan, such as nucleolar volume\(^81\) and telomere length\(^82,83\), and these are promising candidates for inclusion in a multivariate predictor of the rate of human ageing.

Ageing in animals has proved highly malleable in response to environmental and genetic interventions. Various regimes of dietary restriction (DR) are particularly effective, with increased lifespan and a remarkably broad improvement in health during ageing in diverse species, including rodents\(^20,68,84\). Two studies\(^85,86\) of DR in rhesus monkeys had slightly
different outcomes, probably because of differences in the composition of the control diet, the
degree of restriction and the timing of food provision\textsuperscript{87}. Lifespan increased under DR in one
study, while in both there were major improvements in health in the DR animals, with
reduced plasma triglycerides, diabetes, cardiovascular disease, sarcopenia, incidence of
neoplasms and brain atrophy, the most relevant health parameters in ageing humans. Multiple
genetic interventions can also induce broad improvements in health in laboratory animals\textsuperscript{88}.

For instance, reduced activity of the insulin/insulin-like growth factor (IGF)/mammalian
target of rapamycin (mTOR) signalling network can extend lifespan in yeast, worms, fruit
flies and mice\textsuperscript{21}, and genetic variants in candidate orthologous genes, or their gene expression
patterns, in humans can be associated with survival to advanced ages\textsuperscript{41,42,89-91}. As for dietary
protein, any benefits of modulating the activity of the IIS/mTOR signalling network in
humans may depend upon age\textsuperscript{71}. The network detects nutrition and stresses, and matches
costly activities such as growth, metabolism and reproduction to current status. Systems
mediating major life history choices in response to environmental cues thus play an important
role in ageing. Despite the complexity of the ageing process, with multiple hallmarks and
interactions between them, its effects can clearly be ameliorated in animals. Importantly, these
interventions have also proved capable of combatting the pathology of in models of age-
related diseases, including cancer, neurodegeneration and cardiovascular diseases\textsuperscript{92-94}. It
remains to be seen whether human ageing will show a similar degree of malleability in
response to interventions that have proved effective in animals, but a major conclusion from
ageing in model organisms is that delaying, or even preventing, age-related diseases is a
realistic prospect. Furthermore, while no intervention so far studied has improved all aspects
of health\textsuperscript{95}, interventions that extend lifespan generally also prevent more than one age-related
condition simultaneously\textsuperscript{94} (Figure 4).
Anti-ageing interventions that have proved effective in laboratory animals are starting to be assessed for their feasibility, effectiveness and safety in humans. Although DR increases predictors of healthy ageing in human volunteers, it is not a realistic public health intervention, because of poor compliance with even mild (90%) DR regimes. However, more modest dietary interventions may be more realistic. For instance, modulation of the protein content of the diet can both prevent over-consumption of protein-poor diets and avoid the elevated risk of cancer from high protein diets. The amino acid content of protein also determines its value to the animal and can be modulated to reduce total protein consumption. Timing of food intake can also be important. DR rodents are usually fed once a day and consume their limited food ration as soon as it is supplied, with a protracted fasting period until the next day. This fasting period may be at least as important as reduced food intake in promoting healthy ageing. Indeed, trials in middle aged humans with a fasting mimicking diet, low in protein, carbohydrate and calories, but high in unsaturated fats, had beneficial effects on biomarkers of health, such as blood pressure and levels of circulating IGF1, with particularly strong effects in individuals most at risk of disease. The time of day at which food is consumed can also have substantial metabolic effects. These more nuanced interventions would be more feasible than DR itself in humans, but need exploration of adversity when older individuals are included.

