The quest for genetic determinants of human longevity: challenges and insights

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Abstract | Twin studies show that genetic differences account for about a quarter of the variance in adult human lifespan. Common polymorphisms that have a modest effect on lifespan have been identified in one gene, *APOE*, providing hope that other genetic determinants can be uncovered. However, although variants with substantial beneficial effects have been proposed to exist and several candidates have been put forward, their effects have yet to be confirmed. Human studies of longevity face numerous theoretical and logistical challenges, as the determinants of lifespan are extraordinarily complex. However, large-scale linkage studies of long-lived families, longitudinal candidate-gene association studies and the development of analytical methods provide the potential for future progress.

Cohort
A designated group of individuals who are studied over a time period.

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Human lifespan is of vital importance, both for individuals and society. In the past century, most Western countries have experienced large increases in mean life expectancy, from around 50 years to around 75–80 years. This has been due to a marked reduction in early life mortality during the first half of the twentieth century, followed by a less recognized almost twofold reduction in mortality at ages above 70 in the past 50 years[1]. These changes are rapid on an evolutionary timescale, and suggest that important factors causing variation in lifespan are unlikely to be of genetic origin. However, within a given birth cohort in a given country there is still a large variation in lifespan (FIG. 1). Clarifying to what extent this variation is related to genetic differences among individuals and understanding the roles of specific genetic factors in this variation is central to the understanding of human ageing and lifespan, including exceptionally long lifespan, which is known as longevity. The ultimate aim of this research is to provide targets that can be used in the prevention and treatment of disabilities and diseases that occur with increasing age.

Lifespan is the outcome of complicated processes that might involve thousands of genes and non-genetic factors. Many researchers prefer to study specific diseases that are associated with increased mortality, and some argue that even these outcomes are too broad to study. They suggest instead that the focus should be on intermediate phenotypes that predispose to disease, or on physiological outcomes that vary with age and predict lifespan. However, findings from animal studies have provided evidence that individual genes can have a significant effect on lifespan. Furthermore, human genetic studies have shown that common polymorphisms in one gene — apolipoprotein E (*APOE*) — influence lifespan, probably mainly through their association with disease[2]. This, together with familial recurrence patterns for longevity, has given cause for optimism that it will be possible to identify other genetic variants that affect lifespan. However, although the past few years have seen the identification of many candidate genes for involvement in human lifespan, only the role of *APOE* has so far been consistently confirmed.

Here we provide an overview of the genetics of human lifespan and discuss how the genetic factors that underlie variation in lifespan might be successfully identified in the future. Many challenges face researchers trying to identify genetic variants that are associated with human longevity, and we examine different approaches that can be taken to overcome them, along with a discussion of the most promising candidate genes that have been investigated so far. We begin with a brief overview of the study of longevity in animal models, from which most of our understanding of the biology of longevity has originated, and discuss how this knowledge can be applied to the study of human lifespan.

Theories and insights from model organisms

A short lifespan and the ability to control both environment and genotype have made invertebrates especially useful for studying genetic variants that are associated with longevity[3,4]. A substantial proportion of the variation in invertebrate lifespan is heritable (estimates range from 20% to 50%) under controlled environmental conditions in the laboratory[5], and hundreds of genetic variants that lead to life extension have been identified (see the Science of Aging Knowledge Environment web site and REFS 3,4). Single mutants in *Caenorhabditis elegans* can reduce mortality threefold[6,7] and combinations of variants lead to as much as a sixfold extension in lifespan, increasing to almost eightfold when combined with dietary restriction[8].

Several general facts about the biology of longevity have become clear from animal models (BOX 1): most of the genes involved are pleiotropic, specify increased stress resistance and result in increased robustness in older animals. Animal studies have also revealed a down side to increased lifespan: many long-lived mutants are slow-growing, with reduced fecundity and fertility, and fail to compete in a changing environment[9]. There seem to be direct trade-offs between higher fertility and rapid development on one hand, and increased stress resistance and longer lifespan on the other[10]. Finally, animal studies have revealed that there is a major stochastic component to lifespan, such that genetically identical individuals that are grown in a common environment do not have the same lifespan, which is one reason why the heritability of lifespan is moderate[11,12].

Animal studies have also provided insights into the types of gene that can be involved in the regulation of lifespan. The first longevity mutant to be identified was the *C. elegans* gene *age-1* (REF. 13) that encodes phosphatidylinositol 3-kinase (PI3K) (REF. 14), which has a key role in a signalling pathway that is homologous to the mammalian insulin–IGF1 (insulin-like growth factor 1) pathway (FIG. 2). This pathway ultimately targets the transcription factor DAF-16 (FOXO), which regulates the expression of numerous downstream genes that mediate stress resistance, innate immunity, metabolic processes and toxin degradation[15,16]. Mutations that affect this pathway show notable effects on longevity in both invertebrates and mammals; several mouse longevity mutants alter key components of the insulin–IGF1 pathway, with one of the strongest lines of evidence being the increased lifespan of mice that are heterozygous for the IGF1 receptor knockout[17].

A second large class of life-extension mutants in the nematode affect mitochondrial function, the so-called Mit mutants. Starting with the identification of *clk-1*, and now involving about a hundred distinct loci, numerous Mit mutations result in life extension, typically of 20–40% and sometimes more[3,18]. Many of these mutants interact with the insulin–IGF1 pathway mutants to cause life extension beyond that observed in single-gene mutants alone[3,4].

Longevity genes have also been identified in other animal models. Two key examples are *sir-2* and *Tor* (*Target of rapamycin*), which were identified in yeast and *Drosophila melanogaster*, respectively. *sir-2* encodes an NAD-dependent protein deacetylase, which might mediate the lifespan-extending effects of dietary restriction, whereas *Tor* encodes a protein that is involved in sensing amino-acid availability (FIG. 2). The effects of these genes on lifespan indicate a link between nutrient intake and longevity, and both genes might be involved in the life-extension effects that are mediated by dietary restriction. Other longevity genes, including *methuselah* (*mth*) and *I'm not dead yet* (*Indy*) (originally identified in flies), and klotho (*Kl*) (the only such gene identified first in mice), are the subjects of intensive investigation, although their specific roles in modulating lifespan have yet to be determined.

