Genome-wide approaches to understanding human ageing

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Abstract

The use of genomic technologies in biogerontology has the potential to greatly enhance our understanding of human ageing. High-throughput screens for alleles correlated with survival in long-lived people have uncovered novel genes involved in age-associated disease. Genome-wide longevity studies in simple eukaryotes are identifying evolutionarily conserved pathways that determine longevity. It is hoped that validation of these 'public' aspects of ageing in mice, along with analyses of variation in candidate human ageing genes, will provide targets for future interventions to slow the ageing process and retard the onset of age-associated pathologies.

Keywords: *mortality, life span, genomics*

Introduction

The study of the biology of ageing (biogerontology) has seen a reawakening in recent years. Modern molecular techniques are being applied in an effort to understand both the changes that occur as people age and, perhaps more importantly, to identify the genes that determine how quickly these age-associated changes progress. In addition, as life expectancy has increased in developed nations, an ageing population has contributed to a great societal (and financial) interest in understanding the human ageing process and in ameliorating age-associated physical and cognitive declines.

The genomics revolution, in particular, has begun to have a profound impact on the way that biogerontologists approach the study of ageing. Global gene expression profiling has been used to characterise transcriptional changes associated with age and longevity, as discussed in several recent reviews.[1–6] Proteomics and metabolomics technologies are also now being applied to ageing-related problems[7,8] and, as these technologies continue to mature, will certainly be used more extensively. One particularly important application of these technologies will be the identification of diagnostic biomarkers of ageing and ageing rate.[9–12]

This review will describe the use of genomics methods to identify genes that influence human ageing. Two types of approaches will be discussed: genome-wide studies of allelic variants that correlate with longevity in people and high-throughput life span studies in lower eukaryotes. The synthesis of these approaches is beginning to uncover highly conserved aspects of the ageing process and to identify candidate gene targets for future intervention into human ageing and age-associated disease.

Searching for allelic variants that determine longevity in humans

The long life span enjoyed by most people presents a difficulty for researchers wishing to study the genetic and environmental factors that influence ageing and age-associated disease in people. The lack of accepted biomarkers of ageing rate means that there is no diagnostic test which can be used to determine whether a particular mutation or environmental change is likely to have an impact on longevity.[13] The search for such biomarkers is an ongoing process. For now, however, alternative, methods are being developed to address these questions.

One approach for identifying genetic features that influence longevity is the study of individuals that achieve extreme longevity.[14,15] Centenarians represent just such a group, with approximately 1 in 10,000 people reaching their 100th birthday.[16] There is substantial evidence that genetic components influence human longevity and that centenarians are people who have escaped the common age-associated diseases which account for a large fraction of the mortality in the overall population.[15,17,18] In a pioneering effort to identify genetic polymorphisms over-represented among centenarians, Puca and colleagues used linkage analysis to scan the genomes of 308 individuals belonging to 137 sibships displaying extreme longevity.[19] From this analysis, significant linkage

was noted for a locus on chromosome 4,[19] which was subsequently mapped to the gene coding for microsomal triglyceride transfer protein (*MTP*).[20] Alleles of *MTP* are associated with abetalipoproteinaemia and familial hypobetalipoproteinaemia in humans.[21,22] It therefore seems likely that the longevity-associated allele identified by Puca and colleagues represents an allele that is protective against heart disease.[20]

An alternative to studying extremely long-lived cohorts is to identify genetic polymorphisms that change in frequency across a population as a function of age. For example, a high-throughput single nucleotide polymorphism (SNP)-typing approach — where allele frequencies are determined for a large number of SNPs from individuals of many different ages — has the potential to uncover alleles that influence longevity. Under such a design, alleles that result in disease susceptibility should decrease in frequency with donor age, while alleles that are important for longevity should increase in frequency. This approach was used successfully to identify an isoleucine to valine polymorphism in the protein kinase A (PKA) anchoring protein AKAP2, which correlates with decreased longevity and cardiac disease.[23] *PKA* activity has been linked to ageing in simple eukaryotes.[10,24–26] Mice with altered levels of PKA have phenotypes consistent with an ageing-related role, such as decreased adipose tissue, protection against obesity and elevated expression of uncoupling proteins.[27–29] Surprisingly, no additional large-scale searches for age-related changes in SNP frequencies have been described. As technologies for SNP discovery and quantitative analysis continue to improve, this approach may warrant further attention.