Increasing attention is focussing on pharmacological manipulation of mechanisms of ageing in animals, with a view to direct translation to humans to prevent age-related disease. Development of new drugs to ameliorate human ageing would pose challenges for clinical trials since, in the absence of validated biomarkers of risk, a large, random, initially healthy population would have to be treated over a long period. At present, repurposing of existing drugs with a good safety profile is therefore a more realistic short-term prospect than de novo drug development. Because mechanisms of ageing discovered in animals are proving
important in human age-related diseases, many are already targets of drugs licensed to treat these diseases\textsuperscript{106,107}. There is thus an opportunity to widen the use of existing drugs used to treat single, age-related diseases to prevent multimorbidity. For instance, the licensed drug sirolimus (rapamycin) inhibits mTOR complex 1, part of the nutrient- and stress-sensing network, and can extend lifespan in model organisms including mice, where it improves many, but not all, aspects of health during ageing, and protects against cancer\textsuperscript{92,108}. As in elderly mice, the poor immune response of elderly humans to immunisation against influenza can be enhanced by pre-treatment with the related drug everolimus\textsuperscript{109}. The antidiabetic drugs metformin and acarbose can also extend lifespan in laboratory animals, and are currently registered for clinical trials against ageing itself, which has not previously been recognized as a valid target\textsuperscript{105,110-112}. The doses of drugs that are effective in preventing the effects of ageing in animals are often lower than those used clinically, so that side-effects may be reduced, and may be further prevented by making drugs such as rapamycin more specific to their therapeutic target\textsuperscript{113} and by adjustment of dosing regimes\textsuperscript{113,114}.

Other recent discoveries about animal ageing are showing promise for translation to humans. Cellular senescence, a hallmark of ageing in both laboratory vertebrates and humans (Table 2), is a permanent cell cycle arrest, with resistance to cell death and secretion of bioactive molecules, the senescence-associated secretory phenotype (SASP). Cellular senescence is important during both development\textsuperscript{115} and wound healing\textsuperscript{116}, where it plays a key role in tissue remodelling, but in these contexts the senescent cells are removed by macrophages. During ageing, senescent cells persist. Their presence can cause tissue damage, and they are implicated in the aetiology of human age-related diseases, including atherosclerosis, osteoarthritis and cancer\textsuperscript{77,117,118}. Selective removal of senescent cells, or disruption of the SASP, can restore tissue homeostasis and increase healthspan and lifespan in mice\textsuperscript{117-120}.  

\textbf{Table 2}
Although more work in animals will be needed to assess the long term effects and side-effects of this type of intervention, research is already directed to the possibility of improving the quality of tissues for transplantation, such as kidneys, by prior removal of senescent cells\textsuperscript{121} and clinical trials are underway for the treatment of osteoarthritis and glaucoma. A promising approach to emerge from work on animals is epigenetic reprogramming of aged cells to rejuvenate tissues\textsuperscript{122}, which has extended lifespan in a mouse model of premature ageing\textsuperscript{123}. The myriad of microorganisms present in the gut, the ‘microbiome’ is increasingly implicated in the health of the gut itself and of other organs during ageing\textsuperscript{124,125}. Although most work thus far has been descriptive rather than experimental\textsuperscript{126}, transfers of the microbiomes from young to middle aged turquoise killifish resulted in an increase in lifespan and a delay in behavioural decline relative to fish that received a transfer from middle-aged fish\textsuperscript{127}. The composition of the human gut microbiome shows marked individual variation and is labile to many environmental factors, including habitual diet, medication and long-term residential care\textsuperscript{128}. Faecal transplantation from lean donors to metabolic syndrome patients can improve insulin sensitivity\textsuperscript{126,129} and probiotic studies in humans, following positive results in mice and safety assessment in human, are underway\textsuperscript{130}. Further experimental studies in animals are also needed to explore the role of the microbiome in ageing and age-related disease, and to use the findings to inform the design of trials in humans. The systemic, circulatory environment has also proved to play a key role in ageing. Experiments where the blood systems of mice were conjoined (parabiosis) showed that impaired function of stem cells in multiple aged tissues could be slowed or even reversed\textsuperscript{131}. Similar results came from transfer of blood or plasma, and plasma proteins from human umbilical cord have recently been shown to rejuvenate hippocampal function in old mice\textsuperscript{131}, suggesting that there may be evolutionary conservation of the effector molecules between mice and humans. Identification of these is a high priority for research. The practical accessibility of both the human microbiome and blood system makes therapeutic manipulation a particularly attractive
approach, but research in animals is needed to establish the long-term consequences and possible side-effects.