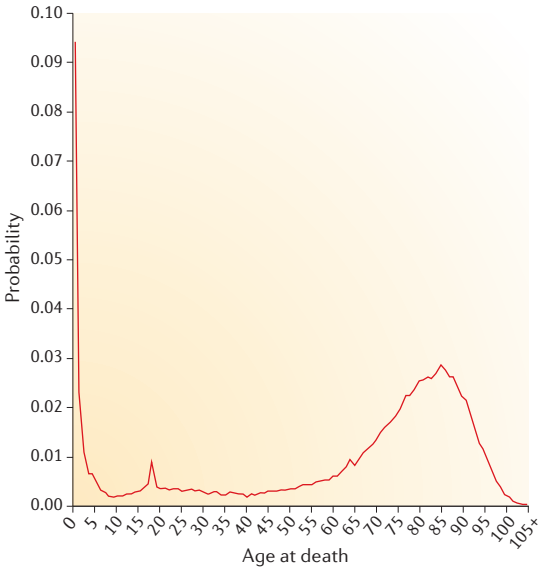

Figure 1 | **Large variation in lifespan within a birth cohort.** The distribution of age at death is shown for a twentieth century Western population that did not experience any world wars — the Swedish female 1900 birth cohort. Data are from The Human Mortality Database.

Caution should be used when investigating human candidate genes that are identified by their orthology to those that have been highlighted by animal studies, as it is unlikely that every longevity gene found in model organisms will correspond to a human longevity locus. For example, in *C. elegans* even disruption of homologues of genes that cause severe human disease, such as frataxin (*FXN*), can result in life extension[19].

Importantly, animal studies have shown that mortality is affected at some ages but not all[7]: the *age-1* mutant reduces late-life mortality as much as 14-fold, but *spe-10* mutants only lower reproductive phase mortality and *clk-1* only affects late-life mortality[7], so human longevity genes might also be age-specific.

In providing these insights, invertebrate studies have motivated the search for human genes that are involved in longevity and have provided candidate genes, while also revealing challenges that must be kept in mind when carrying out these studies.

Pleiotropy
The action of a single gene on two or more distinct phenotypic characters.

Box 1 | Generalities from studies of lifespan-increasing invertebrate genes

- All the longevity genes that have been identified have primary roles in other physiological processes and especially in signal transduction (FIG. 2). It therefore seems that natural selection does not select for genes that cause ageing, but rather ageing occurs as a result of pleiotropic effects of genes that specify other processes[24].
- Most life-extension effects have been found to result from hypomorphic or nullomorphic mutations, which can be interpreted to mean that the wild-type gene shortens lifespan under laboratory conditions. Such genes specify a process that has a negative effect on longevity, and therefore blocking their expression increases longevity. These genes might be called 'gerontogenes' and should be distinguished from 'longevity-assurance genes' for which nullomorphs result in life shortening.
- Where tested, gerontogene mutants show decreased 'fitness' and fail to compete with wild-type animals. These mutants show trade-offs between fitness components such as speed of adaptation to a new environment or fertility schedule[9,10].
- Most longevity mutations also increase the ability to handle stress, such as oxidative stress and starvation[102]. Stress resistance therefore seems to be a public mechanism of ageing[24,102], that is, one that is shared by different species.
- The longest-lived individuals and strains are also the most robust and disease resistant, showing extensions not only of life but also of health (ability to move and react) well into ages at which wild-type controls are dead[3,11].
- Where mortality has been ascertained using populations of several hundred if not thousands of individuals, longevity mutants can alter either or both initial mortality and the slope of the age-dependent increase in mortality[3,6,7], and mutations can affect mortality at some ages, but not at others. All aspects of longevity and mortality seem to be modulated by genes and contribute to variation in longevity.
- Manipulations of more than 100 genes have been found to increase longevity in *Caenorhabditis elegans*. This is in contrast to initial expectations that a few rate-limiting targets modulate ageing[103].

Human lifespan as a heritable trait

Lifespan phenotypes. Studies of life duration can focus on several phenotypes, which are important to understand before considering genetic studies of human lifespan. The most direct measure is individual lifespan, but this can only be studied directly in extinct (or nearly extinct) cohorts, meaning that cohorts should be born at least 100 years ago. Individual lifespan after adolescence is often studied because infant and childhood deaths are likely to have distinct causes (for example, prematurity or congenital malformations). Lifespan researchers are also usually interested in excluding the effects of sex and cohort — that is, the similarity in lifespan for two same-sex twins that arises from having the same sex and year of birth is not the focus. Therefore, the deviation from the sex-specific and cohort-specific mean is often used.

Early deaths (adult deaths before 50–60 years old, depending on the study) are of particular interest because they represent a loss of many years of life and often have significant social consequences. Because of genetic diseases, such as those that are associated with early-onset cardiovascular disease, one might expect a stronger genetic component to early death compared with death at older ages. On the other hand, violent deaths comprise a higher proportion of early deaths than later deaths, which could reduce the genetic component.

Late deaths (after 90–100 years old, depending on the study) are of interest because they could be a marker of successful ageing. The clustering of late deaths in families with many extremely long-living individuals has provided support for a familial component to longevity[20,21]. But is this genetic? On one hand, the accumulation of unique environmental exposures during a long life might be the main determinant of lifespan and health at older ages, predicting decreased heritability at older ages[22]. Alternatively, evolutionary biologists have argued that the reduced selective pressure against deleterious genetic mutations that are expressed only late in life predicts an increase in genetic variance among the oldest[23,24].

Finally, age-specific susceptibility to death, known as frailty, can be studied. Frailty is likely to have a higher heritability than lifespan *per se* as it is more plausible that one inherits a level of susceptibility to death than a fixed lifespan[25,26].

Genetic epidemiology of human lifespan. Twin studies have consistently found that for cohorts born around 100 years ago, approximately 25% of the variation in lifespan is caused by genetic differences[27,28] (FIG. 3a). Recent combined analyses of ~20,000 twins born in Nordic countries between 1870 and 1910 confirm this, but they also show that the genetic influences on lifespan are minimal before the age of 60 and only increase after that age. This finding provides support for the search for genes that affect longevity in humans, especially at advanced ages[29]. The results are comparable in the various Nordic countries, but other settings lack similar data to provide heritability estimates. Countries with larger socio-economic differences might be expected to have lower heritability estimates owing to larger environmental variance.

The genetic contribution to phenotypes other than lifespan *per se* has also been studied. Adoption studies have suggested a genetic component to some causes of premature death. The only large adoption study that has been published shows a correlation between Danish adoptees and their biological parents, especially for death that is due to vascular causes[30]. However, a later extension of this study found smaller effects[31], and twin data[29] indicate that the overall genetic effect on premature death is minimal.

