Genomics of longevity: recent insights from research on centenarians

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ABSTRACT
An ever larger portion of the world population survives into advanced old age, and that has been a steady trend for the last century. Despite some substantial advances in our understanding of the genomic basis of ageing in recent years, healthy ageing remains an increasingly important social task. Initially established in model organisms, many human orthologue genes and pathways associated with longevity have been identified. Ageing is a result from the declining ability of the human organism to maintain homeostatic balance and to regenerate damaged cells and tissues, and is the main risk factor for the prevalent diseases in developed countries. It could be imagined that the genome of very old individuals is purged of disease-causing variants, but recent studies have demonstrated that long-lived individuals carry the same number of disease alleles as young controls. Longevity may however also be explained by the presence of protective genetic factors against age-related phenotypes and diseases, and there is a pertinent need for these to be identified. Centenarians are the extreme phenotype of human longevity and their genomes undoubtedly contain clues about genes and pathways that are involved in longevity. Understanding the genomic basis of ageing, together with knowledge of population ecology and insights from evolutionary biology, will shed light on the biological mechanisms underlying human longevity and hence on the potential of extending healthy human life span.

ARTICLE HISTORY
Received 8 December 2017
Accepted 2 October 2018

KEYWORDS
Centenarians; longevity; genome

Background
Among all distinguishable phenotypes, longevity and healthy ageing are some of the most complex ones [1]. Attaining these desirable phenotypes has also traditionally been a principal challenge of biology and medicine. The dramatic increase in average life expectancy during the 20th century and the consequent steady ageing of the world population, especially in the developed countries [1], has made healthy ageing a pertinent issue that needs a solution. While an understanding of lifestyle and environmental factors will expand our ability to prevent disease and boost the health status in the general population [2], studying the genetic basis of longevity and healthy ageing in exceptionally long-lived individuals offers the potential to illuminate the biology of human ageing [3]. Centenarians are the extreme phenotype of human longevity and can thus be considered the ‘golden standard’ of healthy ageing [3,4].

Ageing comes about as a result of the declining ability of the human organism to maintain homeostatic balance and to regenerate damaged cells and tissues, and is the main risk factor for the prevalent diseases in developed countries, i.e. cancer, cardiovascular disease (CVD) and neurodegeneration [5]. It has also recently been shown that genetic variants associated with these diseases tend to negatively correlate with longevity [6]. The consistent association between processes involved in ageing and the causes of age-related disease suggests that it should be possible to modulate these processes to improve the cellular and system environment of older individuals and thereupon bring about health improvements.

Interestingly, several recent studies have demonstrated that long-lived individuals carry the same number of disease risk alleles as young controls [7–9]. Longevity thus seems not to be compromised by the cumulative effect of risk alleles for common diseases [7]. Longevity may however also be explained by the presence of protective genetic factors against age-related phenotypes and diseases that contribute to population mortality. Recent research has shown that...
multiple protective factors seem to be influencing lifespan and longevity in humans, e.g. with prominent roles for cardiovascular-related pathways [10].

Demonstrating consistent associations between genomic variants and longevity has been elusive [1]. One reason might be that human lifespan is determined by genes whose function is still not fully understood. Also, for age-related diseases, as for longevity itself, there are strong grounds to expect a highly polygenic mix of largely unselected genetic variation that affects an array of complex traits [11]. On the other hand, if genetic variants contribute strongly to extreme longevity, they would be rare since the odds of living to such age are exceedingly small. These considerations offer a possible explanation why even large population-based studies investigating genetic influence on human lifespan have been disappointing, identifying only a few genes accounting for genetic susceptibility to longevity [12]. The recently employed genome-wide analyses (GWASs) have nevertheless identified many genes positively associated with longevity. Still, very few of these findings are significant on genome-wide level, or have been successfully replicated across different populations [7,9,13].

Nevertheless, longevity seems to have a discernible genetic component as demonstrated by studies on long-lived human twins and centenarian siblings. Of the overall variation in adult lifespan approximately 20–30% is accounted for by genetic factors [14–16]. As individuals get older, however, this genetic component becomes increasingly important [17]. The genetic influences on lifespan are minimal prior to age 60 but increase thereafter [15], and the heritability of longevity becomes a key factor after the oldest fifth percentiles of survival [15,18], e.g. for centenarians. The genetic influence is most prominent in families in which survival to high ages clusters [19], and studies of cohorts of family members have been initiated in the hope of finding protective mechanisms delaying the ageing-related phenotypes and disease onset in outstandingly old subjects [20,21].