**Integrating research into ageing in animals and humans**

The increasing pace of discovery of mechanisms of ageing in animals, burgeoning practical efforts to characterise and predict the phenotypes of human ageing, together with the recent appearance of databases of electronic health records (EHRs)\(^1\), biobanks and more focussed long-term cohort studies, is opening new opportunities to discover the mechanisms underlying the diversity in physiological deterioration, multimorbidity and frailty and to intervene to attenuate or prevent them. Further progress will be facilitated by collaboration between scientists working in these different fields. This will align efforts to test the effects of feasible interventions in humans and animal models on ageing biomarkers, hallmarks, multimorbidity and frailty at the individual level. Direct and standardised measures of end-life multimorbidity itself are needed, in both animals and humans. Measures of healthspan and of age-specific multimorbidity, while informative, do not directly assess the duration or extent of multimorbidity at the end of life. Few such studies are conducted, because they demand longitudinal information on individuals until they die, but they will be necessary to assess effects of interventions on compression of morbidity.

The results of research into ageing in animals and humans are producing major dividends. Global public health efforts to increase human healthspan will increasingly focus on lowering the risk of obesity, smoking, high alcohol intake, physical inactivity, hypertension and LDL cholesterol, and success in doing so should yield widespread reductions in diabetes, cardiovascular disease, cancer and, possibly, dementia. Repurposed drugs are also a promising approach to maintain human health during ageing, and new clinical trials are
underway with candidates including rapamycin\textsuperscript{109}, and metformin\textsuperscript{105}. Drugs that kill senescent cells (senolytics), or that block the SASP also show great promise, to induce repair of damaged tissue. If successful, the osteoarthritis and glaucoma trials may be extended to primary prevention among elderly people if a consensus can be reached on surrogate endpoints of cartilage degradation and retinopathy. Ideally, preventative drug treatment in humans would start later in life, to minimise the duration of possible side-effects of long-term medication use. However, clinical trials do not, in general, include older people, and evidence that drugs are effective, at which doses, and whether they have the expected profile of side-effects among the elderly, is needed but is often lacking. For instance, levothyroxine, widely used to treat older adults with slightly underactive thyroid function, has proved ineffective in older people\textsuperscript{133}. The mechanisms leading to this lack of efficacy in late life could be investigated in laboratory animals. Polypharmacy is a major problem in older people, and model organisms could be used to find ways of minimising its effects. Therapies based on cellular reprogramming and systemic factors from young plasma also show great promise for application in tissue regeneration.

Interventions into the ageing process will often be most effective when they are targeted at those people most at risk. When establishing risk of rapid physiological decline and age-related disease, and monitoring the response to interventions, blood is the most practically accessible and, hence, most commonly investigated, tissue, but it is much less commonly used in animal studies. It will be important to develop blood-based biomarkers of risk, of ageing hallmarks and of responses to candidate interventions in animals. Mice are commonly used in studies of ageing and age-related disease, but other mammalian species may be more suitable for work on specific conditions, such as rats for thyroid function and blood pressure. Most laboratory mice are also inbred, with marked strain peculiarities, and more outbred animals would more closely mirror the individual heterogeneity that is typical for human populations,
although this problem is not confined to work on ageing. Some promising new models are also appearing that allow for parallel cell biological studies of animal and human ageing. Direct reprogramming of primary fibroblasts from individuals of different ages can maintain age-specific transcriptional profiles and decreases in nucleocytoplasmic compartmentalization, potentially opening the way to studying age-related cellular changes in vitro\textsuperscript{134}. Organoids can also provide a 3-dimensional context for studying interactions of different cell types with each other and with the extracellular matrix\textsuperscript{135}. These systems will facilitate \textit{ex vivo} work on human ageing with more realistic material than conventional tissue culture.