There is also evidence that longevity clusters in some families. Perls and co-workers found that the chances of survival until 80–94 years old for siblings of centenarians were about four times as high as those for siblings of individuals who died at 73 years of age[20] (FIG. 3b), and even higher values were reported later by the same group[32]. In addition, a study that was based on Mormon genealogies found an increased recurrence risk for siblings for surviving to extreme ages, although the estimate was lower than those from the studies by Perls and colleagues[33]. Similarly, an investigation using the population-based genealogy in Iceland found that first-degree relatives (parents, siblings and offspring) of probands who live to extreme old age (≥95 percentile) are twice as likely as controls to survive to the same age[21]. Finally, Schoenmaker *et al.*[34] found mortality rates to be about 30% lower than in the general population for first-degree relatives of exceptionally long-lived siblings in Holland. However, such studies can only provide an upper limit for the genetic influence, because clustering can be due to both genetic factors and a shared family environment.

Recurrence risk
The likelihood that a given condition that is diagnosed in one or more family members will recur in other family members or in subsequent generations.

Proband
A subject who is ascertained on the basis of their phenotype; probands are often used to identify affected families for genetic studies.

In terms of frailty, a study of Nordic twin pairs estimated the heritability of this trait as approximately 50% (REF. 25). Further analyses[1] suggested that about half the variation in lifespan after 30 years old might be due to survival attributes that are fixed for individuals by the time they reach this age; a third to a half of this effect is predicted to be due to genetic factors, and a half to two-thirds to non-genetic survival attributes (related to, for example, socio-economic status or nutritional and disease history). The model indicates that the importance of survival attributes might increase with life expectancy.

The genetic architecture of human longevity

There are definitely many rare mutations that have large negative effects on lifespan, which are best illustrated by segmental progeriod syndromes that mimic premature ageing, such as the monogenic disorders Werner syndrome[35] and Hutchinson–Gilford disease[36,37]. However, the epidemiological studies described above indicate that common genetic variants with large effects on human longevity are unlikely to exist, as revealed by the low recurrence risk for exceptional longevity within families. One important question is whether families that show clustering of exceptional longevity have rare mutations that are unique to the family and that increase their chances of living to very old ages, and whether these mutations tend to be in the same genes in different families that show this type of clustering. Another key question is how many common genetic variants with moderate effects on lifespan might be identified. The strong evidence for effects of common *APOE* variants on lifespan has generated considerable optimism for finding other common variants. However, the genetic architecture of lifespan probably involves many rare variants with small effects, and the complexity of this phenotypic trait is likely to have contributed significantly to the slow progress in this area.

Genetic study designs in longevity research

Challenges to genetic studies of human lifespan. Epidemiological and demographical analyses have identified numerous factors that are associated with survival at all ages. But why some humans live to extreme ages — some even in relatively good health — is largely unknown. One of the most astonishing results from studies of centenarians is how diverse they are[38]. The few environmental factors that have been shown to be associated with extreme survival are avoidance of heavy smoking and severe obesity, and relatively high educational attainment. In addition, American and Japanese studies have indicated that psychological factors are important, helping centenarians to cope with morbidity and disability. Some studies have indicated that centenarians have escaped major diseases[39], but others have shown that centenarians often have multimorbidity[38,40], indicating that there are multiple ways to achieve exceptional longevity. These factors, together with the probable genetic architecture of human longevity, have influenced the approaches that are used to identify genetic variants that affect this trait. Here we discuss the implications of these

Segmental progeroid syndromes
Syndromes that mimic normal ageing and affect multiple organs and tissues.

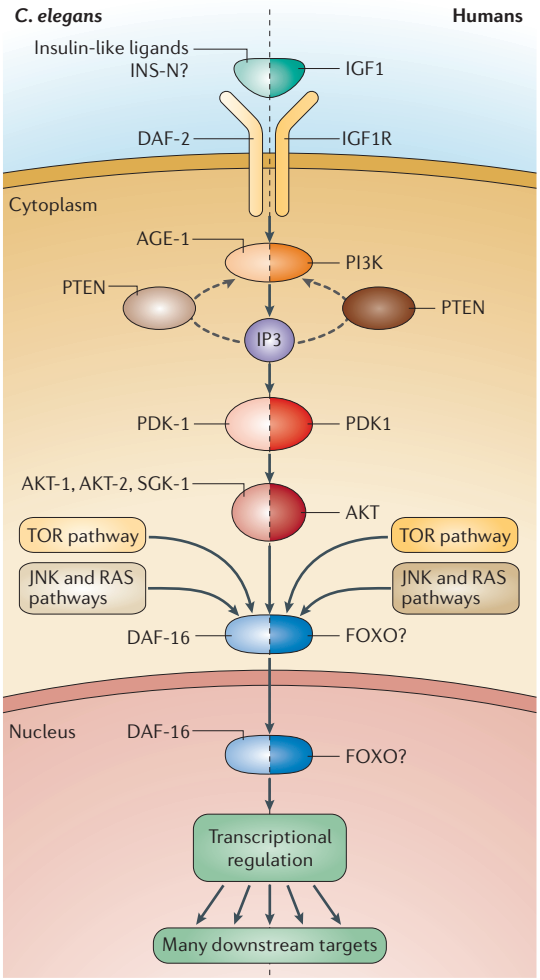

Figure 2 | **Some of the molecular pathways that lengthen lifespan in *Caenorhabditis elegans* and the corresponding components in humans.** The *C. elegans* insulin–IGF-1 (insulin-like growth factor 1) like signal-transduction pathway is shown on the left, and human homologues of the proteins that are involved are shown on the right. This pathway involves a cascade of phosphorylation events that ultimately regulate the nuclear translocation of DAF-16 (REF. 10). INS-N is an unknown insulin-like peptide, and DAF-2 is its cell-surface receptor, which has tyrosine kinase activity. AGE-1 encodes a phosphatidylinositol 3-kinase (PI3K). IP3 is phosphatidylinositol-3,4,5-trisphosphate (for simplicity, it is shown here as part of the pathway, although it is actually a membrane component), which is produced as a result of AGE-1 activity and activates PDK-1. PTEN is a phosphatase with IP3 substrate activity and suppresses AGE-1. PDK-1 is an IP3-dependent kinase that activates AKT-1, AKT-2 and SGK-1, which are serine/threonine kinases. DAF-16 is a forkhead class transcription factor that is homologous to the FOXO class of human transcription factors, and is probably orthologous to human FOXO3A. The target of rapamycin (TOR), JNK and RAS pathways also feed into the insulin-like signalling pathway at the level of DAF-16 regulation. TOR is a kinase that responds to intracellular amino acids, especially leucine, among other activities; RAS and JNK are involved in numerous signal-transduction cascades in mammals. Numerous other genes in which mutations lead to life extension in *C. elegans* (for example, *sir-2* and mitochondrial genes) are not shown.