Difficulties in using humans to study human longevity

Both examples of age-correlated polymorphisms highlighted above (*AKAP2* and *MTP*) demonstrate one of the difficulties associated with identifying genes that influence the rate of ageing from genetic studies of longevity in people: the profound effect of a relatively small number of age-associated diseases on human mortality. A majority of deaths in developed nations occur as a result of a relatively small number of age-associated diseases, including cardiovascular disease, cancer, stroke and diabetes. Thus, one of the prerequisites for achieving extreme longevity is a reduced risk for these diseases, and polymorphisms conferring reduced risk to one or more of these diseases can have a significant effect on individual longevity. In the examples of *MTP* and *AKAP2*, the observed effects on longevity are almost certainly due to an altered risk of cardiovascular disease.[20,23] Does this mean that individuals with the longevity-associated allele of *MTP* are ageing more slowly? Not necessarily. The risk for one or more age-associated phenotypes is reduced; however, there is no evidence that many or all age-associated phenotypes are also retarded in such individuals. Other alleles have also been correlated with life span in people (Table 1), and in almost every case can be attributed to delayed onset of one, or a few, age-associated disease(s).

A second difficulty in population-based studies of human longevity is controlling for population-specific factors.[50] For example, the prevalence of specific age-associated diseases is variable in different populations. This can be due to any combination of cultural (eg diet), historical (eg famine), environmental (eg exposure to toxic chemicals) or genetic components, and can have a profound impact on the types of genotypic variants that influence survival to old age. Recently, it has been suggested the role of *MTP* as a longevity-related locus may be specific for the cohort used in the study from which it was initially identified.[50,51] Simply designing an appropriately controlled human longevity study is challenging, and care must be taken to avoid population stratification. Thus, it will be important to test any candidate human longevity locus in multiple populations to determine the generality of the correlation.

A third potential barrier to identifying genetic variants that have a significant impact on human ageing may be that such variants carry a large selective disadvantage and have been culled from the gene pool. To date, no genetic variant has been definitively shown to slow the rate of human ageing, although rare mutations can accelerate at least some aspects of the ageing process, resulting in progeria syndromes.[52] Abundant evidence from model organisms, however, suggests that mutations in single genes can dramatically slow the rate of ageing and the onset of many (perhaps all) age-associated phenotypes. On the surface, it may seem surprising that, if such 'master regulators' of ageing exist in people, no alleles have been identified that confer extreme longevity. The examples from simpler eukaryotes, however, also demonstrate quite clearly that these types of mutations often come with significant fitness and reproductive costs.[53–55] Thus, strong longevity-enhancing alleles in genes that influence ageing rate are likely to have been selected against during recent human evolution, perhaps making their detection by large-scale polymorphism studies impossible.

Genome-wide approaches to identifying 'public' pathways of ageing

The use of model systems for ageing-related research provides an avenue for getting around many of the difficulties associated with human studies. Both mice and rats are commonly-used mammalian models for ageing and longevity studies. Several simple eukaryotic models have also been developed, including the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*.[56] Perhaps the greatest advantage afforded by model

Table 1. Selected genes for which polymorphisms have been reported to correlate with human longevity. Obvious life span-shortening disease alleles have been excluded. It should be noted that, in many cases, the reported correlation with longevity has been disputed by subsequent work.