Further understanding of human ageing is coming from analysis of EHRs, biobanks and the detailed genetic and phenotypic data from clinical and longitudinal cohort studies. These can capture any features of human ageing that are not recapitulated by laboratory animals. EHRs can be interrogated for the age spectrum of health conditions, and for their associated molecular characteristics in populations with more detailed phenotyping. Expression of genes, proteins and metabolites associated with age-related diseases can provide more mechanistic insights, including the role of mechanisms of ageing discovered in animals (\textbf{Figure 4}). An initial correlation between, for instance, an elevated level of a protein and incidence of a health condition can be interrogated for causality by Mendelian randomisation\textsuperscript{136,137}, in which the random assignment of genetic variation to individuals at the zygote stage constitutes a natural experiment. Experimental studies in human cells, organoids and animals can then be used to probe the mechanistic links between the protein and the condition. Data resources, such as the druggable genome\textsuperscript{138}, can be used to determine if the protein is potential drug targets of approved or novel drugs that could delay or prevent the condition. These approaches would benefit from standardised protocols to biobank older people at general practitioner and hospital visits, in order to obtain a more representative sample of the elderly.
population than is currently available from biobank and cohort studies, and a better basis for monitoring the response to therapy. Novel assays using metabolic imaging now allow non-invasive recording of metabolic health status\textsuperscript{139}. The accumulated longitudinal data and biospecimens already collected in cohort studies can also be used to estimate the individual rate of change in specific biomarkers and outcomes. Robust biomarkers emerging from such systematic research can then be used as surrogate endpoints to indicate whether anti-ageing interventions are likely to have beneficial effects on clinical outcomes.

The expanding proportion of unhealthy elderly people in many populations is indeed a global challenge to society. The success of any intervention to combat multimorbidity will be limited by the wish of individuals to reduce its effects and hence their compliance with preventative measures. However, for the willing, lifestyle adjustments and preventative drug treatments are already at hand, with a battery of promising new interventions on the near horizon.
Acknowledgements

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Table 1. Loci emerging from genome-wide association studies of discrete and continuous lifespan-related phenotypes in human studies.

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<th>Closest gene(s)</th>
<th>Discrete phenotypes</th>
<th>Continuous phenotypes</th>
<th>Replication within publication</th>
<th>Replication between publications</th>
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<td>Yes</td>
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<td>HLA-DQA1/HLA-DRB1</td>
<td>None</td>
<td>Parental lifespan; Parents attained age</td>
<td>Yes</td>
<td>No</td>
<td>Inflammatory</td>
<td>142,143</td>
</tr>
<tr>
<td>ATXN2</td>
<td>None</td>
<td>Parents attained age</td>
<td>No</td>
<td>No</td>
<td>Multiple</td>
<td>142</td>
</tr>
<tr>
<td>FURIN</td>
<td>None</td>
<td>Parents attained age</td>
<td>No</td>
<td>No</td>
<td>Cardiovascular</td>
<td>142</td>
</tr>
<tr>
<td>EPHX2</td>
<td>None</td>
<td>Parents attained age</td>
<td>No</td>
<td>No</td>
<td>Cancer</td>
<td>142</td>
</tr>
<tr>
<td>PROX2</td>
<td>None</td>
<td>Parents attained age</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>142</td>
</tr>
<tr>
<td>CLESR2/PSRC1</td>
<td>None</td>
<td>Parents attained age</td>
<td>No</td>
<td>No</td>
<td>Cardiovascular</td>
<td>142</td>
</tr>
</tbody>
</table>

We included only studies that showed one or more genome-wide, significant associations with lifespan-related phenotypes (P < 5 × 10⁻⁸), with the exception of the RAD50/IL13 locus (P = 5.42 × 10⁻⁷), which was based on the number of linkage disequilibrium-independent markers on the genotyping array (Immunochip) used in the study. We excluded studies that were based on results from cohorts that were also included in more recent and larger studies. within publication refers to replication of a locus in different cohorts in the same publication. between publications refers to replication of a locus in different cohorts in different publications.
### Table 2. Hallmarks of ageing investigated in human studies.