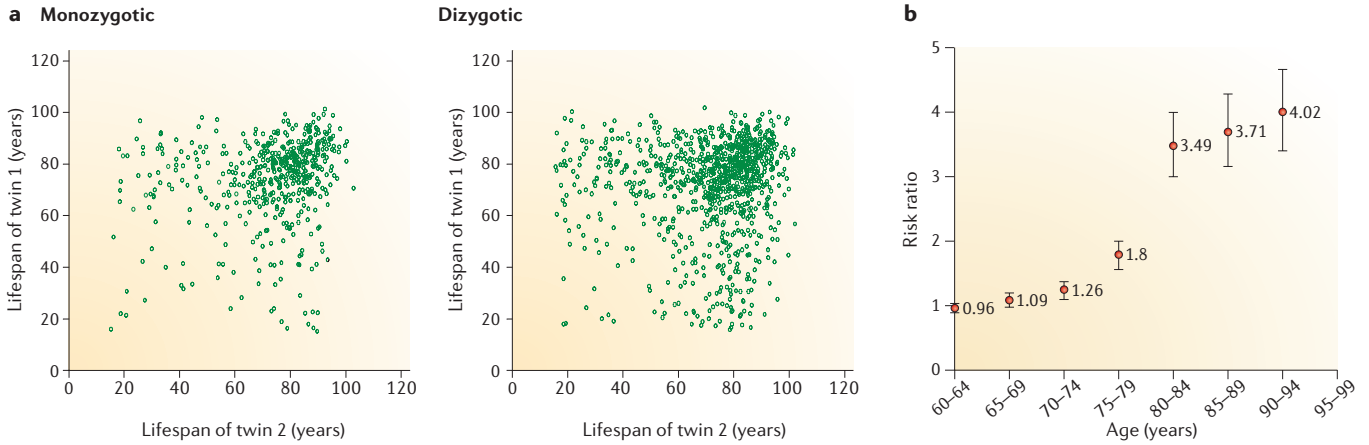

Figure 3 | **Epidemiological evidence for a genetic component to variation in human lifespan. a** | Similarity in lifespan for monozygotic and dizygotic Danish twins of the same sex from cohorts born between 1870 and 1900 and who survived to at least the age of 6. Each dot in the graphs represents a twin pair. The pattern indicates that approximately one-quarter of the variation in lifespan can be attributed to genetic factors. Data are from REF. 27. **b** | Similarity in longevity between siblings in a US population. The relative chance of survival until the age of 60–94 for siblings of centenarians versus siblings of individuals who died at 73 years of age[20].

issues for the various study designs that are commonly used in human genetic studies.

Linkage analysis. Linkage analysis is the traditional means of genetic mapping in humans. In longevity studies, genome-wide linkage scans are often hampered by a lack of availability of multi-generational DNA from long-lived individuals, and by the phenotypic heterogeneity of increased lifespan as a trait. In addition, traditional linkage studies require very large sample sizes to identify genetic regions that are involved in complex multifactorial phenotypes such as lifespan[41]. It has been estimated that mapping a rare, dominant genetic variant that reduces the yearly risk of death by half using non-parametric linkage analysis would require a sample of more than 600 long-lived sibling pairs to ensure acceptable power[42,43]. In the case of recessive genes, the power is greater, and recessive genes with smaller effects can be identified with similar sample sizes. So, although the importance of the power issue might depend heavily on the true genetic architecture of longevity, results from small-scale sib-pair investigations should be interpreted with caution and should be subject to independent replication. Considering these challenges it is not surprising that only few linkage studies have been carried out and that consistent results are lacking[44,45].

Case–control studies. As an alternative to linkage studies, candidate-gene association studies can be carried out to identify genetic determinants of extreme survival by comparing the genotypes of centenarians at specific loci with those of younger cohorts. The advantage of this method over linkage is that variants with small effects can be detected; however, biological knowledge is required to nominate plausible candidate genes.

Another factor that might limit the success of these centenarian studies is a lack of appropriate control groups, as cohort-specific characteristics might confound comparisons between centenarians and younger cohorts. Conclusions that are drawn from such studies (for example, that a genetic variant decreases in frequency with age) are also dependent on a stable population with little migration into or out of the population (that is, no population stratification)[46].

Finally, case–control studies might suffer from publication bias. Many reported associations are often found in subgroups (defined by geographical region or sex), and usually without an *a priori* hypothesis about which polymorphism is advantageous and what the biological basis of this might be. Such results are therefore probably chance findings, and consequently most genetic associations fail to be replicated in independent studies[47].

Longitudinal studies. Longitudinal studies, where a cohort of individuals is followed over time, are less prone than case–control studies to biases that are associated with the selection of controls. However, there are important practical issues that make such studies difficult. A longitudinal study of an elderly cohort until the age of 100 is a logistical challenge, as in order to conduct a longitudinal study of 200 centenarians one must examine 25,000 80-year-olds at baseline or 7,000 90-year-olds, given the current mortality rates in a country like Denmark.

However, Denmark has provided an example of a longitudinal study resource that might provide important insights into the genetics of human longevity. The complete Danish 1905 cohort was assessed in 1998 (REF. 48), when there were 3,600 individuals still alive from this cohort. Of these individuals, 2,262 participated in a survey that included an interview, physical and cognitive tests, and collection of biological material. The participants were 92–93 when they entered the study and intuitively this seems to already be a highly selected population of individuals who are close to becoming centenarians. However, being 92–93 is only half way to becoming a centenarian in terms of selection: only about 1 in 20 of

Linkage analysis
Mapping genes by typing genetic markers in families to identify chromosome regions that are associated with disease or trait values within pedigrees more often than are expected by chance. Such linked regions are more likely to contain a causal genetic variant than other genomic regions.

Non-parametric analysis
Non-parametric approaches are statistical procedures that are not based on models or assumptions pertaining to the distribution of the quantitative trait.

Association studies
Studies in which a genetic variant is genotyped in a population for which phenotypic information is available (such as disease occurrence, or a range of different trait values). If a correlation is observed between genotype and phenotype, there is said to be an association between the variant and the disease or trait.

the 1905 cohort reached 92–93 years old, but only about 1 in 20 of these survivors celebrated their 100th birthday. Hence, this cohort provides a powerful opportunity to study the determinants of survival in the second leg of the long trip to becoming a centenarian.