**Pathways and genes associated with longevity: a brief overview**

Despite yielding inconsistent results, population studies on genetic factors associated with longevity have identified no fewer than a few dozen candidate human longevity genes. These encode a vast variety of cellular functions and they seem to be part of at least four broad physiological processes: metabolic control, resistance to stress, gene dysregulation and genetic stability. More specifically, the genes considered to contribute to longevity are genes preventing or delaying cellular senescence and the consequent ageing of the organism, and seem to be genes involved in DNA repair [22], telomere conservation [23,24], heat shock response [25] and the management of free radicals’ levels [26].

Most genetic variants seem to have very limited effect on longevity, and only their cumulative effect can give a consistently appreciable effect [26]. Thus a limitation of previous analyses may have been that they have mainly been searching for single mutations instead of cumulative effects (reviewed in Ref. [16]). Genome-wide association studies (GWASs) are in this respect the optimal design for genetic architecture that includes many common causal loci of small effect (a polygenic architecture). Here, we briefly present some of the pathways that were demonstrated to be involved in ageing, as well as genes that have repeatedly shown association with longevity and the vast majority of which have been replicated in GWASs.

**IIS**

Nutrient-sensing pathways promote growth during development and contribute to fecundity, while their down-regulation can increase the lifespan in yeast, *Caenorhabditis elegans* and mice [27]. Lifespan has repeatedly been shown to be influenced by calorie restriction, a process which induces the expression of nitric oxide synthase (eNOS) [28], which in turn is regulated by the insulin-like-growth-factor-1/insulin signal (IIS) [29,30]. However, IIS is not only modulated by calorie restriction and physical exercise; genetic manipulation of IIS has also been shown to have profound impact on the rate of ageing [30]. Individuals who maintain high insulin sensitivity are less at risk of CVD, and centenarians have been shown to maintain high insulin sensitivity [31]. Mutations impairing insulin/IGF-1 signalling have been found in centenarians in a number of studies. Such are for example variants in the AKT gene, found in three different centenarian studies [32], and FOXO3A variants present in centenarians from not less than seven different human populations [33–36]. FOXO3A is also a tumour suppressor gene, the protein produced by this gene likely triggering apoptosis [37]. The association of FOXO3A variants with longevity is reportedly stronger in people aged ≥ 95 years and particularly in centenarians [36,37]. Variants of FOXO3A and FOXO1, another transcription factor regulating the insulin/PI3K/AKT signalling, have also been found in nonagenarians, albeit with a lower
frequency compared to the centenarians [34,35]. Owing to its composite function, variants of the FOXO3A gene seem to show principal association with general health into old age and hence extreme longevity, in contrast to other genes reviewed here that appear to be specific to disease susceptibility. The effect of FOXO3A variants has also been shown not to be sex-specific [37].

Target of rapamycin (TOR) signalling, similar to insulin/IGF-1 signalling, is also a nutrient-sensing pathway [38]. Intertwined with IIS, it is another evolutionary conserved pathway that has been shown to play a role in modulating the rate of ageing in different eukaryotic organisms [15]. Inhibition of this pathway through either genetic manipulation or by direct administration of rapamycin has been demonstrated to be effective in extending the lifespan in mice [27]. A study on centenarians has also shown that cellular processes implicated in ageing are rapamycin targets [39]. This pathway is however activated through different nutrient sensors, and its signalling inhibition seems to cause life extension independently of IIS [40]. Currently available evidence indicates that rapamycin extends lifespan by suppressing cancers [41].

Downregulation of components within these nutrient-sensing pathways causes a physiological shift towards cell protection and tissue maintenance [42], testifying the crucial role nutrition plays in modulating life span. It has long been known that dietary restriction is one of the undisputed factors contributing to an increased life span and postponement of age-related disease in metazoans [43]. Experiments with different diets in rhesus monkeys have also shown that dietary restriction delays disease onset and extends healthy lifespan [44].

**APOE**

Apolipoprotein E (APOE) is a major cholesterol carrier that is engaged in lipid metabolism and injury repair in the brain [45]. The ε4 allele of the APOE gene has been associated with both Alzheimer disease (AD) and CVD [46]. The associations with AD and CVD may be related to inflammatory reactions, elevated lipid levels and oxidative stress [1,46,47]. GWASs have shown that the ε4 allele of the APOE gene is depleted in centenarians [9,13,47]. The ε2 allele of this gene has been shown to be associated with an increased likelihood of longevity in Italian and Japanese cohorts [48]. SNPs near the APOE locus are the only variants to have attained genome-wide significance in GWA studies of longevity [13,49,50]. The effect of rare variants on longevity may also be important [50], and further studies are needed to elucidate the role of rare APOE variants on longevity and healthy ageing. A GWAS meta-analysis identified the TOMM40/APOE/APOC1 locus as being significantly associated with reaching ≥90 years of age [13].