Gene	Gene function	Reference
3' *APOB-VNTR*	Apolipoprotein B	30
5HTT	Serotonin transporter	31
ACE	Angiotensin-converting enzyme	32
APOE	Apolipoprotein E	32
CETP	Cholesteryl ester transfer protein	33,34
GH1	Growth hormone	35
IGF-1R	Insulin-like growth factor (IGF)-I receptor	36
IL-10	Interleukin 10	37
KLOTHO	Insulin/IGF-I-repressing hormone	38–40
MTND2	Mitochondrial NADH dehydrogenase	41
MTP	Microsomal triglyceride transfer protein	20
NR3C1	Glucocorticoid receptor	42
PI3KCB	Phosphatidylinositol 3-kinase catalytic subunit	36
PPARG	Peroxisome proliferator-activated receptor gamma 2	43
SHC1	SHC-transforming protein 1	44
SIRT3	Sirtuin protein deacetylase	45
TH	Tyrosine hydroxylase	46
TLR4	Toll-like receptor 4	47
TP53	Tumour suppressor p53	48,49

Abbreviations: NADH = the reduced form of nicotinamide adenine dinucleotide; SHC = Src homology 2 domain-containing.

Table 2. Potential conserved determinants of longevity. Genes shown have been reported to increase life span when mutated in more than one organism.

Gene family	Description
Catalase	Increased catalase activity increases life span in yeast[62] and mice[63]
Insulin/insulin-like growth factor-I receptor	Mutation of insulin and insulin-like growth factor-I receptor genes increases life span in worms,[64,65] flies[66] and mice[67,68]
Rpd3	Deletion of *RPD3* increases life span in yeast[69] and flies[70]
Sch9/Akt	Decreased *SCH9/AKT* activity increases life span in yeast[25,26,62,71] and worms[72–74]
Sir2	Increased expression of Sir2-family proteins increases life span in yeast,[75] worms[76] and flies.[77] A variant allele of a human homolog, *SIRT3*, is reported to correlated with longevity[78,45]
Superoxide dismutase	Increased superoxide dismutase activity increases life span in yeast[62] and flies[79]
TOR	Decreased *TOR* activity increases life span in yeast,[26,80] worms[81,82] and flies[83]

organisms for ageing-related studies is that the relatively short life spans of these organisms allows for controlled longevity studies to determine whether a particular mutation or environmental change alters ageing rate. In particular, interventions that increase population life span are of interest because they either slow the rate of ageing, delay the onset of ageing or both. Several dozen single-gene mutations that significantly increase life span have been identified from studies in model organisms.[57] More recently, genome-wide screens for longevity have been carried out in both yeast and worms, resulting in a wealth of data and a better understanding of the degree to which the genetic basis of ageing has been conserved.

A question that is often raised when considering the usefulness of model organisms in ageing research is whether the ageing process has been sufficiently evolutionarily conserved that the mechanisms of ageing are shared between lower eukaryotes and people, or even between non-human mammals and people. While this question is impossible to answer at this time, there is reason to think that at least some aspects of ageing are highly conserved.[58–60] For example, all of the model organisms used for ageing-related research display an approximately exponential increase in mortality with age (Gompertz-Makeham-like mortality), consistent with the idea that important aspects of the underlying ageing process is conserved.[61] Perhaps the most compelling reason to think that ageing is highly conserved is the recent identification of several conserved determinants of longevity — orthologous genes (or similar environmental changes) that determine ageing rate in evolutionarily divergent organisms (Table 2). These conserved longevity determinants are likely to regulate 'public mechanisms of ageing'[84] that have been maintained

through evolution and which determine longevity in response to environmental cues, such as nutrient availability.