What has been found so far?

Using the approaches described above, many candidate genes have been investigated for putative associations with human survival or longevity. As well as candidates that have been identified in animal models, the main categories of candidate genes are those that are involved in disease, ageing-related genes (in particular, immune-system-regulating genes)[49,50] and genes that are involved in genome maintenance and repair (in particular, those that are involved in premature ageing syndromes such as Werner syndrome)[35]. As mentioned above, many initially positive findings have not been replicated, probably owing to issues of study design and publication bias. With these issues in mind, some of the most investigated and/or biologically most plausible candidates are discussed below; an extended list of candidate genes that have been investigated is given in TABLE 1.

Cardiovascular genes. *APOE*, which is the only gene with common variants that have consistently been associated with longevity, has an important role in regulating lipoproteins. The protein is found as three isoforms, APOE2, APOE3 and APOE4, which are encoded by different alleles and interact differently with specific lipoprotein receptors that alter circulating levels of cholesterols. *APOE4* has repeatedly been associated with a moderately increased risk of both cardiovascular disease and Alzheimer disease, whereas *APOE2* is protective[46,51,52]. Not only is *APOE4* a risk factor for these diseases *per se*, but *APOE4* carriers are also more susceptible to damage after some environmental exposures. For example, they have an increased risk of chronic brain injury after head trauma[53]. Furthermore, individuals with atherosclerosis, peripheral vascular disease or diabetes mellitus have a substantially higher risk of cognitive decline if they also carry the *APOE4* variant[54].

In contrast to other candidate genes, cross-sectional studies of *APOE* allele frequency differences between age groups have been remarkably consistent. *APOE4* frequency varies considerably between populations of younger adults (about 25% among Finns, 17–20% among Danes and about 10% among French, Italians and Japanese) but in all these populations the frequency among centenarians is about half these values. However, although these changes in *APOE* allele frequency with age are substantial (FIG. 4), they are compatible with a situation in which *APOE2* carriers have an estimated average mortality risk in adulthood that is only 4–12% less than for *APOE3* carriers, and *APOE4* carriers have a risk that is only 10–14% more than for *APOE3* carriers throughout adulthood[55]. This would make *APOE* a 'frailty gene' that slightly influences the yearly mortality rather than a 'longevity gene' that 'ensures' a long life. Similarly, in the longitudinal Danish 1905 cohort, the *APOE* genotype has been shown to have a small but statistically significant effect on the probability of surviving as a well-functioning nonagenarian[56].

Recently, a study of US Ashkenazi Jewish centenarians, their offspring, and Ashkenazi controls showed that the –641C allele in the *APOC3* promoter is present at a higher frequency in centenarians and their offspring compared with controls, and that the –641C homozygote status is associated with a favourable profile for lipoproteins and other cardiovascular risk factors, and with survival[57]. The study has the advantage of being conducted in a relatively homogeneous population, which makes population stratification less likely. However, these results require replication because the study tested associations between 66 polymorphisms (in 36 candidate genes) and numerous cardiovascular-related outcomes.

Another protein that is involved in lipoprotein metabolism, microsomal triglyceride transfer protein (MTTP), has also been implicated in human longevity. A genome-wide linkage scan in long-lived US families provided evidence for a longevity locus on chromosome 4 near the microsatellite marker *D4S1564* (REFS 44), although this observation was not replicated in a French population[45]. Fine mapping of the region identified *MTTP* as the gene that is most likely to be responsible for the linkage peak[45]. Two SNPs have been found to account for most of the variation at the *MTTP* locus[45] and a haplotype that contains both of these was found to have a significantly lower frequency in long-lived individuals compared with a group of younger controls. However, although *MTTP* is an excellent candidate gene as its functions and crucial position in lipoprotein assembly resemble those of APOE, rigorous testing in a large case–control study of German centenarians[58] and a longitudinal follow-up in the Danish 1905 cohort[59] failed to confirm any association of *MTTP* variants with longevity.

Variants in the gene encoding angiotensin I-converting enzyme (ACE) are also biologically plausible candidates for longevity. The cleavage of angiotensin I by ACE produces the octapeptide angiotensin II, which is a potent vasoconstrictor, and polymorphisms in *ACE* have been suggested to be involved in cardiovascular and renal diseases, and in the outcome of physical exercise[60]. The *ACE* genotype has been proposed to be associated with longevity, as a German study found an increased frequency of homozygosity for one *ACE* allele in octogenarians[61], which was to some degree supported in a longitudinal study[62]. This finding could not, however, be confirmed in two large studies of centenarians and younger controls[63,64].

Metabolism-related genes. As described earlier, there is substantial evidence from model organisms that ageing in animals is regulated by an evolutionarily conserved insulin–IGF1 signalling pathway[17] (FIG. 2). Genes that encode components of this pathway are obvious candidates for longevity in humans, but only a few human studies have been reported. The presence of at least one copy of a specific *IGF1R* allele was shown to result in low levels of free-plasma IGF and to be more highly represented among long-lived individuals[65]. The same study also reported that different combinations of *IGF1R* and *PI3KCB* alleles affect

Haplotype
An experimentally determined profile of genetic markers that are present on a single chromosome of a given individual.

free-plasma IGF1 levels and longevity. However, this study was based on a case–control approach and is therefore sensitive to the selection of controls. Van Heemst *et al.*[66] took a different approach, carrying out a longitudinal study of two cohorts of individuals who were at least 85 years of age and assessing various components of the insulin–IGF1 pathway in these cohorts. The study indicated that genetic variation causing reduced insulin–IGF1 activation is beneficial for survival in old age, but this was only found for females. Of the polymorphisms analysed, the association was most pronounced for a SNP in the gene that encodes growth hormone 1 (GH1) but, as emphasized by the authors, this finding needs replication.