<table>
<thead>
<tr>
<th>Hallmark</th>
<th>Description</th>
<th>Change with age/health (observational studies)</th>
<th>Beneficial response (intervention studies)</th>
<th>Causal evidence</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic instability</td>
<td>Accumulation of genetic damage affects DNA integrity and stability.</td>
<td>-Accumulation of somatic nuclear and mitochondrial mutations. -Pathogenesis of cancer and progeroid syndromes.</td>
<td>-Dietary energy restriction and increased exercise reduce oxidative and DNA damage, zinc de/repletion and DNA single strand breaks.</td>
<td>-Mutations cause premature ageing syndromes.</td>
<td>22,76,147</td>
</tr>
<tr>
<td>Telomere attrition</td>
<td>Chromosome caps formed by repeated DNA shortening of telomeric DNA and DNA damage to telomeres.</td>
<td>-Shortening of telomeres in cells/tissues due to cell division and damage associates with organ failure, disease, and mortality. -Accelerated by smoking and obesity.</td>
<td>-Mediterranean and plant-based diet and anti-oxidant supplementation slow down telomere shortening, CDV and mortality. -Bariatric surgery.</td>
<td>-Mutations in telomerase cause familiar premature disease; pulmonary fibrosis, dyskeratosis congenita and aplastic anemia. -Loss of regenerative capacity.</td>
<td>22,147,148</td>
</tr>
<tr>
<td>Epigenetic alterations</td>
<td>Change in DNA methylation, non-coding RNA, histone modification, and transcription.</td>
<td>-Global demethylation and, at promotor regions, hypermethylation and increased variation at polycomb target regions in multiple tissues. -Induced by environmental effects (smoking, stress, trauma, alcohol and sun). -Epigenetic clocks associate with health and disease in prospective studies.</td>
<td>-Folate and polyphenol supplementation</td>
<td>-Dietary energy restriction</td>
<td>22,147,149-151</td>
</tr>
<tr>
<td>Loss of proteostasis</td>
<td>Affected protein folding, degradation and repair by ubiquitin-proteasome and lysosome-autophagy affected synthesis of chaperones.</td>
<td>-Misfolded and aggregated proteins (in cataract) and accumulation of autophagic vesicles in affected neurons in neurodegenerative disease. -Accelerated by obesity. -Autophagy is better maintained in centenarians and their families.</td>
<td>-Dietary energy restriction</td>
<td>-Mutations in PS1 and PS2 cause familial autosomal-dominant AD and result in amyloid deposition, neuronal loss and lysosome pathology.</td>
<td>22,147,152</td>
</tr>
<tr>
<td>Deregulated nutrient sensing</td>
<td>Detect concentrations intra/extra cellular nutrients (glucose, amino acids, AMP, NAD+) by insulin IGF-1 (IIS), mTOR signalling inducing FOXO transcription factors AMPK and sirtuin expression.</td>
<td>-Increased gene expression mTOR and IIS pathways with age in different tissues and with brain disease severity. -Serum IGF-1 decreases with age and associates with sarcopenia. -IIS gene variants associate to long life (85+).</td>
<td>-Low protein intake in cohort study and dietary restriction associate to low serum IGF-1, but not in all studies, and reversion to insulin sensitivity.</td>
<td>-Mutations lowering GH and IGF-1 lower cancer/CVD incidence.</td>
<td>22,94,147,153,154</td>
</tr>
<tr>
<td>Mitochondrial dysfunction</td>
<td>Decreased number with age compromised function upon energy demand, ROS accumulation, lipid peroxidation, impaired clearance of dysfunctional mitochondria (mitophagy).</td>
<td>-Accumulation of ROS and somatic mtDNA mutations, clonal expansion and mosaic respiratory chain deficiency in multiple tissues.</td>
<td>-Dietary restriction stimulates FA oxidation and lowers oxidative damage. -High intensity aerobic interval training in young and old improved</td>
<td>-Mitochondrial mutations cause diseases with multiple ageing symptoms.</td>
<td>22,147,155-158</td>
</tr>
<tr>
<td>Hallmark</td>
<td>Description</td>
<td>Change with age/health (observational studies)</td>
<td>Beneficial response (intervention studies)</td>
<td>Causal evidence</td>
<td>Ref</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------</td>
<td>-------------------</td>
</tr>
</tbody>
</table>
| **Cellular senescence**      | Arrested cell division cycle excretion of protein profile (SASP) adversely affecting tissues affected clearance by inflammasome. | - Accumulation with age in a variety of tissues preceding disease, but controversies exists as to accumulation in healthy donors.  
- Accumulation in pathology (lung, kidney, cartilage) in biopsies and following therapeutic damage.  
- Association of genetic variation at the CDKN2A/B locus and multiple metabolic diseases. | - Senotherapy (clearance of senescent cells) in human cell models in which senescence is induced.  
- Prevention of accumulation of senescence by metformin in human cell models in which senescence is induced.  
- Compounds inducing senescence tested in cancer cells. | - Germ line and somatic mutations in CDKN2A contributes to increased risk of range of cancers.  
- Senolytic drug treatment of human OA cartilage explants and cultures: depletion of senescent cells, chondrocyte proliferation and cartilage ECM growth. | 22,77,147,159 |
| **Stem cell exhaustion**     | Decline of stem cell function regenerative potential.                        | - Observed in pulmonary fibrosis.  
- Loss of satellite cells in muscle and decreased regeneration capacity.  
- Increased frequency of haematopoietic stem cells (HSC) with impaired functionality and clonal expansion with controversy as to health consequences. | - Regenerative medicine on the basis of mesenchymal stem cells, musculoskeletal damage repair. |                                                                                                           | 160-163           |
| **Altered intercellular communication** | Deregulated endocrine, neuroendocrine, neuronal signalling associated with inflamming and decline of adaptive immune system or other inter-organ coordination (such as by the gut microbiome) through blood born factors. | - Inflammaging and gut microbiome composition.  
- Chronic over expression of basal levels of stress related proteins such as heat shock proteins in older patients, ER chaperones, hypoxia factor (HIF1alpha).  
- Poor corresponding adaptive response to stress. | - Gastric bypass  
- Calorie restriction  
- Resistance exercise training |                                                                                                           | 78                |