Only variants of APOE and FOXO3A genes have consistently been shown to be associated with longevity. The heritability accounted by variants in these two genes, however, is only a small portion of the genetic contribution to longevity measured by family heritability studies [14,51]. Therefore, much of the heritability of lifespan remains to be explained.

**APOC3**

APOC3 is a very low-density lipoprotein (VLDL) involved in fat metabolism [52]. A higher frequency of a 5’-UTR APOC3 variant in elderly people was demonstrated in a Russian population [53]. Another variant in the promoter (rs2542052) of the same gene was found to be associated with longevity in a study of the Ashkenazi Jewish population [54]. In this study, the homozygote frequency of this polymorphism was highest in centenarians.

**CHRNA3/5**

The protein encoded by CHRNA3/5 is a subunit of nicotinic acetylcholine receptors (nAchR). It is associated with traits linked to smoking behaviour: nicotine dependence, lung cancer, chronic obstructive pulmonary disease and schizophrenia [10,55]. In a GWAS of parental longevity, the variant rs1317286 was associated with having a centenarian parent [10]. In an another GWAS, the lead SNP rs8042849 was significantly associated with longevity [55].

**Lp(a)**

This gene encodes Lp(a), a risk factor for atherosclerotic diseases such as coronary heart disease and stroke. The lead SNP rs55730499 is significantly associated with longevity at a genome-wide level [55], in line with previous results [10] that rs55730499 influences mortality by affecting susceptibility to cardiovascular events, mediated by increasing Lp(a) levels and susceptibility to cardiovascular events.

**HLA-DQA1/DRB1**

The HLA-DQA1/DRB1 complex is part of the large major histocompatibility complex (MHC). MHC class II
genes encode components of the antigen-presenting apparatus and are the most polymorphic region of the human genome. Genes within the MHC have previously been associated with many autoimmune conditions and other traits, including psoriasis [55], rheumatoid arthritis [56] and multiple sclerosis [57]. Lead SNP rs34831921, associated with smoking behaviour, cardio-metabolism, rheumatoid arthritis and Crohn’s disease, is coupled with survival at genome-wide significance [55]. A study on Sardinian centenarians suggests that \( \text{HLA-DQA1/DRB1} \) haplotypes might have a role in determining life span expectancy and longevity [58].

\[ \text{SH2B3} \]

The \( \text{SH2B3} \) gene encodes a lymphocyte adapter protein (LNK), a regulator in signalling pathways relating to inflammation. Variants in this locus have been associated with a variety of conditions, e.g. rheumatoid arthritis, type 2 diabetes, coronary artery disease, blood pressure and cholesterol levels [59]. In a GWAS study of centenarians, the SNP rs3184504 was found to be enriched in this cohort and having a possible protective function against lung and pancreatic cancer, coronary artery disease, rheumatoid arthritis and diastolic blood pressure [6]. The association of this SNP with longevity has recently been validated [55].

\[ \text{CETP} \]

The gene encoding the cholesteryl ester transfer protein (\( \text{CETP} \)), which is involved in the regulation of high-density lipoprotein levels, has been shown to be associated with exceptional human longevity [60]. CETP polymorphisms confer genetic contribution to centenarians in a Chinese study [61]. The SNP rs5882 of this gene has repeatedly been found to correlate with longevity [62,63].

\[ \text{hTERT} \]

The human telomerase reverse transcriptase (\( \text{hTERT} \)) gene is enriched in centenarians and associated with longer telomere length in a study of Ashkenazi Jews [23]. Longer telomeres are also associated with protection from age-related diseases, better cognitive function and lipid profiles of healthy ageing [23]. A polymorphism of the \( \text{hTERT} \) gene is known to be associated with mortality and chronic morbidity at old age [64]. In a recent GWA study, it was demonstrated that this gene also plays a critical role in ageing by regulating the DNA methylation clock [65].

**Additional considerations on the genomics of ageing**

**Evolutionary perspective of ageing**

Evolutionary understanding has helped illuminate important features of the human life history [66]. However, unlike many other biological processes, the genetic factors that influence ageing may not be under strong selection because wild animals usually die from extrinsic factors, e.g. predation and infection, not ageing. Senescence might then occur as a result of accumulating mutations that would lower fitness at later ages. Surprisingly, however, genetic modulators of ageing rate seem to have been conserved across a wide range of organisms [67]. As reviewed in Ref. [67], among these there are mediators between environmental and physiological factors (i.e. temperature, nutrient status and oxygen availability), and growth and reproduction. In this way, organisms under adverse conditions have the ability to invest in somatic maintenance instead of reproduction [66]. Thus, evolution has provided a choice, depending on the quality of the environment, for life to either allocate its limited resources towards reproducing timely, but then age more quickly, or delay reproduction and allocate resources towards somatic maintenance thereby ageing more slowly [67]. Related to this, dietary (or caloric) restrictions have also been shown to extend the lifespan across a wide diversity of organisms [68].