Table 1 | **Selected human candidate genes for involvement in lifespan determination**

Gene names or genetic defects (symbol)	Protein function	Phenotype	Populations/ study locations	References
Apolipoprotein E (*APOE*)	Ligand for the LDL receptor	Consistently associated with survival and longevity	Multiple	55
Apolipoprotein C3 (*APOC3*)	Major component of VLDL and chylomicron remnants	Associated with cardiovascular risk factors and longevity in one study	Ashkenazi Jews	57
Microsomal triglyceride transfer protein (*MTTP*)	Transport of triglycerides, cholesteryl esters and phospholipids	Inconsistent linkage and association results for longevity	United States France Germany Denmark	44,104 45 58 59
Cholesteryl ester transfer protein (*CETP*)	Transfer of cholesteryl esters	Inconsistent association results for longevity	Ashkenazi Jews Italy	105 106
Angiotensin I-converting enzyme (*ACE*)	Hydrolyses angiotensin I to angiotensin II	Inconsistent association results for longevity	Germany Denmark	61 62,63
Insulin-like growth factor 1 receptor (*IGF1R*)	Energy-status signalling	One study shows association with longevity	Italy	65
Growth hormone 1 (*GH1*)	Growth hormone (insulin signalling component)	One study shows association with survival at age 85+	Holland	66
Catalase (*CAT*)	Catalyses the decomposition of hydrogen peroxide	No association with survival	Denmark	107
Superoxide dismutases 1 and 2 (*SOD1* and *SOD2*)	Catalyses the breakdown of superoxide radicals	One study shows association with longevity	Italy	99
Heat shock proteins (*HSPA1A* and *HSPA1L*)	Protein folding and transport; immune system functions	Various associations but not replicated	Italy Ireland	108 109
Paraoxonase 1 (*PON1*)	Preserves HDL function and protects LDL from oxidative modification	Inconsistent association results for longevity	Denmark Italy and Ireland	110 111
Interleukin 6 (*IL6*)	An immunoregulatory cytokine	Inconsistent, but longitudinal studies show association with longevity	Denmark Finland	79 80
Hereditary haemochromatosis (*HFE*)	Regulation of iron absorption in the intestine	Inconsistent association results for longevity	Denmark France Italy	67 69 112
Methylenetetrahydrofolate reductase (*MTHFR*)	Re-methylation of homocysteine to methionine	Inconsistent association results for longevity	Denmark Switzerland Multiple Ashkenazi Jews	63 113 114 115
Sirtuin 3 (*SIRT3*)	Unknown	Various associations but not replicated	Italy	116,117
Tumour protein p53 (*TP53*)	Tumour suppressor gene	Inconsistent association results for longevity	Holland Italy	66 118
Transforming growth factor β1 (*TGFB1*)	Regulation of proliferation and differentiation; various other functions	One study shows association with longevity	Italy	119
Klotho (*KL*)	A type-1 membrane protein that is related to β-glucosidases; function is still unclear	Heterozygous survival advantage in two populations	Czech Republic Ashkenazi Jews	120 121
Werner syndrome (*WRN*)	Maintenance and repair of DNA; DNA replication	One study shows association with age but not replicated	Mexico and Finland Holland	122 90
mutL homologue 1 (*MLH1*)	DNA mismatch repair enzyme	One study shows association with age	Korea	123
Mitochondrial mutations (Mt5178A, Mt8414T, Mt3010A and J haplotype)	Mitochondrial energy production	Single studies or inconsistent association results for longevity	Japan Italy	81 82–84

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

Phlebotomy
A procedure that involves puncturing a vein to withdraw blood.

Another excellent candidate for a metabolism-related gene that is involved in genetic variation in human lifespan is *HFE*, the gene that is mutated in hereditary haemochromatosis, which is a disorder of iron absorption. In northern Europe, 10–15% of the population are carriers of *HFE* mutations. Initial findings showed that in populations with high carrier frequencies there is an age-related reduction in the frequency of heterozygosity for the most common *HFE* mutation, Cys282Tyr, indicating that carrier status is associated with shorter life expectancy[67]. However, enthusiasm was dampened by a large study that concluded that the clinical penetrance of haemochromatosis for this variant, even when homozygous, was less than 1% (REF. 68). Consistent with this finding, a case–control study of 492 French centenarians[69] showed that patients with mild haemochromatosis seemed to be able to survive into old age without overt symptoms. New data have, however, indicated stronger clinical penetrance for homozygosity[70]. This has revived interest in assessing the potentially small effect of the heterozygous state, which might still be considerable on a population level owing to the high frequency of the mutation in many populations. The mutation is of special interest because of the existence of an effective prevention of the clinical manifestations of the mutation, namely phlebotomy.

Immune system genes. Chronic, low-grade inflammation has been suggested to have a central role in ageing[49,50] and is implicated in the pathology of several age-related diseases, leading to increased mortality[71,72]. The multifunctional cytokine interleukin 6 (IL6) is central to this inflammation, and is overexpressed in many of the stress-related conditions that are characteristic features of ageing, such as rheumatoid arthritis, osteoporosis, Alzheimer disease, cardiovascular diseases and type 2 diabetes, linking IL6 overexpression with increased functional decline and mortality[72–74].

Twin studies have shown that inter-individual variation in IL6 expression has a substantial genetic component[75,76]. Three SNPs and an AT stretch polymorphism have been identified in the *IL6* promoter, and the potential significance of one of these polymorphisms (–174G/C) for both IL6 levels and disease susceptibility has been investigated in a large number of studies of a range of diseases. The results have been conflicting[77,78], but recent independent findings of a modest, but significant, increase with age in the frequency of *IL6* –174G homozygotes indicates that this genotype is associated with longevity — a finding that is currently being investigated further in larger populations[79,80].

Mitochondrial mutations. Variants in mitochondrial DNA (mtDNA) are among the mostly highly favoured candidates for genetic factors that are associated with longevity, as mitochondria have a central role in the oxygen free-radical production — an important factor in ageing processes. A Japanese study found three mtDNA mutations to be more common among centenarians than controls, which was based on sequencing the entire mitochondrial genome in 11 centenarians and 43 controls[81]. In addition, an association between longevity and the C150T mutation in the replication control region of leukocytic mtDNA was found in an Italian study[82]. Another study of inherited mtDNA markers in a case–control study of Italian centenarians and younger controls found all nine of the typical European haplotypes in both age groups[83]. A higher frequency of one haplotype was found in centenarians, but only in the male group from a specific region, and a later Italian study failed to confirm this finding[84].

Premature ageing syndrome genes. Werner syndrome is an autosomal recessive disease that is caused by a loss-of-function mutation in the *WRN* gene, which encodes a member of the RecQ family of helicases[35]. The condition is characterized by the early onset of skin wrinkling, hair greying, cataracts, diabetes and osteoporosis, resembling normal ageing, as well as a higher prevalence of early cancer. Most patients with Werner syndrome die before reaching 50 years of age. The exact molecular mechanisms that lead to the clinical features in Werner syndrome remain to be defined, but available evidence indicates an important role for WRN in several aspects of DNA repair[85].