Hallmarks of ageing as formulated for animal studies with adapted criteria: (1) manifestation during normal ageing cross-sectional (young versus old donor comparison) or longitudinally (repeated measures over time), (2) aggravation associates with pathological condition (accumulates in diseased tissue, prevalent in patients or predicts health deficit prospectively), (3) intervention studies beneficially change aggravation, (4) removal of age-change increases health conditions, or aggravation causes accelerated ageing. There is
no systematic approach yet in recording the hallmarks of ageing in human studies for any of the points 1 to 4 and especially repeated measures in longitudinal studies are missing. Hallmarks may not completely cover all the relevant observations in humans, such as the adaptive homeostatic responses\textsuperscript{78}. Evidence for the causality of the hallmark in human ageing mostly results from mutations causing juvenile forms of age-related disease or ex vivo experimental data, mostly on cell models and sometimes on tissues.
Figure legends

Figure 1. Cumulative survival (upper panels) and mortality rates (lower panels) in men and women based on 100,000 individuals per birth cohort (1850 (red), 1900 (blue) and 1950 (green)) from lifetables from the Netherlands. Note that the Y-axis of the lower panels is based on a log scale.

Figure 2. Disability-adjusted life years (DALYs) per 1000 population for malignant neoplasms (red), diabetes mellitus (blue), Alzheimer disease and other dementias (green) and cardiovascular diseases (purple) in different areas of the world for the year 2015 according to data from the World Health Organization\textsuperscript{164}. One DALY represents the loss of one year of health due to mortality or disability caused by the disease indicated.

Figure 3. Schematic representation of the timing and progression of age-related phenotypes in adult humans, such as loss of bone and muscle mass, gain of abdominal fat and changes in hormone levels. The different coloured lines indicate potential trajectories of functional decline during ageing.