Another plausible mechanism, not mutually exclusive to what was mentioned above, is that natural selection favours variants that increase fitness at a younger age but at the cost of a higher subsequent rate of ageing (pleiotropy). These could for example also be variants encoding key developmental processes essential for growth and reproduction that are necessary early in life, but their continuous activity is detrimental later in life leading to many of the pathological conditions associated with old age [69]. An example of such a pathway is the insulin-like signaling (IIS)/mechanistic target of rapamycin (mTOR) that seems to be conserved across a diverse spectrum of taxa. As discussed above, reduced activity of this nutrient-sensing pathway is also one of the few physiological mechanisms repeatedly shown to be associated with longevity in humans [70].

Nonetheless, longevity is a quantitative trait that is determined by a multitude of genes, and their effect is kept modest by the declined strength of natural
selection. This would complicate the detection of genetic variants that determine longevity at the genome-wide level. Also, as only a small proportion of individuals reach extreme longevity, sample sizes from these cohorts are inevitably small and genetic variants contributing strongly to extreme longevity should be rare. These considerations could explain why GWAS have often failed to demonstrate significant association between genetic variants and longevity.

**Physiological age**

Just as physiological age serves as an indicator of an individual’s general health status, it also gives indication of the remaining healthy lifespan [71]. Physical and cognitive functioning, in particular, besides making essential contributions to quality of life, have been shown to be more strongly associated with survival in the oldest individuals than presence or absence of disease [72].

**Epistasis**

Epistasis, i.e. the modification of the effect of an allele of one gene by that of another gene, can easily complicate efforts to understand the genetic basis and heritability of longevity [73]. Recently, Fuku et al. [74] found evidence of epistatic effects among FOXO3A and APOE, the two genes that have repeatedly and consistently been shown to be associated with longevity. Interactions among mitochondrial and nuclear genes, via e.g. imbalance in cellular energy homeostasis and increased vulnerability to oxidative stress, is likely to affect cellular senescence and ageing [75]. Epistasis might thus, at least partially, explain the missing heritability of this complex phenotype.

**Epigenetic effects**

In addition to other mechanisms, suppressing disease-related genes in individuals that reach advanced age is also likely achieved by epigenetic modifications as they are known to play a crucial role in human disease gene regulation [76]. Recently, epigenetic in situ studies [77] have shown that epigenetic modifications are very sensitive to the ageing process. These, as such, can either be used as a biomarker of the quality of ageing [78], or influence the rate and the quality of ageing [79]. Genome-wide scan has revealed important roles of DNA methylation in human longevity by regulating age-related disease genes [80]. Epigenetic modifications summarize, at least in part, the interaction between the individual genetic background and lifestyle characteristics, and should potentially, along with epistatic processes, account for some of the unaccountable heritability for complex diseases (the missing heritability problem).

Chromatin, the complex of DNA and histone proteins that form chromosomes, can also influence gene expression, over long timescales [81]. Sen et al. [82] established links between common operative ageing pathways and hallmark chromatin signatures that can be used to identify modifiable targets to counter human ageing and age-related disease.

**Gene × environment interactions**

Since longevity is a complex trait with moderate heritability, it is also important to take into account the gene-by-environmental interactions. Genetic signatures are likely different in regard to different ethnicities and specific environmental exposures and therefore the genetic basis of exceptional longevity will vary within these contexts [50]. Fumagalli et al. [83] have reported close association between the variation of a large number of SNPs and environmental variables such as climate, diet regime and pathogen loads (reviewed in Ref. [2]). Individuals also react differently to the environments they are part of, or the environments they construct around themselves. The microbiome is also an example of complex interactions between organisms and the environment. New approaches analysing gene–environment and gene–gene interactions will help us understand the complex mechanisms operating in determining the chance to survive to extreme ages [12].

**Conclusions**

Individuals that have reached advanced ages such as centenarians seem to have just as many disease-associated genetic variants as the average population. Their genetic advantage is possibly due to protective genetic variants that reduce the risk for common age-related diseases such as heart disease, stroke, cancer and Alzheimer’s disease. Efforts should then be first directed at screening for these highly penetrant deleterious mutations that should have caused disease, but have not, and then examine these individuals to identify second site mutations (or perhaps environmental factors) that render them immune to the disease-causing mutations they carry. The genetic basis of lifespan is complex and there is no single genetic factor that predetermines long and healthy life.
 Nonetheless, the continued analysis of the genomes of centenarians should illuminate the processes that contribute to the maintenance of health at extreme ages. Comprehensive analyses are needed to further our understanding of the intricate biological mechanisms underlying human longevity and the potential to extend healthy human life span.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by grants from Bulgarian National Science Fund.

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