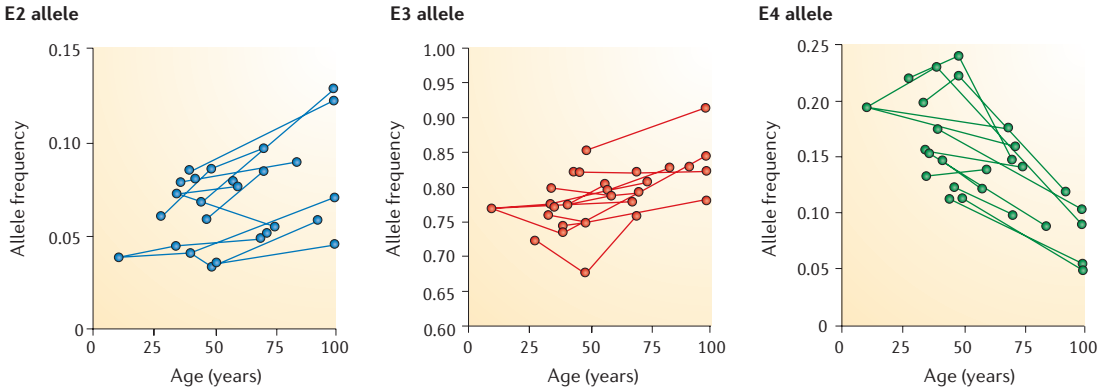

Figure 4 | **The frequencies of apolipoprotein E alleles vary with age.** Frequencies of the three common apolipoprotein E (*APOE*) alleles — E2, E3 and E4 — are shown, taken from data in 13 published studies[55]. Each line connects the frequencies in various age-groups within a given population. Reproduced with permission from REF. 55 © (2000) Wiley-Liss.

It has been suggested that minor deficiencies in WRN function might have a role in ageing in the general population[86], and there are several polymorphisms that might affect the function of this protein[87]. Several groups have carried out case–control studies that compared the frequency of WRN SNPs between patients with age-related diseases and healthy controls. In a Japanese population, an association between a Cys1367Arg variation in the *WRN* gene and atherosclerotic disease was found[88], but this could not be confirmed in Caucasians[85,86].

Box 2 | **Using compositional dynamics to identify variants that are associated with longevity**

If an individual has a particular polymorphism, this is a fixed genetic factor; however, the proportion of surviving individuals who have the polymorphism will increase if the variant enhances survival. This compositional change provides the information that is needed to determine whether an allele increases longevity. Some simple mathematics clarifies the compositional dynamics.

Let N be the number of individuals in a population at a particular age, such as 50 years old. Let π be the proportion of these individuals who have a specific genotype, which could be a particular allele or a set of polymorphisms. Let s be the chance that an individual with this genotype will survive to a specific advanced age, such as 100 years old. The number of individuals with the genotype at the advanced age is $N\pi s$. Let Π be the proportion of individuals who have the genotype at the advanced age. In terms of Π the number of individuals with the genotype at the advanced age is $\Pi N\bar{s}$, where $\bar{s}$ is the probability of surviving to the advanced age among the entire population of N people. Therefore, $N\pi s = \Pi N\bar{s}$. Simplifying and rearranging this equation leads to the key relationship in equation 1.

$$\Pi = \pi \frac{s}{\bar{s}} \qquad (1)$$

Suppose that 1 person in 100 survives from 50 to 100 years old but that 1 person in 10 with the longevity genotype does so. If the proportion of people with this genotype at 50 years old is 2%, then the proportion at 100 years old will be 20%. It is this kind of enrichment that allows the detection of longevity genotypes.

To estimate s using data on proportions of people with a genotype at two ages and demographical data on survival chances, equation 1 can be re-expressed as equation 2.

$$s = \bar{s} \frac{\Pi}{\pi} \qquad (2)$$

If 5% of individuals have the genotype at 60 years old and 15% at 90 years old, and if the chance of surviving from 60 to 90 is 20% (as it was for Danish women born in 1905), then people with the genotype have a 60% chance of surviving from 60 to 90.

Let S be the probability of surviving from some age to a later age for people without the genotype of interest. Then equation 2 can be rewritten as equation 3.

$$S = \bar{s} \frac{1 - \Pi}{1 - \pi} \qquad (3)$$

Here the proportion of people who do not have the genotype is one minus the proportion who do. Because the population is made up of people with and without the genotype equation 4 can be used.

$$\bar{s} = \pi s + (1 - \pi)S \qquad (4)$$

If age-specific death rates for people with the genotype are a factor R of the rates for other people, then s and S are related by equation 5.

$$s = S^R \qquad (5)$$

Suppose that R is 50%. If 1 person in 100 without the genotype survives from 50 to 100 years old, then 1 person in 10 with the genotype will survive.

As indicated by equation 4, for unusual genotypes, S can be approximated by $\bar{s}$. In this case, combining equations 1 and 5 yields equation 6.

$$\Pi \approx \pi \bar{s}^{R-1} \qquad (6)$$

In the table this approximation is used to show how radically the proportion of individuals with the genotype increases with age. The probabilities of survival to various ages are those that prevailed for Danish women born in 1905.

Age	Probability of survival to this age (%)	Probability (%) that an individual has the genotype of interest at a relative risk R		
		$R = 0.90$	$R = 0.50$	$R = 0.25$
50	100	1	1	1
82	50	1.1	1.4	1.7
93	10	1.3	3.2	5.6
100	1	1.6	10	31.6

In addition, in a case–control study (where the SNP frequencies of very old individuals are compared with younger controls) an increased frequency of the 1074Leu allele in Finnish and Mexican elderly populations[89] was identified, but other studies have failed to replicate these findings[90].

Telomere length. The consistent findings of a negative correlation between telomere length and replicative potential of cultured cells, and of decreasing telomere length with age in a number of different human tissues[91], have led to the suggestion that telomeres have a role in cellular ageing *in vivo*, and ultimately in organismal ageing. In *C. elegans*, increased lifespan has been found in worms with long telomeres[92]; in mice, knocking out the telomerase gene had no obvious effect for several generations[93], although this could be due to the extremely long telomeres in the mouse strain that was used.