Figure 4. Ageing is characterized by mechanistic hallmarks that contribute to ageing to different extents in different organisms, and in different cell types within an organism. Hallmarks can influence each other both within cells and at a distance. Different interventions into ageing can affect different constellations of hallmarks, and different constellations of hallmarks can contribute to the aetiology of specific age-related phenotypes and diseases.
Studies aiming to identify genes influencing human lifespan initially explored candidate genes related to human age-related diseases or to amelioration of ageing in laboratory animals. After gene array-based technologies became available, genetic variation in the whole genome was explored in linkage studies of longevity families or genome-wide association studies (GWAS) of population-based older cases and younger controls. The selection of cases for these studies was based on longevity threshold criteria, such as survival of individuals to ages above 90 or 100 years or individuals (or their parents) belonging to the top 10% or 1% of survivors in a population\textsuperscript{140,141,144-146}, or a continuous lifespan parameter, such as age at death of individuals or their parents\textsuperscript{142,143,165}. The candidate gene studies revealed only two loci consistently replicated in independent studies; \textit{APOE}, which is also the only genetic locus that shows robust, genome-wide significance across different GWAS (\textbf{Table 1}), whereby the genetic variant responsible for ApoE ε2 (rs7412) has shown to be protective and the one for ApoE ε4 (rs429358) to be deleterious\textsuperscript{166}, and \textit{FOXO3A}\textsuperscript{41,42} (see LongevityMap\textsuperscript{167} for an overview of results from all candidate gene studies). Genomic locations identified in linkage studies showed no overlap between the different studies\textsuperscript{26}. The GWAS, on the other hand, have thus far identified several genetic variants (\textbf{Table 1}), but the majority of these are disease-related variants influencing early mortality, rather than survival to extreme old ages. One of the loci that was observed only in a single large parental longevity study was also found to be associated with a diversity of age-related diseases\textsuperscript{168}, and contained the \textit{CDKN2A/B} genes, involved in cellular senescence, a hallmark of ageing. Unlike the common genetic variants for age-related diseases identified through GWAS, individuals from long-lived families may harbour more rare genetic variants that can currently not be detected by GWAS. Identification of such genetic variants could proceed by whole-genome sequencing of

\textbf{Box 1: Genetic studies into human lifespan variation.}
individuals from sufficiently large longevity families or by a candidate gene approach, which has already resulted in promising findings for two functional genetic variants in the IGF1 receptor$^{38,169}$. 
Box 2: Biomarkers of the physiological state and biological age of individuals

Biomarkers in human research are used to individual variability in the progress of ageing, as risk indicators for monitoring the response to interventions. Biomarkers can monitor the physiological state of individuals, predict the onset and/or progression of age-related disease, indicate the physiological vulnerability of elderly to poor clinical outcomes and predict mortality. Biomarkers of the risk of age-related diseases have been developed with great success. No consensus has yet been reached on biomarkers of biological age, i.e. the mismatch between chronological age and the stage of an individual along the ageing process. These should meet a number of criteria, such as those defined by the American Federation of Ageing Research (AFAR): they should (1) mark the individual stage of ageing and predict mortality better than chronological age, (2) monitor ageing in a range of systems and not the effects of disease and (3) allow longitudinal tracking (for example by blood tests or imaging techniques), in animals and humans\textsuperscript{170}.

Several biomarkers of physiological state have been developed in humans, including indicators of physical capability (e.g. locomotor function, strength, and balance), and physiological (e.g. respiratory and cardiovascular function and lipid and glucose metabolism), cognitive, endocrine (e.g. thyroid hormones, insulin), and immune function (e.g. IL-6 and CRP)\textsuperscript{33,171}. In addition, several multi-marker indicators have been generated based on assays of multi-organ functionality and molecular characteristics. For early phases of life, the Pace of Aging score was generated\textsuperscript{43}. The functional ability of elderly can be tested by questionnaires, such as those looking at the activities of daily living. Physiological vulnerability later in life, i.e. ‘frailty’ at ages above 80 years is generally described by low physical activity, muscle weakness, slowed performance, fatigue or poor endurance and unintentional weight loss.
About 50 different frailty algorithms are available, the ‘frailty phenotype’\textsuperscript{172} and ‘frailty index’\textsuperscript{173} being the most commonly used clinically. More recent indicators of biological age have been based on age-related changes in the transcriptome\textsuperscript{174}, epigenome\textsuperscript{175,176}, metabolome\textsuperscript{177} and structural neuroimaging\textsuperscript{178}. These await systematic testing and comparison with each other and with traditional parameters, in relation to clinical decisions and intervention studies. Different indicators of biological age (telomere shortening, epigenetic clocks and Pace of Ageing) seem to reflect different aspects of physiological decline\textsuperscript{179}. Since long-lasting cohort studies are rich in ageing phenotypes and clinical, imaging and molecular data collected at multiple timepoints, these could allow systematic comparisons and development of a multivariate mix of marker profiles with the strongest predictive power.
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