Genome-wide studies of ageing in worms

C. elegans has proven to be one of the most important model organisms for ageing-related research, providing the first well-characterised model for the role of insulin/insulin-like growth factor I (IGF-1) in ageing. Several components of this pathway have been shown to regulate longevity in worms, including an insulin-like receptor (Daf-2),[64,65] a phosphatidyl-inositol 3-kinase (Age-1),[85,86] proteins orthologous to Akt kinases (Akt-1, Akt-2 and Sgk-1)[72–74] and a FOXO-family transcription factor (Daf-16).[87,88] Global gene expression profiling by microarray has further elucidated some of the downstream components of this pathway, which are involved in antimicrobial, oxidative and other stress responses.[89,90]

The true power of *C. elegans* as a model for ageing-related research has become apparent with the development of RNA interference (RNAi) as a technology for gene expression knock-down in worms. The typical food source provided to *C. elegans* is live *Escherichia coli* grown on a solid medium. *E. coli* expressing a plasmid-encoded double-stranded RNA corresponding to a *C. elegans* open reading frame (ORF) knocks down expression of the targeted gene.[91] An RNAi library corresponding to 17,000 unique genes (~85 per cent of *C. elegans* ORFs) has been constructed,[92] and two independent genome-wide RNAi screens have been carried out for genes that influence longevity in worms.[73,93–95] Genes that increase life span when knocked down can be grouped into functional categories, the largest being genes important for mitochondrial respiration and genes involved in insulin/IGF-I signalling. Several uncharacterised genes were identified as well, suggesting that important aspects of the ageing process remain uncharacterised, even in simple eukaryotes.

A multi-organism approach to identifying public pathways regulating longevity

Recently, a genomic approach to uncovering genetic determinants of longevity that have been conserved from yeast to mammals has been described, based on the hypothesis that protein families which function to determine ageing rate in both yeast and worms are likely to play a similar role in mammals.[10,96] The first phase of this proposal involves genomic analysis of ageing in yeast.[97] Two types of ageing are commonly studied in yeast: replicative life span, which refers to the number of times a yeast cell can divide prior to senescence,[98] and chronological life span, measured by the length of time a cell can survive in a non-dividing state.[99] High-throughput assays for both replicative and chronological ageing have been developed,[26,100] which will allow for life span determination of approximately 4,800 single-gene deletion strains contained in the yeast ORF deletion collection.[101]

For each yeast ageing gene identified from these screens, the homologous genes in *C. elegans* (if any) will be examined by measuring life span in response to RNAi-mediated knock-down. Those orthologue pairs that determine longevity in both yeast and *C. elegans* will then be candidates for further study in a mammalian system. Conditional and tissue specific knockout of mouse genes orthologous to conserved yeast and *C. elegans* ageing genes will be carried out and selected lines subjected to life span analysis. Due to the costly nature of rodent longevity studies, this is an exceptional method for identifying high-interest candidates. Clearly, any gene family found to determine life span in yeast, worms and mice will be of great interest as a likely determinant of human longevity.

Even prior to life span studies in mice, the identification of longevity determinants conserved in simple eukaryotes, such as yeast and worms, will allow for SNP analysis of human orthologues to determine whether there is a correlation of certain alleles with longevity or age-associated disease. For example, increased expression of Sir2-family proteins increases life span in yeast,[75] worms[76] and flies,[77] and alleles of a human homolog, SIRT3, are reported to correlate with longevity in people[78] (Table 1). Likewise, mutation of the insulin/IGF-I receptor homologues increase life span in worms[64,65] and flies,[66] and it has been suggested that allelic variants in the human IGF-I receptor correlate with longevity.[36] The nutrient-responsive kinases TOR (target of rapamycin), PKA and Sch9/Akt represent additional high-interest candidates, for which human longevity data have not been reported. More subtle effects on human ageing, which may be masked by disease alleles in genome-wide scans, can potentially be uncovered by this type of targeted approach based on knowledge gleaned from model organisms.

Conclusion

The further development and application of genomics methods toward biogerontology has the potential to dramatically enhance understanding of human ageing. Genomic approaches have already uncovered genes important in the onset of human age-associated disease. In simpler eukaryotes, genome-wide studies are rapidly providing a detailed picture of the molecular pathways that regulate ageing. The most effective future studies may come from combining the genes identified in simpler organisms with technologies to rapidly uncover allelic variation in human orthologues which may influence longevity and disease. There is ample reason for optimism that these approaches will enhance our understanding of the molecular biology of ageing and, ultimately, our ability to treat age-associated disease.

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