Telomere length varies among individuals of the same age, and a possible association between telomere length and mortality late in life in humans has been suggested[94]. This study measured telomere length in blood samples that were drawn about 20 years previously from 147 healthy individuals aged 60–97 years. Corrected for age, those individuals with shorter telomeres showed poorer survival than those with longer telomeres. However, a larger independent study has failed to confirm this finding, and showed that telomere measurements fluctuate over time in blood cells[95]. Furthermore, another investigation followed a large sample of elderly twins and singletons and also found that telomere length is not a predictor for remaining lifespan once age is controlled for[96]. This sample provided a unique opportunity to carry out intra-pair comparisons among twins, where genetic effects are controlled for (100% for monozygotic and 50% for dizygotic pairs). However, this comparison also failed to reveal evidence for an association between telomere length and survival among the elderly.

Guidelines for future studies

There are several possible reasons why most of the studies described above have been inconclusive: the probable involvement of numerous genes with small effects and the implementation of small-scale studies, often with cross-sectional designs, are features that will produce many chance findings. Another reason for the inconclusiveness of these studies might be that different variants are involved in lifespan variation in different populations. Bearing these factors in mind, we provide guidelines that we hope will help future studies to provide more conclusive results.

What and whom to study. The genes that influence longevity are those with allelic variants that increase the chance of survival to and at older ages. Therefore, the key information needed to uncover such genes relates to the chances of survival to advanced ages for individuals with or without the alleles of interest (BOX 2). Because many genetic and non-genetic factors affect survival, it is crucial to gather data on and statistically control for the influence of as many of these factors as possible. Most important is information about a subject's age. Because many people live to 80 but only a fraction of them survive past 90 and even fewer to 100, nonagenarians and centenarians are particularly informative about longevity genes. Age misreporting after the age of 90, however, is so widespread in many populations that special efforts might have to be made to validate age[97].

In addition to reliable information on age, many other personal, social and medical characteristics are also informative. These include sex, marital status, place of birth and subsequent residence, educational achievement, occupation, socio-economic status, health behaviour (especially smoking patterns) and history of disease and disablity. Furthermore, all known genetic risk factors should be controlled for in estimating a person's chance of survival to his or her current age or age at death.

This information is also pertinent in choosing candidates for study. Particularly informative would be a male who smoked heavily all his life, who was the eighth child of a poor family in a country with low life expectancy, who left school after 6 years and who has two copies of the deleterious *APOE4* allele, but who is alive and relatively healthy at the age of 102. Such a person would be far more informative than a woman who has never smoked, was the only child of an upper-middle-class Swedish family, became a professor, has two copies of the protective *APOE2* allele and died at 85.

Use of demographical data. For many populations, reliable, long-term time-series of demographical data are available on death rates by age, sex and sometimes other characteristics. If a group that is being studied to find longevity genes is a representative sample of the larger population, then the basic pattern of age-specific survival can be determined from the demographical data. The sample data can then be used to estimate deviations from the general pattern that result from specific genetic variants. This use of demographical data can greatly increase the statistical power of survival studies and reduce the required sample size substantially[98,99]. Nonetheless, it must be emphasized that large sample sizes, with many hundreds and preferably thousands or even tens of thousands of individuals might be needed to uncover alleles that occur in only a few per cent of a population and that only have a modest effect on survival.

Unobserved heterogeneity. Even in a very large study with extensive data on the genetic and non-genetic characteristics of many individuals, some important genetic and non-genetic characteristics will not be observed, either because it is too difficult or expensive to gather the data or because the characteristics are not yet known to be important. If the effect of such hidden heterogeneity is ignored, then statistical estimates of the impact of genetic and non-genetic longevity factors will tend to be biased towards zero and interpretation of the results might be erroneous. This bias will tend to increase with age, as it is well known from epidemiology

Genome-wide association studies
Association studies in which variants across the entire genome are tested for association with a trait of interest. To reduce the amount of genoptying, such studies generally make use of proxy markers (usually SNPs), which, by virtue of falling into blocks of linkage disequilibrium, also provide information about other variants.

and demography that risk factors that affect survival seem to diminish in importance with age. There might be genetic and environmental interactions at older ages that are different from those at younger ages and observed changes in the importance of risk factors might reflect this. The observed changes, however, also at least partially reflect the effect of differential survival of those most resistant to unobserved risk factors. The effect of smoking, for example, seems to become less important and even insignificant at the oldest ages[48]. At least part of the explanation, however, must be that people who smoke and who survive to the age of 80 have compensating genetic or non-genetic factors that make them less sensitive to the hazards of smoking. Similarly, the observed reduction of the harmful effects of the *APOE4* allele among nonagenarians[56] and centenarians is probably, at least in part, an artefact: people with the *APOE4* allele who survive to advanced old age probably have other genes or traits that are protective. Therefore it is crucial to use modern methods of survival analysis and frailty modelling that statistically reduce the effect of unobserved heterogeneity[98–101].

Outlook: the future of human longevity genetics

Although there are many biologically plausible candidates for genes that influence human lifespan, only one finding has so far been replicated. It is hoped that better study designs and analyses that follow guidelines such as those described above will provide more replications in the future.

Large-scale and carefully designed studies will be essential for progress in genetic studies of human longevity. Large international collaborations have recently been established in the European Union (the Genetics of Healthy Ageing project and GenomEUtwin) and the United States (the Long Life Family Study) to identify genetic and non-genetic factors of importance for exceptional longevity. These studies assess long-lived siblings and controls, and some of these also include intermediate phenotypes such as cardiovascular risk factors in their offspring. Such family studies, as well as large cohorts of elderly people who are followed longitudinally, are promising resources for longevity research. These studies are most promising when combined with the use of high-throughput genotyping techniques that make multi-locus analysis (of haplotypes and gene–gene interactions) and genome-wide association studies feasible. Genome-wide association studies have the advantage that they do not depend on biologically plausible candidate genes or knowledge of specific variants.

Large-scale studies are logistically and financially demanding. However, the reason why some humans live to extreme ages are largely unknown, and only a few genetic and environmental factors have been identified. Understanding the genetic basis for longevity is an extraordinarily difficult task, but it has the potential to provide insights into central mechanisms of ageing and disease, which are ultimately hoped to provide targets for the prevention and treatment of late-life disabilities and diseases.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ACE | *age-1* | *APOE* | *clk-1* | *FXN* | IGF1 | IL6 | *Kl* | *mth* | MTTP | *Tor*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer disease | haemochromatosis | Hutchinson–Gilford disease | Werner syndrome

FURTHER INFORMATION

Genetics of Healthy Ageing (GEHA) project web site: http://www.geha.unibo.it
GenomEUtwin: http://www.genomeutwin.org/partners.htm
Long Life Family Study: http://www.biostat.wustl.edu/llfs
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